Vice President of International Association of Neurorestoratology
Chair of Educational& Training Committee of International Association of Neurorestoratology
President of the Romanian Society of Neurology
President of the Society for the Study of Neuroprotection and Neuroplasticity (SSNN)

Dafin F. Muresanu (Romania)

Shifting the paradigm in neurorestoration - from basic concepts to clinical strategies

Nowadays, it is still difficult to find the correct therapeutic approach for brain protection and recovery in stroke, especially because we do not fully understand all of the endogenous neurobiological processes, the complete nature of the pathophysiological mechanisms and the links between these two categories. Endogenous neurobiological processes, such as neurotrophicity, neuroprotection, neuroplasticity and neurogenesis, are central to protection and recovery and represent the background of endogenous defense activity (EDA).Stroke pathological cascades contain a limited number of pathophysiological processes. It is characterized mainly by excitotoxicity, oxidative stress, inflammation, apoptotic-like processes and and important metabolic disturbances.Pathophysiological processes share some common mechanisms with EDA (e.g. excitotoxicity and neurotrophicity together with neuroplasticity have, as a common important driver, the NMDAR activity; inflammation has an important contribution for neuroregeneration, stimulating neuroplasticity, via trophic factors).Postlesional brain regulation is currently better understood. Every lesion in the nervous system triggers in the first minute an endogenous neuroprotective reaction. An endogenous repair process, combining neuroplasticity and neurogenesis follow this as a second answer. All these processes are initiated and regulated by endogenous biological molecules.The biological reality of the nervous system is far more complex. In fact, there is an endogenous holistic process of neuroprotection and neurorecovery that should be approached therapeutically in an integrated way.The current tendency to exclusively frame drug activity in terms of single mechanisms and single focus effect might distract from other paradigms with greater explanatory power and hinder the development of more effective treatment strategies. A change of concept is required in pharmacological brain protection and recovery in stroke therapy. This presentation briefly reviews the current and future considerations in this therapeutic strategy, including an integrated pharmacological approach, focusing on drugs with multimodal activity rather than single mechanism drugs, which usually are chemical drugs. In line with this strategy the current presentation will also highlight the result of CARS Trial, one of the latest double blind placebo randomized controlled trial in the filed.

Founding President, International Association of Neurorestoratology
Founding director, Neurorestoratology Professional Committee of Chinese Medical Doctor Association
Editor in Chief of Journal of Neurorestoratology
Honorary Director and Chief expert, Institute of Neurorestoratology, General Hospital of Chinese Armed Police Forces

Hongyun Huang

OECs——The best neurorestorative cells for neurological damages and diseases

Since olfactory ensheathing cells (OECs) were clinically transplanted for patients with complete chronic spinal cord injury (SCI) in 2001, they have been used in many intractable neurological diseases and damages such as ALS, chronic stroke, cerebral palsy, Alzheimer disease and soon on. Following OEC transplantation, patients with complete chronic SCI can restore some neurological functions and improve their quality of life, such as hand function, walk, and urine control; patients with ALS can stable or improve their neurological functions and improve their quality of life; patients with chronic stroke can markedly improve their quality of life, such as walk and language expression; patients with cerebral palsy, Alzheimer disease also can improve their quality of life. Here we review important preclinical and clinical evidences and summary our team experience to explain why OEC is the best neurorestorative cell for neurological damages and diseases. OECs display unique properties, which share many common properties of astrocytes (GFAP expression), Schwann cells (p75) and oligodendrocytes (O4) and can support olfactory nerve in adult life extend from olfactory epithelium into brain across the boundary between PNS-CNS, which are only a kind of cells to be able to freely migrate between PNS-CNS without any limitation in nervous system. After being transplanted, they can restore, promote and maintain the integrity of impaired or lost neuronal functions and/or structures through mechanisms of neuroprotection, supporting axonal regeneration, remyelination, neurorepair, neuroplasticity, neuromodulation, neurogenesis, angiogenesis, and anti-inflammatory response, and soon on.

Alok Sharma

Vice President of International Association of Neurorestoratology

Chair of Ethical Committee of International Association of Neurorestoratology

Director of the NeuroGen Brain & Spine

An overview of the new regulations for cell therapy in different countries

Cellular therapy has emerged as a promising therapeutic modality as part of Neurorestoratology. Although the evidence of its safety and efficacy is growing rapidly, the regulations for these have not evolved as fast. Many of the countries have no distinction in the regulations for cellular therapy from drugs. Majority of the countries that have separate regulations for cellular therapy do not make a distinction between different types of cell therapies or don't differentiate between cell therapy products for marketing and cell therapy services provided by doctors. However, the scenario is changing and few countries have taken into consideration the intricacies of cellular therapy and adapted their regulations to nurture its growth. Japan took a bold initiative when in late 2014 a partial amendment was made of the older Pharmaceutical Affairs Law (PAL). The most important aspect of the Japanese regulations is that a clear distinction has been made between cell therapy products and cell therapy services and two separate laws have been framed for each. The Pharmaceutical, medical devices and therapeutic products act (PMDAct) is intended to provide a fast track approval mechanism for the marketing of stem cell products. On the other hand The Act on Safety of Regenerative Medicine (ASRM) applies to medical institutions that use cellular transplantation therapeutically in clinical trials or medical practice. In this there is a risk stratification into low risk, medium risk and high risk forms of therapy with different levels of regulations based on the risk. The Korean Regulations too make a distinction between the cell therapy products and cell therapy being offered as a medical service and they have excluded minimally manipulated cells from their 'Review and authorization of Biological products'. The most awaited transformation would be that from the USA. A completely new law called the Reliable and Effective Growth for Regenerative Health Options that Improve Wellness Act (REGROW Act) is under consideration by their Senate. This Act allows for a conditional approval of minimally and more than minimally manipulated cells for autologous use for a period 5 years. India too is in the process of modifying its existing Drugs and Cosmetics act to incorporate new progressive regulations for stem cell therapy. Although other countries in the world do not have such progressive regulations, many countries allow for the use of unproven cellular therapy products and treatments for patients suffering from incurable disorders for which currently no other treatment is available. European medical agency has a provision for 'Compassionate use' of unapproved medical treatments if their safety is sufficiently established. They have also devised a PRIME (PRIorityMEdicines) act that not only allows the use of such products but also creates an opportunity for expediting the research for these products. Hospital Exceptions (HE) act in Europe is also favorable to newer therapies such as regenerative medicines which allow hospitals to provide unapproved therapies under the compassionate use schemes. Compassionate use laws are present in many other countries like USA –Right to try act, Canada – Special access program, Australia – Special access scheme; all of which allow for the use of unproven medical treatments for terminally ill patients. Several other countries are also coming up with newer more favorable regulations. The regulatory scenario in the world is changing in the favor of the growth of cellular therapy field, but slowly. The highlights of these new regulations are the new concepts that are emerging like 1. Conditional marketing approval, 2.Risk Stratification, 3.Post-Hoc efficacy analysis, 4.Presumed efficacy, 5.Patients' right to seek treatment, 6.Distinction between cellular therapies, 7.Distinction between a stem cell product and medical service. 8. Compassionate use. These concepts distinguish the cellular therapy regulations from conventional drug regulations. It is important that all the countries in the world understand and adapt these concepts and design regulations for cellular therapy.

Biography

Dr. Alok Sharma is a Neurosurgeon and presently is the Director of the NeuroGen Brain & Spine Institute, Professor & Head of Department of Neurosurgery at the LTMG Hospital & LTM Medical College & Consultant Neurosurgeon Fortis Hospital in Mumbai, India. He completed his MS and MCh from Seth G.S. Medical College and KEM Hospital of Mumbai University and subsequently trained at the Karolinska Hospital in Stockholm Sweden & the University of Colorado Health Sciences Center Denver USA. He has authored 12 books, edited 2 books, contributed chapters to 8 other books, has 112 scientific publications and has made over 150 scientific presentations nationally and internationally. He has published path breaking results of Stem cell therapy in various neurological disorders. He is founding President of the "Stem Cell Society of India" and Vice President of the "International Association of Neurorestoratology." He is Founder of "The Indian journal of Stem Cell therapy" and on the editorial board of 4 journals. He has been conferred with numerous awards and honors in his distinguished career. His other areas of special interest are Neuroendoscopy, Psychosurgery, Spinal fixations & Revascularization for cerebral ischemia.

Past President of International Association of Neurorestoratology
Director, Int. Exp. CNS Injury & Repair (IECNSIR)
Professor of Neurobiology (MRC)

Hari Shanker Sharma
(Sweden)

Nanodelivery of mesenchymal stem cells in combination with cerebrolysin induces superior neuroprotective effects in alzheimer's disease

Alzheimer's Disease (AD) induced brain pathology is instrumental in causing functional and behavioral disturbances in patients for which there are still no suitable therapeutic strategies are available. Thus, efforts should be made to find novel therapeutic drugs and/or delivery system to reduce brain pathology caused by AD. Several research reports in AD suggest deposition of amyloid- -peptide (A P) within the brain as instrumental in precipitating the pathophysiology of AD. However, this is still unclear whether A P P is directly or indirectly involved in causing pathogenesis of AD. New lines of evidences suggest that A P induced oxidative stress and related molecular consequences could play key roles in AD pathology. Thus, the possibility exists that modulation of A P on brain function may alter the course of AD pathology.Experiments were carried out on Male Sprague Dawley rats (250-300 g, Age 30 to 35 weeks). In these rats A P (1-40) was administered intraventricularly (i.c.v.) in the left lateral ventricle in a dose of 250 ng/10 µl once daily for 4 weeks. After 30 days of the 1st A P infusion, the rats were examined for A P deposits in their brain, gliosis and neuronal loss. In addition these animals were also tested for behavioral disturbances using Rota Rod treadmill, inclined plane angle test and water maze performances using standard protocol. Control rats received saline instead of A P for comparison under identical conditions.We observed distinct ABP deposits within the cortex and in hippocampus as compared to the control group. Increased glial fibrillary acidic protein (GFAP) immunoreactivity, loss of myelin basic protein (MBP) immunoreactivity and increase in albumin immunoreaction were also seen in the experimental group whereas control group did not show any significant changes in these parameters. The cell loss as examined suing Nissl stain was prominent in the ABP infused rats whereas saline treated animals did not show neuronal loss or distortion. The behavioral disturbances on Rota Rod performances and inclined plane angle tests were significantly deteriorated along with the ability to retrieve platform in water maze tests in ABP infused rats. When Cerebrolysin (25 µl) was infused into the left cerebral ventricles daily starting from 1 week after the onset of ABP infusion and terminated 1 week before ABP last infusion, the brain pathology was significantly reduced and the behavioral functions were markedly improved. Whereas, when Cerebrolsyin was started 2 weeks after A P infusion and continued until last infusion of ABP, the pathological changes and behavioral improvements were only mildly affected. At this time period TiO2 nanowired mesenchymal stem cells (MSCs) alone or in combination with cerebrolysin has better neuroprotective effects in AD. On the other hand when TiO2-naonowued Cerebrolsyin was administered together with TiO2-nanowred MSCs under identical conditions starting 2 weeks after A P infusion and continued until the last A P infusion, the brain pathology and deposition of A P in the brain were markedly attenuated, The behavioral function were significantly improved in the animals treated with nanowired cerebrolysin together with nanowired MSCs after A P infusion. Interestingly, nanowired combination of Cerebrolysin and MSCs was also able to induce pronounced neuroprotection and behavioral improvements if the drug was given only for 1 week daily starting from 2 weeks after A P infusion and the A P was continuously infused until 1 week after the termination of cerebrolysin and MSCs administration. These observations are the first to suggest that nanowired Cerebrolsyin and MSCs administration if given during a critical therapeutic time window has synergistically superior neuroprotective ability in attenuating AD pathology.

President of International Association of Neurorestoratology

Iran Professor of Neurosurgery

Director of Brain and Spinal Injury Research center

Hooshang Saberi (Iran)

Role of Granulocyte-Colony Stimulating Factor for Neurological Changes in Traumatic Incomplete Subacute Spinal Cord Injuries

Introduction: Granulocyte-colony stimulating factor (G-CSF) is a major growth factor in the activation and differentiation of granulocytes. This cytokine has been widely and safely employed, in different conditions over many years. In this study we tried to administer the drug for spinal cord injury. Methods: Twenty four patients with spinal cord injury of at least six month duration were included in this study. Patients were assessed by ASIA, SCIM III and IANR-SCIFRS just before intervention and at six month after subcutaneous administration of 5μg/Kg of Granulocyte-Colony Stimulating Factor in the case group and placebo in the control group. Randomization was performed with random block design, the patients and evaluators were blinded in regard to the treatment group. This study was conducted on 24 traumatic SCI patients. Sixteen patients were studied in GCSF group and eight patients in placebo group. The mean (SD) age of the patients was 33.2 year (11). There were 22 male and 2 females in our case. Results: After 6 months of intervention ASIA Impairment Scale (AIS) in control group remained unchanged while in GCSF group 1 AIS B patients improved to AIS C and 2 AIS C patients improved to AIS D. The mean improvement in ASIA motor score in GCSF group was 13.49 scores that was higher than control group (1.5 scores) ($P>0.05$). The mean light touch and pin prick sensory scores increased by 11.00 and 6.75 scores in GCSF group and by 0.5 scores for both sensory indices in control group. Although the increment in sensory scores in GCSF group were higher than control group but it was not significant ($P>0.05$). Evaluation of functional improvement by FRS instrument revealed borderline significant improvement in GCSF group (5.37 scores) compared to the control group (0.00 scores) ($P=0.070$). However no significant difference in functional improvement between the two groups was observed by SCIM instrument ($p=0.129$). Conclusion: Subcutaneous Granulocyte-colony Stimulating Factor administration in Subacute incomplete spinal cord injuries is associated with borderline significant motor improvement. Study in larger number of patients with longer follow up may be necessary to arrive at more detailed results.

Keywords: Spinal Cord Injury, Granulocyte-Colony Stimulating Factor, Neurological Restoration

Shiqing Feng

Elect President of the
International Association of Neurorestoratology
Vice Chairman of Neurorestoratology Professional Committee of Chinese Medical Doctor Association
President of Tianjin Association of Orthopaedic Surgeons

How to Make a Breakthrough in Spinal Cord Injury Repair

Spinal cord injury (SCI) is a catastrophic event that is sudden, unexpected and can be devastating and costly in human and social terms. The SCI can be induced by various causes, including traumatic and non-traumatic ones. With the development of economy and society, the epidemiology of SCI exhibits new features, especially in developing countries such as China. Our research team has investigated the causes of spinal cord injury in Tianjin and found that cervical spinal cord injury accounts for 70% of all cases and most of them happened at the C5 level. All of the current treatments, including operation, rehabilitation, medicine and cell transplantation, have flaws. And the patients who get completely neural functional recovery from SCI were less than 1%. Different form the poor regenerative ability in central nervous system, it is well established that peripheral nerve can get a better regeneration after injury. In PNS, the supporting cells are Schwann cells, which play a very important role in nerve function recovery. However, in CNS, the supporting cells include oligodendrocytes, which disintegrate to axonal regeneration inhibitors, and astrocytes which are main constituents of glia scar forming. The pathophysiologic characteristic of SCI can be divided into two stages, the primary injury and secondary injury. The secondary injury includes a cascade of biological events and is deemed as the main cause of difficulty in spinal cord injury recovery. Our research team has raised the hypothesis that the imbalance of microenvironment is the main cause for limited regeneration after SCI, which includes increased inhibitive factors, such as Nogo and MAG, and decreased promotive factors, such as BDNF, NGF, NT3, etc. Based on this theory, we may re-establish the balance of microenvironment by suppressing inhibitive factors, or promoting promotive factors. For high precision and accuracy, different therapeutic methods should be adopted in different stages. In acute and short-term, immunomodulatory drugs, especially, anti-inflammatory drugs, are recommended. Our research team has found that a latest medicine, nafamostat, can alleviate the local inflammatory reaction and has a synergistic effect with many other treatments. In subacute, using the technology of RNAi to silence PTEN or NGR, we found that neurons can overcome the microenvironment inhibition, which results in the recovery of function. Meanwhile, by application inhibitor of Ras/Raf/ERK1/2, synapse formation can be restored. We have conducted some researches in order to overcome these obstacles and some important advances have been achieved. Valproic acid (VPA) and all-trans-retinoic acid (ATRA) could induce neuronal differentiation and facilitate neurite outgrowth at the expense of astrocytic differentiation in neural stem cells (NSCs). Another important inhibited factor is the imbalance between neurotrophin and its precursor. Using antibody or small molecular inhibitor targeted proBDNF or its receptor-P75NTR, we prevented cortical neurons and DRG from apoptosis as well as growth cone collapse. We proved that Schwann cells can improve SCI microenvironment. By manipulating this rule, we use combinatorial strategies to facilitate the effects of many other methods. At the end, we forecast the further research and make prospects of future work. Through the using of more advanced technology, we may deepen our understanding of this disease, especially the raising of Precision Medicine Initiative, which can guide us to figure out more effect methods and provide more individualized treatment programs to repair it.

Chair of Translational Medicine Committee of International
Association of Neurorestoratology

Wise Young (USA)

Umbilical cord blood mononuclear cell therapy of chronic complete spinal cord injury

We recently complete phase II trials in Hong Kong and Kunming showing that human leukocyte antigen (HLA) matched (>4:6) umbilical cord blood mononuclear cells (UCBMNC) can be safely transplanted into the spinal cord, stimulates fiber growth across the injury site, and improves walking, bladder, and bowel function in people with chronic (>1 year) complete (ASIA A) spinal cord injury (SCI) when combined with intensive walking training. Patients that did not receive intensive 6 hours daily, 6 days a week, and 6 months of intensive walking training did not recover walking. The study also suggested that neither methylprednisolone (MP) nor a 6-week course of oral lithium carbonate improves neurological recovery. These results were quite surprising for the following reasons. First, no previous study had shown improved walking in patients with chronic complete spinal cord injury after cell transplantation or locomotor training. Second, patients recovered walking but not voluntary motor control of the legs when lying prone, suggesting that they have recovered walking by activating their central pattern generator rather than direct activation of their motoneurons. Third, walking recovery did not necessary correspond with bladder and bowel recovery. Even though 60% of the subjects in Kunming recovered bladder and bowel function, this did not correspond necessarily with walking recovery. Based on these findings, we conclude that UCBMNC stimulates long tract spinal cord regeneration but the regeneration was not necessarily associated with locomotor, bowel, or bladder recovery. While many patients recovered walking after transplantation and intensive locomotor training, they did not always recover bowel and bladder function. We are currently planning clinical trials in India, USA, China, Taiwan, and Europe. These trials will be discussse

Vice President of International Association of Neurorestoratology
Chair of Organization Committee of International Association of Neurorestoratology
Full Professor of Immunology& Cellular Therapy
Visitant Professor of Cellular Therapy

Gustavo Moviglia (Argentina)

How to prove the principles of CSCI regeneration using the same technology on blinded people, controlling with OCT and electrophysiology tests as well as vision tests

Introduction: Recently our group have demonstrated, after a Phase 1-2 clinical Trial, that using a combination of three different cellular intravascular implants named MED therapy (Mesenchymal Stroma Cells, MSC, Anti neural Lymphocytes, EC, and Differentiated Stem Cells, DSC) chronic and complete Spinal Cord Injured (ccSCI) Patients may be treated successfully recovering, in a safe mode, the reconnection in between both sectioned spinal cord ends. This reconnection also is reflected in the acquisition of neural functions that effectively improves the quality of life of the patients and their reinsertion into the labor world. Unfortunately, beside some electrophysiological indications, their mode of action cannot yet be fully elucidated. Eye is biologically considered as an extension of the Central Nervous tissue and Chronic aging macular degeneration (AMD), that is the major cause of blindness in the world, have several pathologic features similar to ccSCI. Moreover, the transparence of the pre-retina elements, associated on the modern development of Optic Observation allow us to have a direct access to the way that each kind of cells act repairing the retina and nerve optic damage.Digital biopsy is a novel method based in the study of the OCT (Optical Coherence Tomography) image through the Fournier analysis that allows extra-polarization of the OCT pixels information1. Using this method, pathologist has been able to construct histological images of intraocular tumors without surgical sampling. The method maybe an important tool to analyze the engraftment and further development of MSC, EC and DSC after their ophthalmic intra artery injection. Materials and method 7 patients with retinal atrophy secondary to aging macular degeneration (AMD) and one with traumatic damage of optic nerve were selected to the study. Procedures to obtain the different cell products used for MED therapy may be summarized as follow: autologous adipose tissue was obtained through Lipectomy in an operating room. Cells were processed in a GMP laboratory inside a Biosafety 3 clean rooms. Enzyme dissociation was done using Collagenase Type 4 (Thermo-Fisher), DNAse1 (Thermo-Fisher), and mechanical dissociation was done using GentleMACS dissociator (Miltenyi®) and 70micron plastic grids (thermo-Fisher). Adipose MNC and MSC cells were culture in DMEM TC (Gibco) enriched with rhInsulin, and human platelet lysate. Blood mononuclear cells are obtained through an Leukapheresis using a Spectra-Optia® cell separator. Mononuclear cells were challenged with a pharmaceutical IV grade Brain Lysate (Cerebrolysin®) in DMEM TC (Gibco®). Activated cells were purified from CD8+, CD25+ and CD56+ cells using the Clinimacs immune selection system (Miltenyi®) with the correspondent immune magnetic marked monoclonal antibodies (Miltenyi®). The OCT (OCT 3D 1000, TopCon Inc, Paramus, NJ) images were taken previous MSC implant and one month after it. Results After a month of intervention, 6/7 patients showed, through digital biopsy, quantitative and qualitative analysis, increase of choroidal capillary network as well as improvement of the size and trophism of the retina pigmentary epithelium. At four months after implant they improved the morphology of neural components of the retina. Electrophysiological measurements, as well as clinical studies shows strong parallel with these morphologic. changes are linked to the proposed mode of action of MED therapy. Conclusion The present methodology, that was validated for intraocular tumors analysis, seems to be useful to demonstrate the dynamic changes performed after MED therapy where the main action of MSC implant is neovascularization of the affected area, EC mode of action is to allow the entry of DSC into the nervous part of the retina and DSC to rebuild the nervous tissue itself.

Michael Chopp (USA)

Vice Chair of the department of Neurology

at the Henry Ford Hospital

Replacing stem cells with exosomes for the treatment of stroke, and neurological injury and disease

Stem cell therapy, in general, is based on the supposition that dead or injured tissue can be replaced by exogenously administered stem cells. This has been quite clearly demonstrated, particularly for treatment of stroke in the adult, to be a false premise. Exogenously administered cells, rarely undergo differentiation into parenchymal cells and integrate in substantial numbers into tissue. Cell-based therapies for the treatment of stroke, neural injury and neurodegenerative diseases are essentially catalysts that enhance endogenous restorative mechanisms within the organism. After stroke, the administered cells promote neurovascular remodeling and CNS plasticity by multiple mechanisms including paracrine mechanisms which stimulate parenchymal cells to produce trophic factors which enhance neurological recovery. Stem cells evoke neurological recovery by communicating with the parenchymal cells and other organs to reboot restorative processes. Stem cells send out nanoparticle bilipid containers, called exosomes, which are absorbed by parenchymal and other cells. As cargo, these exosomes contain proteins, mRNA, RNA, lipids and microRNA. And it is the content of the exosomes, to a large extent the microRNAs that communicate instructions to parenchymal cells and to distant organs to initiate restorative processes. Thus, instead of using stem cells to treat neurological disease and stroke, it is reasonable to employ exosomes, the product of the stem cells, to amplify neurorestorative processes. Our data demonstrate that treatment of stroke and traumatic brain injury with the exosome product of stem cells significantly enhance neurological recovery parallel to stem cell treatment. Exosomes may be a more viable, safer, and likely a more effective approach for the treatment of stroke, neural injury and neurodegenerative disease. Keywords: exosomes, microRNA, stem cells, stroke, traumatic brain injury, neurological recovery

Russell J Andrews (USA)

Past Chair of American Association of Neurological Surgeons

Co-Director of International Conference on Neuroprotective Agents

Nanotechniques and the Brain-Machine Interface (BMI)

The brain-machine interface (BMI) – like the communication between two computers - has both hardware and software aspects. The hardware is the electrode that facilitates the charge transfer (for brain electrical activity monitoring and modulating) and the electrode that detects neurotransmitter changes (for brain neurochemical monitoring). The software is the technique used to control the hardware for effective interaction with the brain. The BMI is an essential part of both deep brain stimulation (DBS) for the treatment for neurological disorders such as movement disorders (e.g. Parkinson's disease), epilepsy, and depression and neuroprostheses for the treatment of neurological deficits such as spinal cord injury, blindness, and hearing loss.Nano-enabled electrodes improve electrical charge transfer between the computer "machine" and the brain by orders of magnitude over standard noble metal electrodes (e.g. platinum, silver) through reduction in impedance and enhancement of capacitance. Nano-enabled electrodes also allow the simultaneous monitoring of multiple neurotransmitters in close proximity to each other (i.e. within tens of microns, roughly the diameter of a neuron or an astrocyte) – which standard carbon microfiber electrodes are not capable of doing.On the BMI software side, computational analysis methods (e.g. coordinated reset – CR) allow much more efficient parameters for deep brain stimulation (DBS). This not only extends DBS pulse generator (battery) life, but CR can actually teach the brain to give up the abnormal electrical firing patterns underlying disorders such as Parkinson's disease and epilepsy. For neuroprostheses where an electrode array (either implanted on the cortical surface or closely-spaced scalp electrodes) monitors the changes in brain electrical activity that can be used to drive a prosthetic limb (or to drive the patient's own neurologically disconnected limbs), novel "software" techniques that rely on the brain teaching the prosthesis (rather than the brain learning to drive the prosthesis) can markedly enhance the efficiency of the BMI.Nanotechniques for the BMI not only offer remarkable improvement in the efficacy of treatments such as DBS and neuroprostheses, but also offer the possibility of understanding – at both the cellular and network levels - the electrochemical aberrations underlying devastating neurological disorders such as intractable depression and epilepsy. keywords: brain-machine interface, computational analysis, deep brain stimulation, nanoelectrodes, neuroprostheses

Department of Pharmaceutical Sciences, Jude Children's Research Hospital

Yu Fukuda （Japan）

Innate immunity as a pharmacological target for neuronal protection and repair

Infected or damaged tissues are known to release multiple “alert” molecules such as DAMPs and alarmins, which are recognized by innate immune receptors and, in turn, induce tissue inflammation, regeneration and repair. Recently, an extract from inflamed rabbit skin inoculated with vaccinia virus (Neurotropin®) was found to induce infarct tolerance in mice accepted transient ischemia attack at middle cerebral artery. Here I introduce our recent *in-vitro* works to explore molecular mechanisms underlying such neuroprotection using rat pheochromocytoma PC12 cells.Neurotropin prevented retraction of growth cones of the neurites in PC12 cells with nutrient deprivation. Because the effect was accompanied by activations of intracellular signaling cascades initiated by high-affinity NGF receptor Trk, molecular mechanisms of Neurotropin were further analyzed by focusing on Trk autophosphorylation. As a result, Neurotropin induced association of Trk with GM1 ganglioside, an essential co-factor for Trk tyrosine kinase, and significantly accelerated the time course of Trk autophosphorylation stimulated by NGF, although Neurotropin itself failed to induce Trk autophosphorylation. Since GM1 is known to localize in the lipid rafts, we next examined lipid raft formation in cells treated with Neurotropin. In density-gradient subcellular fractionation assay, a minor lipid raft-like membrane fraction with higher buoyant density and Trk molecules was found to be formed in the Neurotropin-treated cells. Additionally, this membrane fraction contained innate immune receptor TLR4. TAK-242, a specific TLR4 inhibitor, prevented formation of the lipid raft-like membrane fraction as well as neurorestoration by Neurotropin. These observations indicate that Neurotropin controls efficiency of NGF-dependent intracellular signaling through the formation of membrane microdomains providing communications between neurotrophin receptors and innate immune receptors. Neuroprotective mechanism of Neurotropin through innate immune systems may provide a noble pharmacological target for neuroprotection and repair.

Tanaka Hiroyuki (Japan)

Department of Clinical Epidemiology and Health Economics, School of Public Health, The University of Tokyo

The effects of Neurotropin on peripheral nervous system

Neurotropin is a drug widely used in Japan and China to treat chronic pain. Although Neurotropin has been demonstrated to suppress chronic pain through the descending pain inhibitory system, the mechanism of analgesic action in the peripheral nervous system remains to be elucidated. In a chronic constriction injury mouse model, we have revealed new effects of Neurotropin on peripheral nervous system. Neurotropin reduces local mRNA expression of inflammatory cytokines and attenuates the downregulation of myelin basic protein in the injured sciatic nerve. We have also investigated the direct effects of Neurotropin on neurons and Schwann cells. Neurotropin facilitates the neurite outgrowth in cultured neurons and functional recovery in sciatic nerve compression model. Neurotropin also promotes the differentiation in cultured Schwann cells and remyelination in sciatic nerve demyelination model. Neurotropin may have the ability to promote peripheral nerve regeneration.

Chairman of Taiwan Academy of Physical Medicine
Chairman of RehabilitationExecutive Committee of International
Academy of Physical Medicine and Rehabilitation

Simon FT Tang (Taiwan)

Effects of robotic training and Botox injection in stroke patients with hemiplegia

Ambulation is an important part of neurorehabilitation. Walking after stroke is characterized by slow gait speed, poor endurance, and a reduced ability to adapt to the task and the environmental constraints. Positive features of stroke that include hyperreflexia, spasticity, dystonia and enhanced sensitivity to nociceptive input. Chemodenervation with botulinum toxin type A(BTX-A)have been shown to be effective at reducing post-stroke foot and ankle spasticity, while improving the motor control capability. Robotic-assisted gait training(RAGT) have shown positive effects on post-stroke gait pattern. Therefore, the aim of this study is to investigate if combined use of BTX-A and RAGT can improve the outcome of walking ability in stroke patients with hemiplegic gait. The results revealed robototic training will improve gait symmetry when compared to conventional rehabilitation training.

The application of Rewalk system in spinal cord injury patients with paraplegia

Previously, long leg brace was used to assist gait training in spinal cord injury patients with paraplegia (SCI.) In 1990's reciprocal gait orthosis(RGO) was developed for gait training in SCI. However, RGO had never been used widely due to excess energy consumption. Most of the paraplegic patients preferred the use of wheel chair.Rewalk is an exoskeletal robotic device for assisting ambulation in spinal cord injury. This exoskeleton is an external,bipedal lower limb frame that is attached to a central pelvic bar and consist of inbuilt actuator motors located near the hip and knee joints. The user is strapped into the device using soft strapping and a rechargeable battery and computer unit are carried in a backpack. The exoskeleton is controlled by a wireless. A battery of tests included 6 minutes walking, 10 meters walking test, body sway measured by force plate in sitting position and cardiopulmonary function will be assessed before and after 40 hours training of using Rewalk system. (SCI) patients with paraplegia. Seven patients with complete SCI were recruited for applying the Rewalk system, 5 males and 2 females, averaged 31 years old. The level of injuries ranged from T4 to T11. After training for more than 40 hours, 5 patients could walk both indoor and outdoor. The average distance they walked was 770 meters. The average walking speed was 0.4 meter/second. One of the patients, receiving only 22 hours of training and could walk only indoorly with 20 meters in walking distance and walking speed of 0.15 meter/second. One of the patient , whose neurological level was T4, could abmulate with Rewalk system fairly. Rewalk is a novelrobotic system, and most of the patients could walk well with its assisstance after proper training.

Lumig Li

Vice chairman of the Chinese Neuromodulation Society

Chang Jiang Scholars of Chang Jiang Scholars

Deep brain stimulation – from clinical to basic research

Deep brain stimulation (DBS) is now experiencing the fastest development since its emergence and gaining great attention from industry, clinicians, engineering scientists and neuroscientists. Besides its application in treatment of various refractory diseases, including the recent progress in treating Alzheimer's disease and other diseases, it has been explored to unravel the working mechanisms of the human brain in combination with modern imaging technologies, especially the functional magnetic resonance imaging (fMRI).In last 10 years, we developed DBS and rechargeable DBS device in China and got certification form Chinese FDA. Till now, more than 2500 patients implanted our devices in nearly 115 centers in China. Besides the developing work, we also focused on MRI compatible technologies related to DBS. One novel design based on shielding to decrease the lead heating risks will be reported in this talk. Furthermore, DBS is not only a therapy device but also a sensing tool in the era of brain research. We also developed the rechargeable DBS which can record and transmit in real-time the deep brain local field potential during stimulation. With MRI compatibility, sensing capability and its inherent stimulation functionality, DBS will provide a great potential to study the mechanisms of brain disorders and promote the understanding of the brain. The new progress will also be presented in this talk.

Keywords: brain science, deep brain stimulation, magnetic resonance imaging compatible, local field potential, Parkinson's disease

Tiansheng Sun

Vice Chairman of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Director of the Whole Army Traumatic Orthopedic Research Institute

Motor Recovery at 6 Months after Admission is Related to Structural and Functional Reorganization of the Spine and Brain in Patients with Spinal Cord Injury

This study aimed to explore structural and functional reorganization of the brain in the early stages of spinal cord injury (SCI) and identify brain areas that contribute to motor recovery. We studied 25 patients with SCI, including 10 with good motor recovery and 15 with poor motor recovery, along with 25 matched healthy controls. The mean period post-SCI was 9.2 ± 3.5 weeks in good recoverers and 8.8 ± 2.6 weeks in poor recoverers. All participants underwent structural and functional MRI on a 3-T magnetic resonance system. We evaluated differences in cross-sectional spinal cord area at the C2/C3 level, brain cortical thickness, white matter microstructure, and functional connectivity during the resting state among the three groups. We also evaluated associations between structural and functional reorganization and the rate of motor recovery. After SCI, compared with good recoverers, poor recoverers had a significantly decreased cross-sectional spinal cord area, cortical thickness in the right supplementary motor area and premotor cortex, and fractional anisotropy (FA) in the right primary motor cortex and posterior limb of the internal capsule. Meanwhile, poor recoverers showed decreased functional connectivity between the primary motor cortex and higher order motor areas (supplementary motor area and premotor cortex), while good recoverers showed increased functional connectivity among these regions. The structural and functional reorganization of the spine and brain was associated with motor recovery rate in all SCI patients. In conclusion, structural and functional reorganization of the spine and brain directly affected the motor recovery of SCI. Less structural atrophy and enhanced functional connectivity are associated with good motor recovery in patients with SCI. Multimodal imaging has the potential to predict motor recovery in the early stage of SCI.

Professor of Hong Kong University of Science and Technology

Kai Liu (HK)

Intrinsic mechanisms regulating axonal regeneration in adult central nervous system

The failure of axon regeneration in the adult mammalian central nervous system (CNS) attributed to two properties of the adult CNS, the inhibitory extrinsic environment and a diminished intrinsic regenerative capacity of mature CNS neurons. Deleting Pten (phosphatase and tensin homolog) in retinal ganglion cells (RGCs) and corticospinal motor neurons (CSMNs) promotes robust axon regeneration. Importantly, the loss of the regrowth potential of axons is accompanied by a corresponding down-regulation of mTOR activity in neurons upon completion of development. An injury further diminishes neuronal mTOR activity. Our recent findings suggest that Pten deletion promotes regeneration in a chronic spinal cord injury model, and enhancing neuronal activity by melanopsin/GPCR signaling promotes axon regeneration in adult CNS.

Committee member of Neurorestoratology Professional Committee of
Chinese Medical Doctor Association
Director of tissue engineering laboratory of medical center
at China medical university

Qiang Ao

Development and evaluation of new materials for nerve repair

Peripheral nerve injurie is one of the main problems in trauma centers. Treatment of injuries to peripheral nerves is one of the most challenging surgical problems. In cases of simple peripheral nerve disruption, to some extent functional recovery can be attained through tension-free, end-to-end coaptation of residual nerve stumps. In contrast, trauma and surgical procedures, such as tumor resection, often result in peripheral nerve defects. When the gap between proximal and distal nerve end is large, autologous nerve grafts (autografts) were often clinically used to repair the nerve defect. Autologous nerve grafting remains the gold standard treatment in addressing peripheral nerve injuries that cannot be bridged by direct epineural suturing. However, the autologous nerve graft is limited and not readily available, and the process of harvesting autologous nerve graft results in morbidity, such as additional operation injury, recipient nerve difficult to match, donor site denervation, and neuroma formation at the site of harvest, which is like "robbing Peter to pay Paul". Thus, it is necessary to take an alternative to autologous nerve graft to achieve satisfactory functional recovery with little complications, particularly in patients with extensive peripheral nerve injury and insufficient amount of donor nerve for harvest. As a result, a lot of interest has been placed in the development of effective alternatives to nerve autografts in the treatment of peripheral nerve injuries. In the last few decades, researchers have been working to find substitutes for autologous nerve grafts, and have made great progress. Among them, the most promising and most possible alternatives to autologous nerve grafts were acellular nerve allograft and artificial nerve repair conduits.

Molecularly Targeted Cell Preparations in the Therapy of Neurological Diseases: Reality and Future

Andrey Bryukhovetskiy

The development of the molecularly targeted drugs became the major trend of the research in the last decade. The social and economic results of their application are impressive: the general survival of the EU and the US population improved by 25-30 years. The targeted pharmaceuticals rely on the molecular analysis of the abnormal intracellular signal transduction pathways (ICSTP) in the pathological cells in different nosologies, finding the targets (acceptor proteins) on the membrane of these cells of proteins in the structure of the ICSTP. The targeted molecular effect on them can arrest or activate specific ICSTP and initiate or to stop some effector functions of the cells (apoptosis, proliferation, mitosis, ability to invade etc.). The ligand proteins and the ligands represented as monoclonal antibodies (MCA) or iRNA, are the elements of the targeting drugs. When they accurate hit the acceptor proteins, the effector functions of the pathological cells can be effectively maintained and the clinical effect is achieved. However contemporary pharmacological targeted therapy (PTT) has significant flaw that can surpass its advantages. The ligand molecules are not able to affect the acceptor proteins selectively, they affect the ICSTP proteins of almost all cells that they contact when circulating in blood, cerebrospinal fluid (CSF), lymph etc, and this can lead to grave side effects. Hence, for the sake of accuracy of molecular targeting and to minimize the complication from the PTT, it is targeted only at the ICSTP of the pathology-specific proteins. In other words, to specifically affect only pathologically changed proteins of ICSTP and to block them with PTT only the pathologically specific (for example, onco-specific) molecules of pathological proteins should be targeted. Such PTT is not suitable to maintain the systems of healthy cells or to regulate the cell systems with insufficient molecular damages of the ICSTP. This PTT is not suited to maintain systems of healthy cells and to regulate cell systems with minor molecular damage of the ICSTP. Development of the targeted cell preparations for the treatment of neurological diseases can significantly improve effectiveness and safety of the therapy and improve life quality of the patients with the diseases and damages of the brain and spinal cord and to provide unique conditions for the regulation neural tissue with biological processes. Cell targeting therapy (CTT) opens new horizons for the regulation of the effector functions of the damaged cells and vessels of neural tissue. It is conditioned by unique neurobiological phenomena that are characteristic for cell preparations prepared from the stem and progenitor cells (SPC). First, the systems of SPC will always reach pathological cells due to homing and targeted migration (pathotropism) and arrive to the site of injury. Second, the mechanism of cell adhesion will make the SPCs adhere to the pathological cells only. And third, the by-stander effect provides for purposeful effect of ligand proteins and ligands represented as iRNA of the SPCs on the regulated object. Hence, the CTT can be used for therapeutic effect on the specific and non-specific ICSTP, and namely, on the ICSTP that were not involved into the pathogenesis. We propose to consider the opportunities of CTT using the examples of two neurological disorders with different pathogenesis. 1. Glioblastoma Multiforme (GM) and 2. Chronic injury of spinal cord (SCI). The main issue of the CTT in GM is to suppress bproliferation, migration and invasion of the cancer stem cells (CSCs) (CD133+). While the main issue of the CTT for SCI is to activate proliferation, mitosis and regeneration of the neural cells in the site of injury. The author's theoretical and experimental post-genomic research of GM showed that the methodology of the development of molecularly targeted SPCs for this pathology must rely on the fundamental molecular-biological evidence and neurobiological phenomena of GM: 1. The leading role in the GM carcinogenesis belongs to mitotic, proliferative and migration processes in the CSC. 2. Oncospecific proteins take from 57% to 67% in the proteomic structure of the GM CSCs. These are the proteins that are not found in a healthy person and they considerably limit the management of the CSCs by means standard ICSTP. 3. Managing and regulating effect on the migration, infiltrative and proliferative processes in the GM is only possible when the uninvolved in carcinogenesis ICSTP are affected. They are the focal adhesion pathway and integrin signaling pathway (Bryukhovetskiy A.S., 2015) 4. Membrane proteins of these pathways (CXCL1, CD81, TPT1, Cas6 and AXL) can become the main targets for the targeted cell therapy of GM. The ligand proteins that are capable of protein-protein interaction including the follistatin, can suppress proliferation and growth of the GM. The secretome of neural stem cells (NSCs) is analyzed by proteome mapping and profiling of the normalized signal intensity of the proteins the molecules of which can targetedly affect the CSCs in horizontal (the targets inside the ICSTP) or vertical (the acceptor proteins of cell membrane) way. The presence of follistatin in the NSC secretome permits evaluation of these cells as targeted preparation. Our data fully confirm Swedish experimental research (Staflin K, Zuchner T, Honeth G, Darabi A, Lundberg C, 2009) of the follistatin role in blocking proliferation of the GM CSCs. This can also be achieved Эby the pre-processing of the SPCs by low-molecular chemical composites (perturbagens) that can change the expression of specific genes of these cells. Common

acetylsalicylic acid can function as perturbagen. In the case of SCI, the main goal of the CTT is to launch and activate the ICSTP of mitosis, proliferation and regeneration of the damaged cells of the spinal cord. The main effector pathways to activate regeneration and specific molecules for targeting are well known: STAT3 phosphorylation and molecular target STAT3/gp130 imrpove regeneration; axonal growth in peripheral nervous system is associated with mTOR signaling and pTEN deletion molecular target; axonal regeneration in the central nervous system is associated with SOCS3 deletion and JAK/STAT signaling target; PSAF expression and ERK-mediated signaling contributes to acetylating in associated regeneration etc. The main molecular targets of the acceptor proteins for activation of the regeneration pathways (mTOR signaling, JAK/STAT signaling, Myc signaling, PI3K/Akt signaling, Atf3/CREB signaling, RAC1 signaling, STAT3&C-Jun, Rho signaling, Notch signaling, SMAD signaling, eIF5A signaling) can influence the regulation and feedback of the main pathgenetic neurobiological processes that happen in the spinal cord after the injury. The ligand proteins for targeted molecular effect can be detected in the bioinformation computer analysis using the international data bases of protein-protein interactions. The list of the ligand proteins to affect specific target ICSTP of the neural cells is of key importance for the CTT. The analysis of proteomic mapping of the secretomes of the cells helps to detect the cell systems with high concentration of the necessary ligand proteins in the database and further use them for the SCI therapy. Hence, the CTT can significantly improve safety and effectiveness of the therapy of neurological diseases. While standardization and certification of the cell systems that were received from the SPCs by proteome mapping and profiling of their secretomes permit development of the registry of cell preparations with molecularly targeted secretome of specific nosological specialization. Selection of the cell systems with specific ligand proteins improves the effectiveness of the CTT enhancing their neurorestorative capacity in different neurological disorders.

Ntramuscular cell implant in muscles with electromyography recovery in SCI patients previously treated with neural progenitor cells and intensive rehabilitation

Couto

Introduction: Between June 2013 and December 2015 ten previously chronic and complete SCI patients were part of a clinical trial consisted of neural progenitor cells implant (each one received 3 implants, one each 12 months) and intensive rehabilitation program. During 2015 10/10 patients showed Electromyography recovery in muscles that were previously affected by the lesion. Despite this, muscular atrophy caused by previously denervation persisted. Looking forward to improve muscular trophism and contractile capacity we performed intramuscular cell implant in muscles with electromyography recovery.

Method: In January 2016, 10 SCI patients who recovered electromyography register after neural progenitor cell implant received intramuscular cell implant. The implant consisted in a co culture of autologous muscular progenitor cells and effector T lymphocytes. Muscles that recover electromyography activity in response to voluntary order were selected. Using ultrasound guide cells were injected in between muscle fibers. Each muscle received 4-6 implants. All patients continued with the rehabilitation program Results: All implants were well tolerated. Implanted muscles showed: volume increase, improvement in strength and range of motion and ultrasound changes. The first changes were observed after three weeks; they persisted and improved after consecutive muscular implants. Conclusion: In previously denervated muscles, even thought muscular electrical activity associated with voluntary order has been recovery, improving muscular conditions is absolutely necessary to get a functional response. To achieve this goal, intramuscular cell implant seems to be effective. The amount of muscular cell that need to be implanted depends on the muscular volume and the severity of the atrophy.

Anesthesia challenges in patients with incurable neurological disorders during stem cell therapy.

Hema Sriram

Stem cell therapy, a novel treatment, is being developed as a therapeutic option for incurable neurological disorders such as autism, cerebral palsy, muscular dystrophy, spinal cord injury, amyotrophic lateral sclerosis, etc. Different routes of administration are used to inject the stem cells namely intravenous, intrathecal, intralesional, etc which may require anesthetic intervention. In our study, we have given a detailed description of challenges faced with respect to anesthetic intervention during pre transplant work up (MRI Brain, PET CT Brain) and during intrathecal transplantation of autologous bone marrow mononuclear cells. Patients with autism often have behavioural issues such as hyperactivity, aggression which may make it difficult for them to undergo the imaging procedures without anesthesia. Their behavioural issues may also interfere with the IV access during the imaging as well as the transplant procedure. Patients with cerebral palsy have symptoms such as spasticity and seizures which makes the positioning during imaging and transplantation difficult. Patients with muscular dystrophy have concomitant medical concerns such as respiratory, pulmonary and cardiac involvement, which poses many challenges for effective and safe anesthesia management. The involvement of back muscles and the resultant kyphoscoliosis makes subarachnoid access during intrathecal transplantation a difficult procedure. Similar challenges are faced while administering anesthesia to patients with amyotrophic lateral sclerosis with respiratory issues. Most of the patients with incurable neurological disorders are prescribed heavy medications for symptoms like behavioural issues, seizures, spasticity, etc. which may also interfere with the anesthetic drugs. Due to these difficulties it becomes a challenge to manage the anesthesia management during the pre transplant work up as well as intrathecal transplantation of stem cells.

Research Chair in Neuroscience in School of Pharmacy and Medical Sciences in University of South Australia, Adelaide, Australia

Xin-Fu Zhou (Australia)

Pro-BDNF is a potent suppressor of nerve regeneration after spinal cord injury

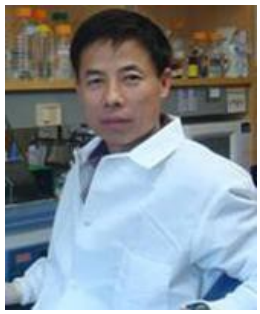
Spinal cord injury is a devastating condition which causes a high rate of mortality and morbidity and results in big social and economic burdens for the society. There is little regeneration of injured nerves in mammals which often results in permanent disability of patients. However the underlying mechanisms why mammalian central neurons do not regenerate are not fully understood. Nerve regeneration is controlled by two opposing signaling pathways. The neurotrophic pathway represented by mature neurotrophins and their receptors activate neurons for neurite growth and extension, and promote synaptic connection and functional recovery. On the other hand, the neurodegenerative pathway represented by the Nogo receptor and their ligands causes neurite collapse and retraction and glial scar formation which inhibits the regeneration and functional recovery. In recent years we found BDNF is a critical factor which regulates neural regeneration and degeneration after spinal cord injury. Peripheral BDNF can promote regeneration of injured spinal axons. After sciatic nerve lesion and spinal cord injury, the expression of brain-derived neurotrophic factor (BDNF) and its precursor, proBDNF, is increased in both dorsal root ganglia (DRG) and spinal cord. ProBDNF is a potent neurite growth inhibitor via activating the p75NTR/RhoA pathway. We have examined whether neutralization of proBDNF by using an anti-proBDNF antibody can promote nerve regeneration after spinal cord injury. We found that endogenous proBDNF is detrimental for the regeneration and functional recovery after spinal cord injury. Neutralization of endogenous proBDNF can promote the functional recovery and increase macrophage infiltration into the inured site. The percentage of NF200 neurons double labelled with fast blue (FB) in the ipsilateral DRG of anti-proBDNF treated rats, was significantly higher compared to the contralateral side and compared to the ipsilateral DRG of the IgG rats. The number of biotin dextran amine (BDA)-labeled fibres in the centre of the lesion site and rostral site was increased in anti-proBDNF treated compared to the IgG rats. In addition, anti-proBDNF treatment reduced the number of activated astrocyte but increased numbers of microglia/macrophages in the spinal cord. The anti-proBDNF treatment also led to more sparing and possible sprouting of serotonergic axons. Our data suggest that the inhibition of endogenous proBDNF establishes more favourable conditions for regeneration. Anti-proBDNF treatment may have therapeutic potential for the injured central nervous system.

Yong Hu (HK)

Committee member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Diffusion Tensor Imaging Reveals the Column-Specific Degeneration in Cervical Spondylotic Myelopathy and Correlates with fMRI

Recent studies have shown the motor and sense deficits in patients with cervical spondylotic myelopathy (CSM) are coupled with microstructural changes of nerve tracts. However, the intrinsic structure-function relationship in myelopathic cord is still unexplored. In this study, we first measure the morphometry, microstructure and functional assessment by combining the conventional T2-weighed MRI, diffusion tensor imaging and blood-oxygen-level-dependent (BOLD) functional MRI, to investigate the relationship between function and structure in healthy and myelopathic cervical spinal cord. Greater microstructural damage was significantly and linearly correlated with enhanced activation in myelopathic cord. These findings demonstrate a quantitative relationship between the extent of structural integrity and functional response in healthy and myelopathic cord, which might provide a promising method to gain additional insight into the role of structural damage and functional reorganization in the spinal cord diseases.



Fabin Han

Committee member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association
PhD in Human molecular genetics from the university of Ottawa，Canada

Transplantation of Human Induced Pluripotent Stem Cells for treatment of Parkinson's Disease in animal studies and clinical trials

Since human embryonic stem cells（hES cells）and human fetal neural stem cells (NSCs) have immune rejection and ethical issues, recent advancements of induced pluripotent stem cells (iPS cells) provide new possibilities for Parkinson's disease (PD) using autologous cells. We isolated human skin fibroblasts from normal individuals and patients with PD, generated iPS cells by transfecting these human skin fibroblasts with retroviral reprogramming factors of OCT4, SOX2, KLF4, and c-MYC and induced iPS cells to differentiate neural stem cells (NSCs) and then into neurons and dopamine neurons in vitro. We found that iPS cells derived NSC transplanted into the striatum of the 6-OHDA-induced PD rats significantly improved the motor functional defects of the rats. iPS cells derived NSCs were found to survive and integrate into the brain of transplanted PD rats, and differentiated into neurons, including dopamine neurons in vivo. Other studies also shown that the iPS cells-derived neurons or neural stem cells improve the motor dysfunctions of rat and monkey PD models. In order to overcome the tumor-formation of iPS cells, the iPSC-derived NCAM(+) /CD29(low) DA neurons were shown to restore motor function of 6-hydroxydopamine (6-OHDA) lesioned rats after transplantation and integrated in the rat brain tissue with robust TH+/hNCAM+ neuritic innervation of the host striatum. These studies provided experimental proof for future clinical application of iPS cells in cell-based treatment of PD. Biography: Dr. Fabin Han is the professor and director, Centre for Stem Cells and Regenerative Medicine, Affiliated Liaocheng Hospital/Taishan Medical University of China. He graduated with his degrees of MD and MPH from School of Medicine and School of Public Health, Shandong University, China. Then he got his PhD in human molecular genetics through University of Ottawa, Canada. He worked as a postdoctoral fellow in molecular genetics at Texas A & M University, USA and Research Scientists in Stem cell Research program in University of Wisconsin at Madison, and Johns Hopkins University, USA. After that he moved back to China to set up his research lab to do genetics and stem cell research in translational medicine. He has been working to identify the disease-causing genes and explore the stem cell therapy for Parkinson's disease and other neurological diseases. He is currently working on generating induced pluripotent stem cells (iPS cells) from patients with Parkinson's disease and Alzheimer's disease to study the molecular mechanisms and iPS cell-based therapy for these diseases. He has published many research papers in Journals of Stem cells, Cytotherapy, Neurology, Movement disorders, Journal of Human Genetics and Toxicological sciences. He is the member of International Stem Cell Research, American Society of Human Genetics, and Society for Neuroscience. He is also the committee members of Chinese Neurorestoratology, Chinese Medical Doctor Association and Chinese Stem cell Engineering, Chinese Medical Association. He is the Reviewer for Cell transplantation, Clinical endocrinology and Journal of Neurorestoratology.

Committee member of Neurorestoratology Professional Committee of
Chinese Medical Doctor Association
Committee member of the International Society for Stem Cell Research

Zhiguo Chen

Cell therapy for Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease with the major pathology being the progressive loss of dopaminergic (DA) midbrain neurons in the substantia nigra. Due to that the lost cells in PD are limited in space and cell type, PD has been considered as an ideal candidate neurodegenerative disease for cell therapy. Autologous DA neurons may circumvent the immune recognition problems associated with fetal midbrain grafts, and recent advance in the reprogramming field has made it possible to obtain patient-specific DA cells. In this talk, I will present data in our lab regarding autologous transplantation of iPSC-derived DA neurons into a monkey PD model, as well as generation of human iNSCs from peripheral blood, which may have a potential for clinical translation.

Standing Member of Neurorestoratology Professional Committee of

The Chinese Medical Doctor Association

Committee member of Nerve Ophthalmology of the Chinese Medical

Doctor Association

Siwei You

Restoration of the Optic Neuropathy

Optic neuropathy refers to disorders involving the optic nerve (ON). Any damage to ON or ON-deriving neurons, the retina ganglion cells (RGCs), may lead to the breakdown of the optical signal transmission from the eye to the brain, thus resulting in a partial or complete vision loss. The causes of optic neuropathy include trauma, ischemia, inflammation, compression, infiltration and mitochondrial damages. ON injuries include primary and secondary injuries. During these injury phases, various factors orchestrate injured axons to die back and disability to regeneration, and these factors could be divided into two categories: extrinsic and intrinsic. Extrinsic inhibitory factors refer to the environmental conditions that influence the regeneration of injured axons. The presence of myelin inhibitors and glial scar, lack of neurotrophic factors and inflammation mediated by injury are regarded as these extrinsic factors. Extrinsic factors need to trigger the intracellular signals to exert inhibitory effect. Properly regulating these intracellular signals have been shown beneficial to ON regeneration. Intrinsic factors of RGCs are the pivotal reasons that inhibit ON regeneration and are closely linked with extrinsic factors. Intracellular cAMP and calcium levels affect axon guidance and growth cone response to guidance molecules. Many genes, such as Bcl-2, Pten and mTor, are crucial in cell proliferation, axon guidance and growth during development, and play important roles in the regeneration and extension of RGC axons. With transgenic mice and related gene regulations, robust regeneration of RGC axons has been observed after ON injury in laboratories. Although various means of experimental treatments such as cell transplantation and gene therapy have achieved significant progresses in neuronal survival, axonal regeneration and restoration of the visual function after ON injury, many unresolved scientific problems still exist for their clinical application. Therefore, more time is needed before the aim of clinical application of these effective therapeutic methods is achieved.

Research Scientist and coordinator of Molecular
Diagnostics at Super Religare Laboratories
post-graduate fellowship in the field of Developmental Neurology and
stem cell research at Tata Institute of Fundamental Research, Mumbai

Nandini Gokulchandran (India)

Stem Cell Therapy in Traumatic Brain Injury

Traumatic brain injury (TBI), one of the most common causes of death and disability in the young population, is often caused by an external physical impact resulting in impairment of physical functions or cognitive abilities. The damage caused to the brain is either focal or diffused depending on the event causing TBI. In chronicTBI, the life expectancy of the affected is normal, but there is high prevalence of the residual disability such as hemiparesis, spasticity, affected cognition, emotion and behavior arising from the injury. This affects the quality of life of the patient and caretaker along with posing an economic burden on the society. In past few decades, extensive research has been conducted to develop a standard treatment for TBI. Stem cell therapy has shown a great potential as a therapeutic strategy for TBI. Stem cells when administered migrate to the damaged areas of the brain and initiate repair and regeneration. They promote angiogenesis, axonal remodelling, neurogenesis, synaptogenesis, neuroprotection. These cells reverse the pathology of TBI by either directly differentiating and replacing the lost or damaged cells or indirectly by paracrine activity. They release various chemokines and cytokines which stimulate endogenous neuroprotection and repair. To study the effect of stem cells in TBI, we administered 14 cases of TBI with autologous bone marrow mononuclear cells, intrathecally. The patients were followed up for 6 months and no major adverse events were recorded in this duration. The Functional Independence Measure (FIM) scale, the SF-8 Health Survey Scoring and the disability rating scale (DRS) were used as outcome measures. At the end of six months, a percentage analysis was carried out for improvement in every symptom. 73% showed improvement in balance, 69% in voluntary control, 60% in memory, 57% in oromotor activities, 55% in lower limb activity and ambulation and gait patterns, 54% in trunk and upper limb activity, 50% in speech, posture and communication, 45% in psychological status, 38% in cognition, 36% in muscle tone and coordination and 33% in ADLs. SF8 scale was performed on 7 patients who met the eligibility criteria of the test. Six months after the intervention, the mean physical component summary (PCS8) score improved from 39.11 to 45.27 and mean mental component summary (MCS8) from 48.44 to 52.55. On the DRS, 6 out of 13 patients showed reduced scores while the scores remained the same in 7 cases. It was observed that these patients showed improved scores primarily in the cognitive component of the DRS. Objective improvements were also recorded on PET CT scan at the end of 6 months in the form of improved metabolism of the brain. These changes correlated to the clinical and functional improvements demonstrated by these patients. The results of this study demonstrated that autologous BMMNCs are safe and efficacious as a therapeutic modality for TBI and have a promising scope to be developed as a standard line of treatment . Graph demonstrating Symptom-wise improvements in chronic TBI patients seen after intrathecal administration of autologous BMMNCs, PET CT Scan showing improved metabolic activity which is indicated by decrease in blue areas after stem cell therapy

Emeritus professor, Baylor College of Medicine Houston Texas, USA

Principal investigator of the Foundation for Movement Recovery, Oslo, Norway

Milan R Dimitrijevic(USA)

Central state of human spinal cord and motor control

Non-patterned electrical stimulation of the posterior structures of the lumbar spinal cord in subjects with complete spinal cord injury, can induce patterned, locomotor-like activity. The rhythmic locomotor activity can be evoked by placing a quadripolar stimulating electrode epidural space or transcutaneous stimulation and applying an electrical train of stimuli-50 Hz, with stimulus strength from 5-9Vto the posterior structures of the second lumbar segment. In this presentation we shall discussed neurocontrol of movement with special emphases on what we learned from externally controlled restored neurocontrol of completely or partially paralyzed volitional movement after posttraumatic spinal cord injury. Thus, in spinal cord motor structures through complete or partial separation. We argue that electrical stimulation of the posterior structures of the lumbar cord can augment clinical and subclinical residual motor aqctivity. We shall demonstrate how sustained stimulation of lumbar below the level of the lesion can induce and/or augment spinal cord motor output in the absence/or augment spinal cord output in absence of brain motor control. The spinal cord as part of the central nervous systems present in all vertebrates and for nearly two centuries has been extensively studied for its anatomical, physiological and biological characteristic. It is understanding conceptual phases. Bell in the early 19 century described the spinal cord as the “way in and way out” to and from the brain. Thus, it was seen for it conducting properties. Sherrington added the concept of reflex activity and their integration. Years later Lundberg brought to conceptual understanding of the spatial cord function and central inputs. Finally through Grillner’s work the ability of the spinal cord to intrinsically generate repetitive, rhythmic activity was recognized. The responsiveness of the human central nervous system can change exercise, injury

Regulation of microglia/macrophage polarization through Myd88 associated signal to enhance recovery from the spinal cord injury in the rats

Fang Kuang
Neurobiology, Institute of Neurosciences, The Fourth Military Medical University

Innate immune reaction plays critical roles in the secondary injury mechanisms of the spinal cord injury (SCI), and resident microglia and infiltrated macrophage are fundamentally involved in the innate immune reaction induced by SCI. Poor recovery from SCI may be, at least partially due to the shortage of M2 polarization of microglia/macrophage, compared to other peripheral tissue injury. However, how to regulate M2 polarization of microglia/macrophage in situ remains an open question. We found that Toll-like receptor (TLR) 4 was significantly increased after rat SCI injury. As myeloid differentiation factor (MyD) 88 serves as adaptor for all TLRs except TLR3 to transform signal of stimulating molecular pattern, we tried to inhibit MyD88 signal to regulate the polarization of microglia/macrophage in the SCI rats with specific inhibitory peptide (MIP). The results showed that blockade of MyD88-dependent pathway significantly enhanced the BBB score, foot print analysis and tissue repair, compared with control peptide. Cell culture experiment indicated that MIP treatment caused significant upregulation of M2 marker, mannose receptor and arginase 1 in N9, a microglial cell line, and the anti-inflammatory cytokine product in the medium. Investigation of MyD88 associated pathway with western blotting showed that inhibition of MyD88-dependent pathway led to the activation of MyD88-independent pathway, that was indicated by the change in the key molecules levels. In addition, MIP treatment induced M2 polarization of microglia/macrophage significantly reduced astroglial necroptosis in the injured spinal cord of the mice. Altogether, our data suggested that MyD88 associated pathways could regulate the polarization of microglia/macrophage and therefore enhance the recovery from SCI, and MyD88 may be a potential therapeutic target for SCI.

.

Magnetofection of Schwann cells (SCs) with PST actuate SCs migrating across astrocyte-SC boundary under an applied magnetic field

Liangliang Huang

Axonal regeneration in the rostrocaudal orientation is essential for functional recovery after spinal cord injury (SCI). SC is a promising cellular candidate for SCI. However, SC can not migrate across the astrocytes-rich interface, resulting in failure of regenerated axons to enter the distal spinal cord. Thus, combinatory strategies are needed not only increase SC migratory ability, but also direct SC migrate to a certain direction. In this study, we first used newly developed magnetofection method to overexpress polysialyltransferases (PST), which induced synthesis of polysialic acid (PSA) on neural cell adhesion molecule (NCAM), resulting in enhanced SC migration. Then, magnetic field (MF) was used to direct the magnetofected SCs to migrate into astrocyte rich domain. Live/dead and CCK8 assay suggested that polyethylenimine-coated superparamagnetic iron oxide nanoparticles (MagNPs) was a kind of low cytotoxicity transfection reagent and the concentrations lower than 8 μg/ml were nontoxic for SCs. TEM showed that MagNPs were internalized 24 h after magnetofection. PCR indicated that MagNPs/PST plasmid ratios 1:4 at 4 μg /ml achieved 1000-fold higher PST expression than control SCs. Western blot showed an obvious PSA-NCAM specific band in PST/SCs. Furthermore, immunofluorescence staining showed that PSA-NCAM was only overexpressed PST magnetofected SC and co-localized with SC specific marker S-100. The inverted coverslip migration assay over astrocytes monolayer found that in the present of MF, much more and much longer for PST magnetofected SC migrated away from the edge of the coverslips compared with other groups. Further analysis showed that the orientation of magnetofected SCs tended to parallel along the direction of the magnetic force. More interestingly, confrontation assay demonstrated that PST/ SCs in contact with astrocytes no longer formed boundaries and aligned preferentially when applied a magnetic field. In a 300μm row on the two type cells boundary, the number of PST/ SCs migrated into the astrocytes domain under a magnetic field was 2.95 and 6.71 times higher than those in the absence of magnetic field and those normal control SCs with magnetic field, respectively. In conclusion, this study was the first report to use magnetofection approach to express PST in SCs. Magnetofection of SCs with PST and magnetic force can synergistically actuate SCs migrating across astrocyte-SC boundary

X-ray therapy promotes structural regeneration after spinal cord injury in a rat model

Dong Liu
the Second Affiliated Hospital of Soochow University

Objectiv：To investigate the therapeutic effects and mechanisms of x-ray treatment on rats following spinal cord injury(SCI). Methods ： Forty-six female Sprague-Dawley rats were subjected to spinal cord injury using the modified Allen weight-drop method. The animals were randomly divided into 6 groups. Two of the animal groups were irradiated with 10 Gy at the lesion site; another two groups were irradiated with 20 Gy; and the last two groups without irradiation were regarded as the sham group. One of the every two groups' animals were euthanized at different time points at 4 and 12 weeks after irradiation. Spinal cord calluses were assessed using kinology, and electrophysiology and histology methods.Results：In all of the groups, the Neurofilament (NF) counts at 14 weeks were found to be higher than that at 6 weeks after SCI. Both 10-Gy irradiated and 20-Gy irradiated groups were higher than those of the sham group at each time point ($P < 0.05$).The myelin basic protein (MBP) count decreased at 14 weeks after SCI in the irradiated groups ($P < 0.05$), but increased at 14 weeks in the sham group($P < 0.05$). Furthermore, the MBP count of irradiated groups was lower than that of the sham group at 14 weeks ($P < 0.05$). The glial fibrillary acidic protein (GFAP) and Nogo-A counts at 14 weeks were higher than those at 6 weeks in all the groups ($P < 0.05$), and there was no statistical significance with kinology and electrophysiology tests in all groups. Conclusions：A self-repair mechanism exists after spinal cord injury, which lasts at least 14 weeks. X-ray therapy promotes the regeneration of the spinal cord system after injury.

Key words：Rat, X-rays, Therapy, Spinal cord injuries

Myelin basic protein induces transplanted neural stem cells migration after spinal cord injury

Zhijian Cheng
The Second Affiliated Hospital of Xi'an Jiaotong University

Abstract: Transplantation of neural stem cells (NSCs) is promising treatment for spinal cord injury. After SCI, the grafted NSCs migrat from injection site into damage area. The mechanisms of modulating migration of transplanted NSCs are still under scrutiny. Myelin debris are generated immidately after SCI and myelin basic protein (MBP) is the second most abundant protein of the myelin sheath in CNS. In this study, we will identity the roles of MBP on the migation of NSCs. We Cultured NSCs from GFP transgenic mice. By using a tranwell chamber, we found that myelin MBP could induce NSCs migration in vitro. Next, to detect the effect of MBP on NSCs migration in vivo, we used MBP-/- mice and compared the migration of NSCs in the spinal cord tissues of wild type and MBP-/- mice. The resulted showed that the migartion of NSCs towards injured area was signifcantly inhibited in the MBP-/- mice compared with wild type mice. Furthermore, we injected nano particles into normal spinal cord and found that the number of migrated cells was significantly higher in particle with MBP group than in particle with BSA group (control group). Finally, we some inhibitors to detect the interaction protein involved in MBP-induced NSCs migration. A pan-FGF receptor, BGJ398 and FGF receptor neutralizing antibody could block the migation of NSCs induced by MBP. Therefore, MBP induces the migration of NSCs through FGF receptor. The migration of NSCs toward injured area may due to MBP released after SCI.

Keywords: myelin basic protein, neural stem cells, migration, spinal cord injury

Embryonic stem cells polarizing macrophages to M2-like phenotype improves locomoter recovery from acute spinal cord injury in mice

Lei Guo
the Second Hospital of Xi'an Jiaotong University

Objective: To investigate the interaction between embryonic stem cells (ESCs) and macrophages, and whether this effect contributes to locomotor recovery in an acute spinal cord injury (SCI) model of mice. Methods: In vitro, day 7 bone marrow-derived macrophages (BMDMs) were incubated with CON-M and ESC-M for 48h in the absence of macrophage colony- stimulating factor (M-CSF) and other exogenous stimuli. Length of BMDMs was measured by Image J, arginase-1 activity was measured by a colorimetric assay and the expression of arginase-1 in the supernatants was detected by ELISA. In vivo, 1 ml of conditioned medium obtained from ESC culture (ESC-M; without direct cell-cell contact) or regular medium for ESC culture (CON-M; without ESCs secretion) was injected intraperitoneally (i.p.) immediately after the contusion operation and then every day until the mice were sacrificed, and the BMS scores for each group were evaluated every two days. Frozen sections were made after the mice were sacrificed at 7 days post-injury, and Arginase-1 (Arg-1) and CD16/32 were used to distinguish between M1 and M2 subpopulation among Iba1+ or F4/80+ infiltrated macrophages. The area of each marker's positive signals was measured by Image J software. Results: In vitro, cells treated with CON-M exhibited the typical bipolar, spindle-shaped morphology of BMDM. In contrast, in the presence of ESC-M, some of macrophages showed a long, single process or bipolar processes, and the quantitative analysis showed that ESC-M treated macrophages exhibited a significantly higher degree of elongation compared to CON-M treated macrophages. What's more, the colorimetric assay indicated that treatment with ESC-M for 2 days significantly upregulated (5.4 fold increase) Arg-1 activity in BMDMs, compared with treatment with CON-M. Nevertheless, the amount of arginase-1 secreted into the culture medium was significantly increased in BMDMs treated with ESC-M compared to the amount present in supernatants of CON-M- treated macrophages. In vivo, at 4 weeks post-injury (termination of the study) the average BMS scores per group were: ESC- M (n=15) 5.4±0.7; CON-M (n=12) 4.5±0.4. Comparing to group treated with CON- M, BMS scores of group treated with ESC-M were higher all the time, and the differences were significant (p<0.05), especially after 7 days post-injury (p<0.01). The measurement data by Image J revealed that there was no significant difference between the total numbers of infiltrated macrophages at epicenter in groups treated with CON-M and ESC-M for 7 days. However, Arg-1 of M2 maker had a lager distribution area in group treated with ESC-M than CON-M (p<0.05), and CD16/32 of M1 maker had a smaller distribution area in group treated with ESC-M than CON- M (p<0.05). Conclusions: This study showed that ESCs induce alternative activation of macrophages (M2 activation) by polarizing macrophages into an Arg-1 high phenotype, providing a new way of targeting macrophage in order to control inflammatory microenvironment after SCI. Moreover, ESCs contribute to locomotor recovery after acute spinal cord injury by i.p. injection of ESC-M, offering a new approach to develop clinical therapy based on ESCs while circumventing the tumor risk involved in stem cell transplantation.

Key words: Spinal cord injury; Embryonic stem cells; Macrophages, Inflammation

miR-214 5p modulate the inflammatory response in LPS-induced BV2 microglia by directly regulating the expression of TSG-6

Hu Yang
Neurology,Tongji Hospital, Tongji Medical College

Objectives: To investigate whether ADSCs with the treatment of TNFa could inhibit the inflammatory response of LPS-induced BV2 microglia through TSG-6 and to compare the difference in microRNA profiles between TNFa-treated ADSCs and control ADSCs through high-throughput sequencing technology and cluster analysis, and then to find the microRNA that might modulate TSG-6 mRNA in ADSCs.Methods: ADSCs were harvested from rats and cultured in vitro, and the 3rd to 5th passage cells were applied to our study. The expression of TSG-6 mRNA and protein in ADSCs were tested by RT-qPCR and ELISA assay. siRNA-TSG-6 and siRNA-control were separately transfected into ADSCs, and the TSG-6 gene silenced ADSCs and control ADSCs were co-cultured with LPS-induced BV2 microglia for 6h. The mRNA level of several cytokines in BV2 microglia, including iNOS, IL-1β, IL-6 and TNFa were tested by RT-qPCR. The total RNA was extracted from TNFa treated ADSCs and control ADSCs and sequenced by Illumina HiSeqTM 2500 according to the manual guidance. Poor quality data was removed from raw reads and clean data was analysed to compare the different microRNA profiles between two groups of ADSCs. The differentially expressed microRNAs between TNFa treated ADSCs and control ADSCs were tested by RT-qPCR. Student's t-test was used for comparison between two groups and One-Way ANOVA was used for comparison among three or more groups using SPSS 20.0 software (SPSS Inc. Chicago, IL, USA).Results: In this study, we found that TNFa treated ADSCs could significantly up-regulate the expression of both TSG-6 mRNA and protein. Furthermore, ADSCs were shown to be able to inhibit the expression of pro-inflammatory cytokines including IL-1β, IL-6, TNFα and iNOS in LPS-induced BV2 cells, but the inhibitory effects were much weaker in TSG-6 gene silenced ADSCs. Through the difference analysis and cluster analysis, totally 35 microRNAs were found to be differentially expressed between two groups of ADSCs, 19 in which were down-regulated in TNFa treated ADSCs. miR-214-5p was identified to have the potential to regulate TSG-6 mRNA. ADSCs transfected with miR-214-5p mimic down-regulated the expression of TSG-6 mRNA and protein while ADSCs transfected with miR-214-5p inhibitor up-regulated the expression of TSG-6 mRNA and protein.Conclusion: ADSCs with the treatment of TNFa can regulate the inflammatory response in LPS-activated BV2 microglia by upregulating the expression of TSG-6 and miR-214 5p can directly regulate the expression of TSG-6.

Key words: ADSCs; TSG-6; Inflammation regulation; BV2 microglia; high throughput sequencing; miR-214 5p

Human umbilical cord mesenchymal stromal cells infected with adenovirus expressing HGF promote regeneration of damaged neurol cells in a Parkinson's disease model

Jinfeng Li

Parkinson's disease (PD) is a neurodegenerative movement disorder that is characterized by the progressive degeneration of the dopaminergic (DA) pathway. Mesenchymal stromal cells derived from human umbilical cord (hUC-MSCs) have great potential for developing a therapeutic agent as such. HGF is a multifunctional mediator originally identified in hepatocytes and has recently been reported to possess various neuroprotective properties. This study was designed to investigate the protective effect of hUC-MSCs infected by an adenovirus carrying the HGF gene on the PD cell model induced by MPP+ on human bone marrow neuroblastoma cells. Our results provide evidence that the cultural supernatant from hUC-MSCs expressing HGF could promote regeneration of damaged PD cells at higher efficacy than the supernatant from hUC-MSCs alone. And intracellular free Ca(2+) obviously decreased after treatment with cultural supernatant from hUC-MSCs expressing HGF, while the expression of CaBP-D28k, an intracellular calcium binding protein, increased. Therefore our study clearly demonstrated that cultural supernatant of MSC overexpressing HGF was capable of eliciting regeneration of damaged PD model cells. This effect was probably achieved through the regulation of intracellular Ca(2+) levels by modulating of CaBP-D28k expression.

Neurorestoration of Microchimerism in Neuromyelitis Optica during Pregnancy

Mangsuo Zhao
Tsinghua-Yuquan Hospital

Neuromyelitis optica (NMO) is an inflammatory disease of the central nervous system which is common in women of childbearing age. Relationship between NMO and pregnancy was not consistent, someone believed that pregnancy would aggravate the conditions of NMO and increased the Expanded Disability Status Scale (EDSS) score, but someone suggested that pregnancy was innocent bystander for NMO. Some doctors in China advised that NMO female were prohibited to pregnancy, and pregnancy in women with NMO should be terminated. Our clinical data showed that the risks of relapse increased during pregnancy, especially in early stage of pregnancy, but severity of disease was milder and prognoses of mothers and fetus and /or infants were good. The imbalance between Th1 and Th2 cytokines during pregnancy may partly explain effects of pregnancy on conditions of NMO, and the increased risks of NMO relapse may be associated with Th2 cell dominating humoral immune response. However, how to explain the relatively milder symptoms of relapse and good prognosis of NMO during pregnancy? It was inferred that pregnancy may have some beneficial factors for NMO. Microchimerism is a small portion of cells in the body which is genetically and antigenically distinct from the host. During pregnancy, bidirectional gene/cell exchange between the fetus and mother happened, and microchimerism is formed in the respective body. The fetal microchimeric cells have the characteristics of stem-like cell and can persist for long-term even for lifetime. Microchimerism appeared in the maternal inflammatory tissues of the autoimmune diseases such as system sclerosis, system lupus erythematosus and xerosis, and it is thought to play a protective role in lupus nephritis and autoimmune thyroiditis. Animal model studies found that microchimerism in rat brain and spinal cord differentiated into glial cells and neurons. Human studies have also found that microchimerism existed in approximately 63% of maternal brains. Therefore, some scholars believe that microchimerism may be beneficial to injured tissue by regenerating and repairing. The effect of microchimerism on NMO during pregnancy remains unclear. We hypothesized that fetal microchimeric cells may offer positive effects on NMO during pregnancy. That is, microchimerism could contribute to the neurorestoration through cell differentiation, secretion of cytokines, regulation of autoimmune reaction, elimination of inflammatory cells, remyelination and so on.

Comparison of Different Microsurgery Techniques for Trigeminal Neuralgia

Zihang Xie
Neurosurgery, Yu Quan Hospital, Tsinghua University,

Objective: To study the influence of different microsurgical manners on surgical outcomes and complications, and to improve the surgical effects for trigeminal neuralgia. Methods: The clinical data of 109 patients with trigeminal neuralgia, treated by microsurgery, were analyzed retrospectively. All patients were divided into 3 groups according to surgical modality: the trigeminal neuralgia decompression group (TND group, 19 patients), the TND and rhizotomy group (rhizotomy group, 55), and the TND and selective lesioning group (lesioning group, 35). The mid-term and short-term effects and complication occurrences were compared between the 3 groups. Results: There were no statistical differences in the frequency of complications between the 3 groups ($P>0.05$). Eighty-four patients were followed-up for 6 to 33 months. The rate of pain disappearance was found to be 94.4% in the TND group, and 100% in both the rhizotomy and lesioning groups; thus, no significant differences were found between these 3 groups ($P>0.05$). Additionally, 50% of the patients in the rhizotomy group and 3.6% of the patients in the lesioning group had facial numbness while no patient was affected with facial numbness in the TND group. There were significant differences between these 3 groups ($P<0.05$). Conclusions: Microsurgery is effective and safe for trigeminal neuralgia. TND, together with stereotactic lesioning, ensures therapeutic efficacy and improves the life quality of postoperative patients.

Culture in hopoxia Promotes DAergic-neuronal differentiation of Nasal OM-MSCs by up-regulation of hypoxia inducible factor-1α

Lite Ge
Neurosurgery, the Second Affiliated Hospital of Hunan Normal University

Backgroud: OM-MSCs have significantly clonogenic activity and could be easily propagated for the purpose of parkinson's disease (PD), how to induce OM-MSCs to differentiate into dopaminergic (DAergic) neurons more efficiently by OECs is an attractive topic. Methods: The induction protocol of hapoxia induction(H-I) group has been designed to generate DAergic neurons from OM-MSCs using a physiological oxygen (O_2) level of 3% and olfactory ensheathing cells(OECs) conditioned medium, while the O_2 level of normal induction(N-I) group stay ambient air level(21%). In order to study the role of hypoxia-inducible factor-1alpha (HIF-1α) in differentiation of OM-MSCs under hypoxia environment, the H-I-R group(H-I group treated with HIF-1α inhibitor before induction) was set for comparsionResults: Compared with N-I group and H-I-R group, the H-I group had a significantly increased proportion of TUJ-1 and TH positive cells through immunocytochemistry and western blot. Further more, the level of DA was significantly increased in H-I group. In whole-cell voltage-clamp test, slow outward potassium current recorded in differentiated cells after 21 days induction. Our results also demonstrate that hypoxia environment can enhance DAergic neuronal differentiation of OM-MSCs by increasing the expression of HIF-1α. Conclusion : Hypoxia promotes DAergic neuronal differentiation of OM-MSCs, the HIF-1α may play an important role for hypoxia-inducible pathways in DAergic lineage specification or differentiation *in vitro*.

Key words： olfactory mucosa mesenchymal stem cells； differentiation； DAergic neuron； hypoxia-inducible factor-1alpha

The potential of human umbilical cord-derived mesenchymal stromal cells as a novel cellular therapy for multiple sclerosis

Shanshan Wang

Multiple sclerosis (MS) is a complex disease of neurological disability, affecting more than 300 out of every one million people in the world. The purpose of the study was to evaluate the therapeutic effects of human umbilical cord- derived mesenchymal stromal cell (hUC-MSC) transplantation in MS patients. Twenty-three patients were enrolled in this study and 13 of them were given hUC-MSC therapy at the same time as anti-inflammatory treatment, whereas the control patients received the anti-inflammatory treatment only. Treatment schedule included 1000 mg/kg of methylprednisolone i.v. 1000mg/kg daily) for 3 days and then 500 mg/kg for 2 days, followed by oral prednisone 1mg/kg/day for 10 days. The dosage of prednisone was then reduced by 5mg every two weeks until reaching a 5mg/day maintenance dosage. Intravenous infusion of hUC-MSCs was applied three times in a 6 week period for each patient. The overall symptoms of the hUC-MSC treated patients improved compared to patients in the control group. EDSS scores were significantly lower and relapse occurrence was much lower than those of the control patients. Inflammatory cytokines were assessed, and the data demonstrated a shift from Th1 to Th2 immunity in hUC-MSC treated patients. Our data demonstrated high potential of hUC-MSC treatment for MS.

Indication and operative technique of intramedullary decompression after spinal cord injury

Jiaxin Xie

Spinal cord injury is a devastating event that often produces severe and permanent disability. Spinal cord injury includes both primary injury and secondary injury. Primary injury could be caused by direct trauma, which is unalterable. The secondary injury is the focus of our surgical intervention. After long-term clinical practice, we proposed early combined surgery of spine and spinal cord. Besides accomplishing internal fixation and laminectomy, we performed intramedullary decompression at the same time, removing necrotic tissues and improving microcirculation, finally achieve complete decompression of the injured spinal cord. If the patient was complete spinal cord injury, we recommend intramedullary decompression. During operation, if we found high tension of spinal dura mater and fluctuation disappearance of spinal cord, we recommend intramedullary decompression. Moreover, if CT or MR indicated there was intramedullary compression by bony fragments or foreign matter, or severe contusion of spinal cord, or intramedullary hematoma or softening region, we also recommend intramedullary decompression. Moreover, we summarized four different intraoperative findings which would require corresponding surgical interventions.

Ensor-based analysis of a closed-skull weight drop TBI model

Javier Allende Labastida
Neuroscience and Cell Biology

Traumatic brain injury (TBI) affects an approximately 150-300 per 100,000 people worldwide, with 2.5 million in the U.S. in 2010. In China there are estimates of 3-4 million TBIs, with a yearly increase of 4.67% from 2001-2007. The main biomechanics of brain damage in TBI (blunt impact and acceleration/deceleration) are recreated in the closed-skull weight-drop injury model developed by Kane et al. However, variability in animal models of TBI and reproducibility among studies and laboratories has been a topic of concern. This project is the first step to address this concern with the implementation of a sensor that allows us to accurately calculate the kinetic energy of the falling weight before impact to quantify one source of variability in the model. Two-month old C57BL/6 male mice were randomly assigned to 3 groups: sham, TBI with a 95g weight and TBI with 150g weight. Mice received mild TBIs using either a 95g or 150g weight dropped from a height of one meter. Velocity of the dropping weight was measured using a sensor located in the distal portion of the guiding tube. Locomotor activity was measured by photo beam activity system 30 minutes after TBI. Brain tissues were collected at 5 days post injury for western blot and immunohistochemistry analysis. We found a direct relationship among the weight, its kinetic energy, and the righting reflex of animals. Particularly, the kinetic energy of weight was inversely correlated to the locomotor activity of the injured animals. Our study is thus the first attempt to optimize the closed-skull weight-drop model by implementing quantifiable measures to monitor and consequently reduce injury variations.

Cytokine change in cerebrospinal fluid of children with mental retardation before and after neural precursor cell transplantation

Hui Yang
Navy General Hospital

Abstract Objective: To investigate cytokine change in cerebrospinal fluid (CSF) of children with mental retardation (MR) before and after neural precursor cell transplantation (NPCT), in an attempt to provide experimental clues for the clinical treatment of MR with NPCT. Methods: Included in this study were 28 MR children who received twice NPCT in our hospital. CSF was collected at both NPCT to screen out cytokines by ELISA. In addition, the content of insulin-like growth factor 1 (IGF-1) in CSF was further assayed to see whether there was any correlation between IGF-1 change and the short-term therapeutic effect of NPCT. Results: Of all cytokines detected in CSF, only IGF-1 was increased significantly after NPCT compared with that before NPCT($P<0.05$). 15/28 MR children achieved the short-term therapeutic effect, in whom the content of IGF-1 after NPCT was significantly higher than that before NPCT ($P<0.05$), while there was no difference in IGF-1 change in the remaining 13 MR children without short-term therapeutic effect ($P=0.657$). There was significant difference in IGF-change between the two groups of patients ($P<0.05$). Conclusion: IGF-1 may be one of the mechanisms contributing to the therapeutic effect of NPCT. Key words: mental retardation; neural precursor cell; transplantation; cytokine; IGF-1

Functional analysis of lncRNA in schwann cells after peripheral nerve injury

Bin Pan
Tianjin Medical University General Hospital

Background: Long non-coding RNAs (lncRNAs) are widely accepted as key players in various biological processes. However, the roles of lncRNA in peripheral nerve regeneration remain completely unknown. Method: A microarray analysis was performed to detect expression of lncRNAs in the distal segment of sciatic nerve after injury. Bioinformatic approaches were performed to investigate the functions of these dysregulated lncRNA. Schwann cells were isolated and cultured. Cell and Molecular biology in combination with lncRNAs overexpression were performed. Result: We identified more than 2000 mRNAs and lncRNAs that were differentially expressed in the distal end of sciatic nerve after injury. Bioinformatics approaches and PCR verification predicted 15 lncRNA-mRNA pairs that may participate in biological processes related to peripheral nerve injury. We selected NONMMUG014387 which was predicted to target CTHRC1 for further analysis. PCR and western blotting indicated that after nerve injury, an consistent change tendency existed between CTHRC1 expression and NONMMUG014387 expression. Transwell migration assays showed that transfection of Schwann cells with the NONMMUG014387 significantly increased their migration compared with the control. In addition, No changes in cytotoxicity and proliferation were found after Schwann cells were transfected. qRT-PCR and western blotting showed that the expression of endogenous CTHRC1 mRNA and protein were increased in primary Schwann cells transfected. The important components of Wnt/PCP pathway was also increased. Surprisely, we observed significantly lower level of mir-9. In the previous study, miR-9 was also comfirmed to inhibit Schwann cell migration by targeting Cthrc1 following sciatic nerve injury. Considering that both NONMMUG014387 and Cthrc1 mRNA are targets of miR-9, and that NONMMUG014387 was coexpressed with Cthrc1 after peripheral nerve injury. we speculated that NONMMUG014387 and Cthrc1 mRNA may act as a pair of ceRNAs that are linked by miR-9. However, further investigations are needed to comfirm this hypothesis. Conclusion: our results indicated after peripheral nerve injury, NONMMUG014387 promte migration of schwann cell by targeting Cthrc1 and activating Wnt/PCP signaling pathway, thus offering a new approach to peripheral nerve repair.

Hypoxic and Ischemic Effects on Gene and Protein Expression Level of Paracrine Factors by Human Olfactory Mucosa Mesenchymal Stem CellS

Ting Yuan
Neurosurgery, Second Affiliated Hospital of Hunan Normal University

Objective: Human olfactory mucosa mesenchymal stem cells (hOM-MSCs) secrete paracrine factors that may exert a protective effect on the cerebral ischemia. This study was done to determine the hypoxic and ischemic effects on the mRNA and protein expression level of paracrine factors by hOM-MSCs. Methods: The hOM-MSCs were cultured with 5% or 20% serum and under either normoxic (21% O2) or hypoxic(3% O2) conditions. Expression of mRNA and protein for vascular endothelial growth factor (VEGF), nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and matrix metalloproteinase-2 (MMP-2) were determined by RT-qPCR and Western blot, respectively. Results: Hypoxia reduced gene expression for VEGF(5% serum),GDNF,BDNF(5% serum) and NGF(5% serum) and increased it for BDNF(20% serum), MMP-2(5% serum) and NGF(20% serum). Ischemia lowered gene expression for VEGF(hypoxia), GDNF, BDNF(hypoxia), MMP-2(normoxia)and NGF(hypoxia) and increased gene expression for VEGF (normoxia), BDNF(normoxia), MMP-2(hypoxia) and NGF(normoxia).The protein level of these factors were almost in a line with gene level. Conclusion: These data demonstrate that serum and oxygen levels have a significant effect on the gene and protein expression level of paracrine factors by hOM-MSCs which will affect how hOM-MSCs interact in vivo during cerebral ischemia.
Keywords: human olfactory mucosa mesenchymal stem cells; paracrine factors; hypoxia; ischemia; cerebral ischemia

Change of myelin ultrastructure and Iba1 level in sciatic nerve of rat experimental autoimmune neuritis model treated with human immunoglobulin

Xiaojing Yuan

Department of Rehabilitation, Taian City Central Hospital, Shan Dong;

Purpose:To explore ultrastructure and protein expression in sciatic nerve of rat experimental autoimmune neuritis (EAN) model treated with human immunoglobulin. Methods: Subjects were randomly divided into drug group (Intravenous immunoglobulin<IVIg> group)(n=15) , model group(n=15) and normal control group(n=15). The former two groups were immunized with mixture of P0 peptide 180-199, Mycobacterium tuberculosis, and incomplete Freund's adjuvant to establish EAN. IVIg group received injections of 100 mg/100 g body weight of IVIg once daily for 2 days when neurological symptoms (ie, tail drooping, scoring 1 point) appear post-immunization. Model group received no therapy post-immunization.Rats given saline isolution were used as a normal control group. Index Observation: Electrophysiology, sciatic nerve ultrastructure and immunofluorescence histopathology were observed at the neuromuscular severity peak and on day 42 recovery post-induction. Cell-specific protein markers were used for immunofluorescence histopathology staining to characterize sciatic nerve cells: Iba-1 (microglia), S100 (myelin), and neurofilament 200 (axon). All rats were weighed and scored daily until day 42 post-immunization. Results: The expression of Iba1 overlapped with S100 on myelin was verified. In contrast with the S100, Iba1 is positive correlation with disease progression. IVIg can reduce Iba1 expression, increase S100 expression at the neuromuscular severity peak post-immunization, and promote myelin (swelling, vesicular disorganization and separation) repair and axonal regeneration under electron microscope.Conclusion: Schwann cells might have the dual role of myelination and macrophages immune, IVIg can promote myelin repair and axonal regeneration.
Keywords: experimental autoimmune neuritis, sciatic nerve, myelin ultrastructure, Intravenous immunoglobulin

Induction of neuronal differentiation from Endogenous Neural Stem Cells ameliorates excessive astrogliosis and promotes functional recovery in adult mice following spinal cord injury

Yan Hao
Tianjin Medical University General Hospital

Direct manipulation of the endogenous neural stem cells (eNSCs), mainly existed surrounding the spinal central canal, poses an ideal approach to repair damage and replenish cells lost after spinal cord injury (SCI). However, the gliogenic microenvironment after SCI drives differentiation towards astrocytes, which contributes to glial scar formation and block neuronal repair. Previously we were able to guide human NSCs (hNSCs) to generate motor neurons (MNs) in vitro by regulating the PI3k/AKT or JAK/STAT3 signaling pathway. Here we asked whether bFGF combined with of PI3K and STAT3 regulation would enhance MN differentiation in vivo. Adult Nestin-Cre:YFP bi-transgenic mice were used to trace the fate of the eNSCs after SCI and treatment and randomly divided into 4 groups (Sham, SCI+vehicle, SCI+bFGF, SCI+FH+LS). After contusion SCI, FGF2 and PI3K/STAT3 inhibitors were delivered by intrathecal infusion. Such treatments dramatically increased the YFP/NeuN co-expressing cells 4-week post injury. Additionally, both GFAP+/YFP- and GFAP+/YFP+ cells significantly decreased in treated mice compared with any other treated SCI mice. YFP and ChAT double labeled cells in treated group suggested that these new MNs derived from eNSC. More solid evidence from CTB retrograde and BDA anterograde tracing showed that new born MNs reconnection to target muscles and regenerated axon cross epicenter. More excitingly, the BMS score in the bFGF/inhibitor group was significantly higher than any other groups, accompanied by improved rearing events, traveling distances and resting time. In summary, MN differentiation from eNSCs and suppression of excessive astrocytes can be regulated by FGF2 together with PI3K and STAT3 inhibitors and leading to dramatically functional improvement after SCI. Our novel findings suggest that the gliogenic microenvironment after SCI can be manipulated to allow endogenous spinal cord NSC to generate neurons. Therefore, eNSCs which is more close to clinical application can be attractive candidates as an alternative to cell transplantation to facilitate neural repair after SCI.

The Application of FA Values in Diffusion Tensor Imaging Combined with Fiber Tracking Technique in the Early Spinal Cord Injury

Fang Wang
the Second Affilated Hospital, Xi'an Jiaotong University

The aim of this study was to evaluate the characteristics of magnetic resonance diffusion tensor imaging (DTI) in acute spinal cord following a thoracic spinal cord injury (SCI), and to determine the optimal time of examination. Sprague-Dawley rats were used as experimental animals and contusion injuries were made at the T10 vertebral level. The rats were divided into control, mild injury, moderate injury, and severe injury groups. Spinal magnetic resonance DTI was scheduled at 6, 24 and 72 hours (h) post-SCI, and the DTI parameters such as fractional anisotropy (FA) and apparent diffusion coefficient (ADC) were calculated, and the diffusion tensor tractography (DTT) of the spinal cord was also generated. We observed a significant decrease of FA in all the three injured groups, and the FA at 24 h post-SCI exhibited the greatest decrease among different set times. For ADC, only the group of severely injured rats saw a significant decrease at 24 and 72 h compared with the control group. DTT showed interruption of nerve fiber tracking in the injured groups. This study demonstrates that FA can differentiate various grades of SCI in the early stage, and 24 h after injury might be the optimal time for identifying injury severity

Human fetal brain nrural stem cells for studying zika virs-associated neuropathology

Erica L McGrath

Zika virus (ZIKV) infection has recently been recognized as a major threat to human health. Particularly, the linkage between ZIKV infection and microcephaly raises a huge concern for a serious health problem worldwide. To date, little is known about the mechanism underlying ZIKV-associated neural damage, including microcephaly. Since a normal brain is developed from neural stem cells (NSCs) and their differentiated neural cells, abnormal brain development, such as microcephaly, is most likely associated with the abnormal function of these cells. Yet, it is unknown whether and how human fetal brain NSCs or their progenies are susceptible to ZIKV infection, whether different strains of ZIKV infect NSCs at the equal efficiency, and whether such infection affects the normal functions of NSCs that are important for the development of human brain. Recently we have established a human fetal brain NSCs-based *in vitro* system to study ZIKV infection in neural cells. We found that various strains of ZIKV, including a strain from the Mexican outbreak in spring of 2016 (strain: Mex I-7), directly infect human NSCs *in vitro*. Additionally, we found that ZIKV infection alters NSC proliferation and differentiation. To the best of our knowledge, this is the first study examining clinically relevant ZIKV strains from recent outbreaks in an *in vitro* human fetal NSC culture to study the neuropathological and developmental effects of ZIKV. This system will facilitate further studies to characterize different strains or modified ZIKV, to understand ZIKV-induced pathological changes, or to develop therapeutic strategies and screen drugs to ameliorate ZIKV-mediated neural damages.

Semen Cassiae extract on rat model of chronic glaucoma intraocular pressure and visual effect of nerve protection mechanism

Yi Wang
phthalmology, Affiliated Hospital of Tai'shan Medical University

Puopose: To investigate the Semen Cassiae extract on rat model of chronic glaucoma intraocular pressure lowering mechanism and to observe the protective effect of the optic nerve. Methods: 72 male SD rats (mean age: 8 weeks), making the use of multi-wavelength yellow green laser for corneal limbus (360 degree angle) photocoagulation, corneal laser spot is 60~ 80, the left eye as control. The rats were randomly divided into experimental group and control group, with 36 rats in each group. The experiment group was treated with the extract by intragastric administration (5g/kg), with the same amount of 0.9% Sodium Chloride Solution was gavaged in the control group. 1d before laser ablation, 1d, 7d, 14d, 21d, 28d, 42d, 56d, 70d, 84d after laser ablation, using Icare tonometer to monitor intraocular pressure. After Laser srugery, 4, 8, 12W production in rat retina flatmount ocular, Nissl staining, retinal ganglion cells (RGCs) quantitative detection. Some specimens were prepared frozen section, hematoxylin eosin staining, the pathological changes of model eyes angle and trabecular meshwork were observed by optical microscope. Results: Except two rats death after anesthesia accident, the IOP of remaining 70 rats was significantly higher than that before operation,.The control group rats intraocular pressure than the same period of the experimental group compared with the model eyes, IOP, trabecular space become narrowing, a decrease in the number of retinal Nissl staining cell count and optic nerve axon count. Conclusion: Semen Cassiae can reduce intraocular pressure by increase of chronic glaucoma rat models of metalloproteinase expression and broadening the trabecular meshwork, and has neuroprotective effect in retina.

Professor in Cellular Neuroscience

Director School for Mental Health and Neuroscience

Harry W.M. Steinbusch

(Finland)

Brainstem Dysfunction in Neurodevelopmental and Neuropsychiatric Disorders -Moving from the Forebrain to the Brainstem

Despite the fundamental role of the brainstem in regulating vital functional abilities such as arousal, breathing, autonomic nervous system activity as well as regulating all higher cerebral functions via neurotransmitter projections systems originating in the brainstem, the role of the brainstem has received relatively little attention in most neuropsychiatric disorders. Besides the dorsal and median raphe nuclei complex comprising mainly serotonin-producing neurons, the brainstem also contains noradrenalin, dopamine and histamine-producing nuclei, i.e. resp. the locus coeruleus, the substantia nigra and the mamillary bodies. The brainstem is furthermore the relay station of afferent and efferent projections between the autonomic nervous system in the peripheral body and higher cerebral brain regions. The current presentation aims to review the neuroanatomy of the brainstem as well as the current status on findings, derived from a wide range of studies using molecular, cellular and imaging technologies, of brainstem involvement in neurodevelopmental (i.e. autism, schizophrenia) and neurodegenerative disorders (Alzheimer's and Parkinson's disease). Over the past decades, the incidence of age-related, neurological and psychiatric disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), but also depression has considerably increased. Mood disorders are strongly related to the exposure to stress. The hippocampus and other forebrain structures are the apex of the stress hormone control mechanism and damage to them may be one way in which stress hormone secretion escapes from inhibitory control in depression. In turn, stress, probably through toxic effects of glucocorticoids, decreases neurogenesis and cell survival while antidepressants enhance these processes in experimental animals. Therefore, since treatment strategies are not yet available, primary prevention in these age-related and stress related neurological disorders is of importance. As mentioned before most of the focus on neurobiological questions on above mentioned disease are related to forebrain structures since they are often associated with cognitive dysfunction. The brainstem is a highly neglected brain area in neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's (PD) disease and frontotemporal lobar degeneration. Likewise, despite a long-standing recognition of brainstem involvement, relatively few studies have addressed the exact mechanisms that underlie brainstem autonomic dysfunction. Improved insight in the cellular and molecular characteristics of brainstem function is pivotal to study the developmental origins. As brainstem dysfunction also poses health issues in several other, neurodegenerative, disorders (like AD and PD), progress in these neurological fields will benefit from scientific advancement in the current proposal as well. In the area of depression, several observations have been made in relation to changes in one particular brain structure: the Dorsal Raphe Nucleus (DRN). The DRN is also related in the circuit of stress regulated processes and cognitive events. In order to gain more information about the underlying mechanisms that may govern the neurodegeneration, e.g. amyloid plaques, neurofibrillary tangles, and impaired synaptic transmission in AD, a rat dissociation culture model was established that allows mimicking certain aspects of our autopsy findings. We observed a similar phenomenon in brains from patients suffering from neurodegenerative disease since this also related to changes in BDNF levels. The ascending projections and multitransmitter nature of the DRN in particular and the brainstem in general stress its role as a key target for AD/PD research and autonomic dysfunction. It also points towards the increased importance and focus of the brainstem as key area in various neurodevelopmental and age-related diseases.

Zuo Luan

Vice chairman of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Secretary-General of the People's Liberation Army's Academy of Pediatrics

Feasibility of Paracrine factors from Human adipose stem cells for Infants with moderate/severe Hypoxic-Ischemic Encephalopath

Our objective was to assess feasibility and safety of intraspinal injections of paracrine factors from human adipose stem cells (hASCs) to neonates with Hypoxic-Ischemic Encephalopathy (HIE). We conducted an open-label, multi-center randomized clinical trial at seven Intensive Care Nursery. Neonates diagnosed with HIE were randomly assigned to either standard therapy or intrathecal injections of paracrine factors from hASCs in 12, 24, 48 hours after birth. The paracrine factors were obtained from cultured human adipose stem cell .We recorded all patients'baseline characteristics，pre-and post-infusion vital signs, neural manifestations and adverse events such as fever, infection, seizures coursed by intervention. Neurobehavioral testing were undertaken before participants were enrolled and reexamined at the 12 and 18 month of age. Neonatal behavioral neurological assessment （NBNA）at 14 and 28d were recorded as hospital outcomes. At the 12 and 18 month after birth, we compared survivals'Bayley Scores, Peabody Development Measure Scales，and Gross Motor Function Measure Scale for children diagnosed Cerebral Palsy for statistical analysis.72 patients were enrolled and all received standard therapy .44 Infants were assigned to experiment group.Clinical characteristics were similar between groups.Vital signs including heartbeats and oxygen saturation were similar before and after intrathecal injections. NBNA score of 14d after birth group was similar in experiment group （ 33.76 ± 4.652 ） and control group （ 32.25±2.364 ）， (95%CI:0.539-3.566， P=0.146).28d NBNA score of hASCs paracrine factors recipients（37.1±2.178）significantly higher than the conventional group （ 35.71±2.758 ） (95% CI:0.136-2.658， P=0.03).In the experiment group， death rates were 6 of 44 neonates (13.6%);11 patients (5 moderate and 6 severe)didn't reach the time of 12-18month follow up, statistical analysis of long-term outcomes were from 33 patiens;4/33(12.1%) patients had development delay,and 3/33(9%) had psychomotor retardation or cerebral palsy.No clinically important complications occurred in the experiment groups except:5/44(11.3%) patients had low –grade fever or irritability in the first 24h after intrathecal injection.Most data of control group for long-term outcome are being collected.In an analysis of these partial data，paracrine factors derived from hASCs was associated with a reduced risk of brain injury. More following data for neurobehavioral outcomes are being collected and analyzed.

Keywords: paracrine factor，human adipose stem cells，Hypoxic-Ischemic,Encephalopathy，newborn

Institute of Fundamental and Clinical Immunology

Lab of Cellular Immunotherapy

Elena Rem Chernykh (Russia)

Application of monocyte-derived macrophages as novel therapeutic opportunity for cerebral palsy

Over the past two decades, intensive research in neuro-immunology demonstrated the potential beneficial role of monocyte-derived macrophages in central nervous system (CNS) repair. Macrophages have been shown to exist as polarized populations, including M1 and M2 subsets. Whereas M1 macrophages are powerful inflammatory cells that phagocytize pathogens and clear cell debris, M2 macrophages down-regulate the inflammatory responses and help with angiogenesis, neurogenesis and neuroplasticity through the production of anti-inflammatory cytokines and growth/neurotrophic factors. In injured nervous tissue, local microglia and newly recruited macrophages can polarize toward M2 cells and promote functional improvement. The pathophysiology of cerebral palsy (CP) is still largely unclear. Nevertheless, inflammatory responses have been suggested to play a pivotal role in CNS impairment in CP. Numerous experimental studies have demonstrated that various types of stem cells capable of down-regulating inflammatory response reduce brain damage and return motor functions in brain injury models including hypoxic ischemia. Initial clinical studies also demonstrated the safety and clinical benefit of stem cell transplantation in children with CP. Of note, several lines of evidence suggest that clinical effect of stem cell-based therapy is largely mediated by monocytes/macrophages. Given the potent anti-inflammatory activity and high regenerative potential of M2-macrophages, these cells may be an alternative source for cell transplantation. Previously, we demonstrated safety of intrathecal injection of M2 macrophages in 16 children with severe CP. The present study aimed to evaluate the tolerability and clinical efficacy in expanded group of patients with moderate to severe CP. The study group included 46 children with median age of 4,0 years old. The primary outcome measure was functional improvement of motor activity (66-item GMFM test). PDMS-FM test, Ashworth scale, questionnaire for evaluation of cognitive functions).safety, which included assessment of mortality of any cause, immediate cell-related adverse reactions and long-term side effects and comorbidities. The secondary outcome measures were safety and improvement of fine motor activity and cognitive functions as well as decrease of spasticity (using PDMS-FM test, questionnaire for evaluation of cognitive functions and Ashworth scale). M2-like cells were generated by cultivating peripheral blood monocytes in low growth factor conditions. Deficiency of growth factors resulted in deprivation apoptosis of non-adherent cells and their engulfment by monocytes induced M2 phenotype of generating macrophages. These cells differed from the M1 cell by higher expression of M2-associated and proapoptogenic molecules, lower production of pro-inflammatory cytokines and chemokines and reduced capacity to stimulate T-cell proliferation. Along with EPO, bFGF, EGF, G-CSF and BDNF (also produced by M1 cells) M2 macrophages produced higher concentrations of IGF-1 and VEGF. Intrathecal injection of autologous M2-cells (in median dose of 12, 2 x 10^6 cells) did not induce any serious adverse events. After 6 months GMFM score increased from 19.9 ± 5.4 to 73.2 ± 9.5 (Pu<0.01), PDMS-FM score improved from 1.0 ± 0.31 to 5.3 ± 0.64 (Pu<0.01), and Ashworth score decreased from 3.6 ± 0.14 to 2.7 ± 0.21. An improvement of cognitive activity (from 1.6 ± 0.29 to 4.6 ± 0. 42, according to Questionnaire assessment) and reduction of seizure syndrome was registered as well**.** Therapy with M2-macrophages did not induce the increase of serum IFN- γ, IL-17, and IL-4, but resulted in enhancement of BDNF. The data obtained suggest that cell therapy with M2-macrophages is safe, does not induce adverse effects and comorbidities and is accompanied by significant improvement of motor and cognitive activities in CP patients.

Committee member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Committee member of Asia Pacific Association Pediatric Neurological

Suqing Qu

Analysis of Adverse Events Related to 720 Cases of Neural Stem/Progenitor Cell Transplantation

Background： The safety issue of cell transplantation is not clear.Methods： 720 cases of NSCs/NPCs transplantation to treat a variety of brain injury in children and to discuss the safety issue of cell transplantation through a retrospective analysis of adverse events. Results： 166 (or 23.1%) cases had postoperative crying and irritability, of which 49 cases exhibited exciting and slight asleep disorders. 69 (9.58%) cases had vomit, mostly occurred once or twice within 12 hours post-surgery. The ratio have dropped to 3.15% by delaying the time to eat and change the posture of feed. 53 (7.36%) cases had cerebrospinal fluid leakage. A total of 84 (11.67%) cases had fever (37.5-39.5℃) not resulting from respiratory tract infection, then dropped to 3.75% for have improved the methods of cell culture and cell collection. One month postoperatively, 568 (78.8%) cases received the EEG test, which showed improvement in 153 patients, abnormal changes in 74, and no changes in 341 cases. 6 cases (0.83%) had intracranial hemorrhage, but left no CNS sequelae other than the primary diseases. Follow-up head MRI or CT on a total of 59 patients (8.18%) during the period of 6 months to 7 years post-operation revealed no signs of tumorigenesis. Only one case with cerebral palsy died of severe pulmonary infection within one month after the surgery. Conclusions： we did not find serious and irreversible operation-related, cell-related adverse events, or tumorigenesis after the transplantation.

Ali Otom (Jordan)

Vice-President of International Association of Neurorestoratology

Chairman of Membership Committee of International Association of Neurorestoratology

Senior Consultant in Spinal Injuries & Musculoskeletal Medicine.

Director of the Royal Rehabilitation Center-King Hussein Medical Center

Current Trends in Neuropathic Pain With Special Reference to Spinal Injury

Neuropathic pain is a serious consequence of many neurological disorders, such as spinal cord injury, neuropathy, multiple sclerosis and stroke. It develops as a result of lesion or disease affecting the somatosensory nervous system.Chronic neuropathic pain is considered a disease not a symptom. Clinically it is characterized by spontaneous ongoing or shooting pain and evoked amplified pain response following noxious or non-noxious stimuli. Pathophysiological mechanisms are complex and remain a challenge for proper management, although recent research identified different pathophysiological pathways which reflected in improvement of new treatment strategies. A better understanding of neuropathic pain and its underlying mechanisms will lead to more effective and mechanism-based approach. Treatment goals include reducing baseline pain and pain exacerbations, ensure a balance between efficacy and safety, and follow individual tailored mechanism-based treatment approach including psychosocial intervention and improvement in quality of life. This talk will discuss the latest update on mechanism and treatment approaches.

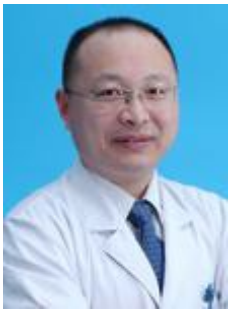
Mahsa Ghajarzadeh (Iran)

Brain and Spinal Cord Injury Research Center, Neuroscience Institute,
Tehran University of Medical Sciences,

Tehran, Iran

Emotional intelligence in patients with Spinal cord injury (SCI)

Objectives: to evaluate depression and EI in SCI patients in comparison with healthy subjects.Methods: One hundred and ten patients with SCI and 80 healthy subjects (the patient's relatives were enrolled. All participants were asked to fill valid and reliable Persian version Emotional Quotient inventory (EQ-i) and Beck Depression Inventory (BDI). Results:Mean age of patients was 28.7 and mean age of controls was 30.2 years. Spinal cord injury in 20(18.3%) were at cervical level, in 83 (75.4%) were thoracic and in 7 (6.3%) were lumbar.Mean values of independence, stress tolerance, self-actualization, emotional Self-Awareness, reality testing, Impulse Control, flexibility , responsibility and assertiveness were significantly different between cases and controls. Mean values of Stress tolerance, optimism, self-Regard, and responsibility were significantly different between three groups with different injury level. Most scales were not significantly different between male and female cases. Conclusion: Emotional intelligence should be considered in SCI cases as their physical and psychological health are affected by their illness.
Key words: SCI, emotional intelligence, IRAN.



Lin Chen

Standing Member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Secretary-general of the 1st, 2nd & 3rd of the International Association of Neurorestoratology

Chromaffin cell transplantation for neuropathic pain after spinal cord injury: a report of two cases

Abstract: Neuropathic pain (NP), a common secondary complication following spinal cord injury (SCI), presenting at or below the level of injury is largely refractory to current pharmacological, physical, and surgical treatments. Cell transplantation is a potentially powerful approach for the alleviation of chronic pain. Cell therapies including stem cell have been used for alleviating the neuropathic pain induced by SCIs. Olfactory ensheathing cells (OECs), bone marrow mesenchmal stromal cell(BM-MSC), umbilical cord mesenchmal stromal cell (UC-MSC), neural stem/progenitor cells transplantations alleviated the symptoms of neuropathic pain and resulted in subsequent motor recovery after SCI. Intrathecal transplantation of embryonic stem cell-derived spinal gabaergic neural precursor cells attenuates neuropathic pain in a SCI rat mode. Previous studies have demonstrated the promising value of adrenal chromaffin cell act as mini-pumps that release amines and peptides for alleviating chronic pain. The paper presents two gentlemen suffering from severe central neuropathic pain after thoracic SCI. Aborted fetus (12 gestational weeks) adrenal taken under sterile conditions was quickly placed at 4 ° C Hanks solution. The adrenal cortex and medulla were repeatedly washed and blunt dissected. Separated medullary tissue were moved to plates and cut into pieces, then collected into a centrifuge tube. They were added 2ml 0.25% trypsin and placed in 37° C water bath digestion, taken 20 minutes after repeated pipetting and then 2ml stop solution to stop digestion. Stainless steel filter to collect all filtrate in a centrifuge tube and centrifuged at 1,500 rpm / 5min, the supernatant was discarded, trypan blue cell counts. Cells were cultured for 3 days with 10% fetal bovine serum and 37 ° C in 5% carbon dioxide incubator. Lumbar puncture (LP) were performed routinely. After cerebrospinal fluid 10ml were released slowly, 1 million chromaffin cells + 5ml normal saline + dexamethasone injection 5mg were injected slowly. Six months after chromaffin cell intrathecal injection, their pain relieved significantly. The results are consistent with previous studies showing the therapeutic efficacy of chromaffin cell transplants in NP, and support the use of this treatment strategy for the management of intractable chronic pain due to SCI, although one of the major limitations for widespread application of grafts is the availability of donor tissue or cells in adequate quantities.

Keywords: chromaffin cell, cell transplantation, neuropathic pain, spinal cord injury

Ping Wu (USA)

Professor in the Department of Neuroscience &
Cell Biology John S. Dunn
Distinguished Chair Professor in Neurological Recovery University of
Texas Medical Branch Galveston, Texas

Neural stem cell and alcohol abuse

Alcohol has been shown to alter neural stem cells (NSCs) in the adult brain. However, little is known about how the dependence of NSC response to alcohol is related to sex and brain region. To better understand these differences, an inducible transgenic mouse model was used to track the fate of adult endogenous NSCs following alcohol consumption. We observed distinct patterns of survival, proliferation and differentiation of NSCs in different brain regions after short and long-term alcohol consumption. We found that chronic alcohol consumption profoundly affected the survival of NSCs in the subventricular zone of the lateral ventricle (SVZ), and also NSCs in the subgranular zone of the dentate gyrus (SGZ) and tanycyte layer of the third ventricle (TL). Significant differences between male and female mice were further discovered in the SVZ and TL NSCs. Thus, adult brain NSCs respond to alcohol consumption in a sex-, time and region-dependent manner.

Committee member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association
Committee members of the Young and Middle-aged Neurosurgery Exchange Association

Hongyan Han

Novel Combined Bypass Surgery for Moyamoya Disease

Objective:We reviewed our 3.5-year experience with a novel bypass procedure, superficial temporal artery to middle cerebral artery (STA-MCA) anastomosis and encephaloduro-myo-arterio-pericranio-synangiosis (EDMAPS), for moyamoya disease regarding its clinical effects and cerebral hemodynamics. Methods:This study included a series of 109 patients with moyamoya diseas. The main clinical manifestations of these patients presented as headache, memory deterioration, transient ischaemic attacks, cerebral infarction, or intracranical haemorrhage. They were performed STA-MCA anastomosis and EDMAPS on 184 hemispheres. In addition to conventional STA-MCA anastomosis and indirect bypass for the MCA territory, the medial frontal lobe was revascularized using the frontal pericranial flap through medial frontal craniotomy. Cerebral hemodynamics before and after operation were analyzed with magnetic resonance imaging, computed tomography perfusion(CTP), computed tomography angiography, and/or cerebral angiography. The improvement of the main symptoms was analyzed. Results: CTP studies on 107 patients 1 week postoperation revealed that cerebral blood flow markedly improved in direct bypass territories. Perioperative complications： Brain haemorrhage or infarction occured in 1 case respectively. During the follow-up period of 4～42 months, 43 (81.1%) of 53 patients who had the symptoms such as headache, memory deterioration, aphasia improved significantly. 5 of 56 patients who suffered from TIA had 11 TIA postoperatively. 105 procedures of 76 patients had CTP study during 3～18 mths postoperation, their CBF and TTP at corresponding area of ACA were compared with preoperation, and found significantly improved.Cerebral angiography performed on 21 patients showed well bypass in the area of operation ,and 17 patients showed that the pericranial flap functioned well as donor tissue for indirect bypass. Conclusion:The clinical data of this series of patients strongly suggest that STA-MCA anastomosis and EDMAPS using a frontal pericranial flap is a safe and effective surgical procedure to improve the neurofunctions in moyamoya disease by improving cerebral hemodynamics in both the MCA and ACA territories.

Key words**:**Combined bypass surgery; Pericranial flap; Moyamoya disease; Cerebral hemodynamics

Stephen Skaper (Italy)

Councillor of the International Association of Neurorestoratology

Editorial Board Member of Neuroscience

Co-ultramicronized palmitoylethanolamide/luteolin promotes oligodendrocyte development, precursor cell survival and improves outcome in experimental autoimmune encephalomyelitis

Oligodendrocytes, the myelin-producing cells of the central nervous system are responsible for ensheathment of axons. Oligodendrocytes have limited ability to repair damage either to themselves or to other nerve cells as in multiple sclerosis (MS), a chronic neuroinflammatory demyelinating disorder of the central nervous system with a strong neurodegenerative component. MS lesions are characterized by the presence of a diminished pool of undifferentiated oligodendrocyte precursor cells (OPCs) which are unable to mature into myelin-producing oligodendrocytes. In such settings, an important strategy may be to replace the lost oligodendrocytes and/or promote their maturation or proliferation. N-palmitoylethanolamine (PEA), an endogenous fatty acid amide signaling molecule possesses analgesic, anti-inflammatory, and neuroprotective actions. Recent studies show a co-ultramicronized composite of PEA and the flavonoid luteolin (co-ultraPEALut, 10:1 by mass) to be more efficacious that PEA alone in improving outcome in experimental models of spinal cord and traumatic brain injuries. Here, we examined the ability of co-ultraPEALut to promote the progression of OPCs into a differentiated phenotype. OPCs were isolated from newborn rat cortical mixed glial cell cultures and maintained under conditions which favor either differentiation (Sato's medium) or proliferation (serum-free medium with fibroblast growth factor-2 and platelet-derived growth factor-AA ('SFM')). When maintained in Sato's medium co-ultraPEALut (10 μ M) treatment of OPCs stimulated, in a time-sependent manner their morphological development, total protein content and gene expression for the major structural myelin proteins myelin basic protein (MBP) and proteolipid protein, the enzyme 2',3'-cyclic nucleotide 3'-phosphodiesterase (thought to mediate process outgrowth in oligodendrocytes and play a critical role in the events leading up to myelination), as well as genes coding for enzymes involved in cholesterol and fatty acid synthesis and antioxidant defense (catalase). Under these conditions, co-ultraPEALut also increased the content of MBP at the protein level. OPCs, maintained in an undifferentiated state (SFM) displayed improved survival capability in the presence of co-ultraPEALut and down-regulation of *Apoe*, whose deletion reportedly leads to a later time of peak symptoms/disease severity and less severe demyelination/axonal damage in myelin oligodendrocyte glycoprotein (MOG35-55)-induced experimental autoimmune encephalomyelitis in female C57BL/6 mice. Importantly, co-ultraPEALut improved the clinical score in this experimental autoimmune encephalomyelitis mouse model, which is often used as a chronic monophasic model of MS. Hence, strategies intended to promote endogenous remyelination in MS patients should focus on both enhancing the long-term survival of OPCs and on stimulating these cells to proliferate and differentiate into remyelinating oligodendrocytes. Within this context, co-ultraPEALut may represent a novel pharmacological approach.

Standing Member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

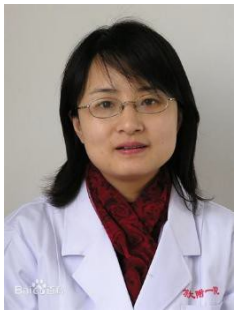
Committee member of Science and Technology Committee of Guangzhou Military

Ming Lu

Hypoxic microenvironment promote olfactory mucosa-derived mesenchymal stem cells (OM-MSC) to differentiate into dopaminergic neurons induced by olfactory ensheathing cell-conditioned medium

Backgroud OM-MSCs have significantly clonogenic activity and could be easily propagated for the purpose of parkinson's disease (PD), how to induce OM-MSCs to differentiate into dopaminergic (DAergic) neurons more efficiently by OECs is an attractive topic. Methods The induction protocol of hyCCpoxia induction(H-I) group has been designed to generate DAergic neurons from OM-MSCs using a physiological oxygen (O2) level of 3% and olfactory ensheathing cells(OECs) conditioned medium, while the O2 level of normal induction(N-I) group stay ambient air level(21%). In order to study the role of hypoxia-inducible factor-1alpha (HIF-1α) in differentiation of OM-MSCs under hypoxia environment, the H-I-R group(H-I group treated with HIF-1α inhibitor before induction) was set for comparsion.Results Compared with N-I group and H-I-R group, the H-I group had a significantly increased proportion of TUJ-1 and TH positive cells through immunocytochemistry and western blot. Further more, the level of DA was significantly increased in H-I group. In whole-cell voltage-clamp test, slow outward potassium current recorded in differentiated cells after 21 days induction. Our results also demonstrate that hypoxia environment can enhance DAergic neuronal differentiation of OM-MSCs by increasing the expression of HIF-1α.Conclusion Hypoxia promotes DAergic neuronal differentiation of OM-MSCs, the HIF-1α may play an important role for hypoxia-inducible pathways in DAergic lineage specification or differentiation in vitro.

Key words： olfactory mucosa mesenchymal stem cells； differentiation； DAergic neuron； hypoxia-inducible factor-1alpha



Qun Xue

Committee member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Contributing editor of Chinese Clinical Physicians Journal of the Chinese Medical Association

The therapeutic mechanism of C3H10T1/2 Stem cells in Experimental Autoimmune Encephalomyelitis (EAE):B7H4 plays a regulatory role in immunization and microenvironment

Mesenchymal stem cells (MSCs), with the capacity of differentiating into multiple lineages, unlimited ability of self-renew, and immune-modulating effect, open new prospects in the management of autoimmune diseases, such as Multiple sclerosis (MS). However, the mechanisms need to be clarified. Our previous studies showed human bone marrow derived mesenchymal stem cells and mice mesenchymal stem cell line C3H10T1/2 (C3H10) constitutively express the negative co-stimulatory molecule B7H4, which mediate their immune modulatory function on T cells in vitro. This experiment, we object: to study the possible therapeutic mechanism of MSCs mediated by B7-H4 in vivo. Method: The lentiviral vectors with mouse B7-H4 target shRNA were transfected into C3H10 (C3H10-B7-H4).The MS mice model, Experimental allergic encephalitis (EAE) was stetted up using peptide MOG35-55 in complete Freund's adjuvant (CFA); C57BL/6 mice(n=50) were divided into five groups: the control group（ n=10 ）, EAE group（n=10）, C3H10 group（implanting C3H10 cells）（n=10）, C3H10-NC group（implanting C3H10-NC cells ）（n=10 ）, and C3H10-B7-H4 group （implanting C3H10-B7-H4 cells ）（n=10 ）. The neurological function impairment score was measured every day. 6 days after the immunization, about 1×106 C3H10 cells, C3H10-NC cells and C3H10-shRNA cells were injected to each EAE mouse respectively. In the acute and chronic aggravating stage of the disease, the expression of B7-H4、PD-L1 on peripheral blood cells were measured by flow cytometry. The expression of sB7-H4 、 IL-2 、 IL-17 、 IFN-γ 、 IL-4 in plasma were detect by ELISA. Flow cytometry was used to detect the expression of B7-H4、PD-L1 on spleen. Pathologic analysis, such as H-E staining, fast-blue staining, immun-influorescent staining were used to analysis the inflammatory infiltrating, demyelination degree in the slices of the spinal cord from each group. Result: The inhibitory effect of C3H10 on spleen lymphocyte secreting B7-H4、IL-2、IL-17、IL-4、IFN-γ was partly revised in vitro. Control group showed no impairment, the onset time of EAE group was earlier than C3H10 group and C3H10-shRNA group; and the nerve function score was higher than that of the other groups. The onset time and neural function impairment score of C3H10-B7-H4 group were between the C3H10 group and EAE group. The proportion of PD-L1+ CD19+B cells and B7-H4+ CD19+B cells in peripheral blood and spleen cells derived from C3H10-shRNA group were between that of C3H10 group and EAE group. There was no significant difference of IL-4 expression among the five groups. While the expression of soluble B7-H4 in the plasma from EAE group was lower than that of normal group, and the expression of IL-2, IL-17, IFN-γ in the plasma from EAE mice were higher than those from the all other groups; those from the C3H10-B7-H4 group were lower than that from the EAE group but were higher than that from the C3H10 group. Tissue histopathology also confirmed the effectiveness of C3H10 transplantation via decreasing cellular infiltration and demyelination, and changing the microenvironment in the spinal cord. Conclusion: This study confirmed that the expression of B7H4 on C3H10 cell can influence its biological characteristics, and plays an important role in stem cell therapy by suppressing the immune response and creating a moderate microenvironment.
Keywords: experimental autoimmune encephalomyelitis (EAE); Mesenchymal stem cells (MSCs); B7H4; C3H10 T1/2

Hamid Mobasheri(Iran）

PhD in Biophysics, University of East Anglia, UK

M.Sc. in Molecular Biology and Biophysics

School of Biological Sciences, University of East Anglia, UK.

Post Doc. University of East Anglia, UK

Biophysical approaches in neurorestarotology at atomic, molecular and cellular levels

The repair of damaged neurons in central nervous system has been challenged for ages with no definite cure, due to their complex and sophisticated nature. Though, different genes, proteins, lipids and involved cells in the immune system have been targeted and manipulated to restore lost activities, we are still far away from complete cure. It seems some aspects were hidden under the simplified pictures and protocols and certain constructive aspects have been missed out. The bioelectric, bioelectronic and bioimpedence nature of the signal conducting nervous system plays major roles in defining temporal and spatial status of individual atom and molecules whose distortion cause disintegration, malfunction, and failure of the damaged host cells and tissues. Accordingly, further to the cellular and chemical treatments applied so far, taking physical approaches and using electrical, magnetic and electromagnetic fields might improve the situation and provide us with efficient treating outcomes. Some machines such as SQUID, Magneto therapy gadgets, TENS, and so on, have already shown progressing results. Here, the effectiveness of intrinsic and external therapeutic physical field stimulation through SSEP, SCEP and other means on the restoration of the lost activities of neurons in the CNS will be discussed. Our studies have already shown the susceptibility of the constituent molecules of the cells to the imposing fields. The disrupted integrity of the membrane has been restored by sealing and restoration of the membrane-cytoskeleton binding affinity. The devastated spatial and temporal dynamics of cytoskeleton constituent molecules were re-established and paved the path for the restoration of the intracellular traffics and cell motility. The approaches presented here relies on the very potential of the damaged cells to re-establish their structural organization and restore the lost function in the first place, and triggering differentiation and directing the delivery of the required drugs if the former approaches were not efficient enough.

Zhouping Tang

Committee member of Neurorestoratology Professional Committee of

Chinese Medical Doctor Association

miR-214 5p modulate the inflammatory response in LPS-induced BV2 microglia by directly regulating the expression of TSG-6

Objectives: To investigate whether ADSCs with the treatment of TNFa could inhibit the inflammatory response of LPS-induced BV2 microglia through TSG-6 and to compare the difference in microRNA profiles between TNFa-treated ADSCs and control ADSCs through high-throughput sequencing technology and cluster analysis, and then to find the microRNA that might modulate TSG-6 mRNA in ADSCs.Methods: ADSCs were harvested from rats and cultured in vitro, and the 3rd to 5th passage cells were applied to our study. The expression of TSG-6 mRNA and protein in ADSCs were tested by RT-qPCR and ELISA assay. siRNA-TSG-6 and siRNA-control were separately transfected into ADSCs, and the TSG-6 gene silenced ADSCs and control ADSCs were co-cultured with LPS-induced BV2 microglia for 6h. The mRNA level of several cytokines in BV2 microglia, including iNOS, IL-1β, IL-6 and TNFa were tested by RT-qPCR. The total RNA was extracted from TNFa treated ADSCs and control ADSCs and sequenced by Illumina HiSeqTM 2500 according to the manual guidance. Poor quality data was removed from raw reads and clean data was analysed to compare the different microRNA profiles between two groups of ADSCs. The differentially expressed microRNAs between TNFa treated ADSCs and control ADSCs were tested by RT-qPCR. Student's t-test was used for comparison between two groups and One-Way ANOVA was used for comparison among three or more groups using SPSS 20.0 software (SPSS Inc. Chicago, IL, USA)Results: In this study, we found that TNFa treated ADSCs could significantly up-regulate the expression of both TSG-6 mRNA and protein. Furthermore, ADSCs were shown to be able to inhibit the expression of pro-inflammatory cytokines including IL-1β, IL-6, TNFα and iNOS in LPS-induced BV2 cells, but the inhibitory effects were much weaker in TSG-6 gene silenced ADSCs. Through the difference analysis and cluster analysis, totally 35 microRNAs were found to be differentially expressed between two groups of ADSCs, 19 in which were down-regulated in TNFa treated ADSCs. miR-214-5p was identified to have the potential to regulate TSG-6 mRNA. ADSCs transfected with miR-214-5p mimic down-regulated the expression of TSG-6 mRNA and protein while ADSCs transfected with miR-214-5p inhibitor up-regulated the expression of TSG-6 mRNA and protein.Conclusion: ADSCs with the treatment of TNFa can regulate the inflammatory response in LPS-activated BV2 microglia by upregulating the expression of TSG-6 and miR-214 5p can directly regulate the expression of TSG-6.

Key words: ADSCs; TSG-6; Inflammation regulation; BV2 microglia; high throughput sequencing; miR-214 5p

Committee member of Orthopaedic branch of Chinese Medical Association

Vice president of the Xiangya hospital

Jianzhong Hu

New prospects for spinal cord microvasculature and neural network imaging: From 2D to 3D

The development of a reliable method for high-spatial resolution three-dimensional (3D) imaging of the microvasculature has attracted increasing interest in the field of neurovascular research. While in the past, the neurovascular imaging was typically confined in two-dimension (2D), the destructive histology method, which was the gold standard for tissue evaluation. However, the 3D nature of the neurovascular architecture was failed to obtained. The understanding of the morphological changes of the neurovasculature in 3D during the diseases development can provide us further insights into the various pathological conditions. Based on Synchrotron Radiation micro-computed tomography (SRμCT), a new prospect for spinal neurovascular imaging has been shifted from 2D to 3D. In this study, we not only present a framework for ultrahigh resolution digitalized mapping of the angio-architecture in rat spinal cord, but also provide a series of delicate 3D anatomical analysis of the vascular structure ranging from cervical, thoracic to lumbar spinal cord. In the same time, the morphological changes and regeneration of the microvascular network after spinal cord injury (SCI) was systematically analyzed in our study. Subsequently, the pro-angiogenetic effect of microRNA21 after SCI was confirmed. In addition, we image the 3D distribution of the micrometric axons and neuron somata in the rat spinal cord. In conclusion, the present research confirmed that SRμCT could has the potential to serve as a powerful imaging tool for evaluation of the morphological changes in the 3D neurovascular architecture of the central nervous system in preclinical experiment.
Keywords: SRμCT; spinal cord injury; three dimension; angioarchitecture; neural network

Department of Tissue Engineering and Applied Cell Sciences,
School of Advanced Technologies in Medicine,
Tehran University of Medical Sciences

Somayeh Ebrahimi Barough
（ Iran）

LY294002 small molecule promote motor neuron differentiation of human endometrial stem cells cultured on electrospun biocomposite Polycaprolactone/Collagen scaffolds

Small molecules as useful chemical tools can affect cell differentiation and even change cell fate. It is demonstrated that LY294002, a small molecule inhibitor of PI3K/Akt signal pathway, can inhibit proliferation and promote neuronal differentiation of mesenchymal stem cells (MSCs). The purpose of this study was to investigate the differentiation effect of Ly294002 small molecule on the human endometrial stem cells (hEnSCs) into motor neuron like cells on polycaprolactone (PCL)/Collagen scaffolds. hEnSCs were cultured in a neurogenic inductive medium contain 1μM LY294002 on the surface of PCL/Collagen electrospun fibrous scaffolds. Cell attachment and viability of cells on scaffolds were characterized by SEM and MTT assay. The expression of neuron-specific markers was assayed by real-time PCR and immunocytochemistry analysis after 15days post induction. Results showed that attachment and differentiation of hEnSCs into motor neuron-like cells on the scaffolds with Ly294002 small molecule were higher than that of the cells on tissue culture plates as control group. In conclusion, PCL/ collagen electrospun scaffolds with Ly294002 have potential for being used in neural tissue engineering because of its bioactive and three-dimensional structure which enhances viability and differentiation of hEnSCs into neurons through inhibition of the PI3K/Akt pathway that manipulation of this pathway by small molecules can enhance neural differentiation.

Key words: PI3K/Akt signaling, small molecule, differentiation, motor neuron cells, PCL/Collagen scaffol

Qunyuan Xu

Advisory Board member of International Association of Neurorestoratology

Councillor of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Honorary President of the Chinese Anatomical Association

Angiogenic microspheres promote neural regeneration and motor function recovery from spinal cord injury in the rat

In order to create an effective approach to improve symptoms after spinal cord injury, the sustained co-delivery of a group of angiogenic factors encapsulated by biodegradable and biocompatible PLGA microspheres into the contusion injury in the dorsal thoracic cord was performed in the rat. The vascular endothelial growth factor (VEGF), angiopoietin-1 and basic fibroblast growth factors (bFGF) were used as angiogenic factors for local vessel formation and neural regeneration in the injury sites. The behavior improvement and neural regeneration in the cord-injured rats were significantly seen after implantation of those factors carried by PLGA microspheres. As for the vehicles for those ahgiogenic factors, the effects by using PLGA microspheres were even better than that by using transgenic cells. Our resuls have shown that angiogenic factors released in a sustained pattern in the injury epicenter can markedly stimulate angiogenesis and neurogenesis *in situ*, leading to faster recovery of neurologic function in rats with spinal cord injury. And the PLGA mirospheres are promissing biomaterials for treatment with the spinal cord injury.

Keywords: VEGF, Ang1, bFGF, PLGA microspheres, angiogenesis, spinal cordinjury

Vice President of International Association of Neurorestoratology
Chair of Educational & Training Committee of International Association of Neurorestoratology
Chair of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Xijing He

Anti-inflammatory and immunomodulatory mechanisms of neural stem cell in spinal cord injury

Background: Neural stem cell (NSC) has been proposed to enhance spinal cord injury function recovery. However, lacking is a detail understanding of the mechanisms by which NSC exerts their therapeutic plasticity. In this study, we investigated the anti-inflammatory and immunomodulatory properties of NSC in SCI-induced neuro-inflammation by IP injection of NSC-conditioned media (NSC-M) into SCI model. Methods: We examined the effects of systemic administration of NSC-conditional media on neuro-inflammation in spinal cord injury (SCI) and the interaction between NSCs and macrophage in vitro and vivo. Results: NSC-M was able to significantly improve motor function and improve lesion healing. In addition, NSC-M demonstrated significant anti-inflammatory potential in vitro and in vivo, reducing inflammatory cytokine production in both activated macrophages and injured spinal cord tissues. NSC-M was also able to reduce the expression of inducible nitric oxide synthase (iNOS) within the spleen of injured animals, indicating an ability to reduce systemic inflammation. Thus, we believe that NSC-M offers a possible alternative to direct stem cell engraftment for the treatment of SCI. Conclusions: The results of this study suggest that NSC-M have the ability to modulate inflammation-associated immune cells and cytokines after SCI and further refinement could offer an alternative to neural stem cell engraftment. NSC-M offers an exciting opportunity for treatment of SCI.

Key words: Neural stem cell, Spinal cord injury, macrophage, inflammation cytokine

Liming Cheng

Committee member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Committee member of Clinical Medicine Teaching Steering Committee of the Education Ministry

Strategies of endogenous neural stem cells mobilization for spinal cord injuries reconstruction

Though the surgical procedures, neurotrophic factors, HOP therapies and modern rehabilitation provide better clinical efficacies, spinal cord injuries are still challenges for medicine due to the nerve regeneration and neural circuits reconstruction. Our recent studies found that administration of VEGF and bFGF active CD133+ ependymal NSCs and revealed the existence of dormant ependymal neural stem cells throughout the CNS and the signals pathways. Another job reported that neurotrophin-3 coupled chitosan biomaterial elicited robust activation of endogenous NSCs in the injured spinal cord and enhanced endogenous neurogenesis could be a potential strategy for treatment of spinal cord injuries. At the same time, physical stimulation play very important role in the neurogenesis regulation. Based on the molecular pathogenesis of spinal cord injuries, we will reveal the lineage and activation factors of the endogenous NSCs during spinal cord injury reconstruction though single-cell transcriptome and weighted gene co-expression network analysis (WGCNA) in the animal models. In addition, we explore the mobilization and neurogenesis mechanism of ependymal NSCs with physical stimulation including ultrasound, magnetic and electric field and get the optimum parameters. What's more, physical stimulation tolerance biomaterial and nano materials coupled with mobilization factors will be transplanted into the injured spinal cord for nerve regeneration and neural circuits reconstruction. A novel strategy based on endogenous NSCs for treatment of spinal cord injuries could be clinical translated with physical rehabilitation, which promote the CNS treatment and related health industry development.

Member of The Chinese medical association orthopaedic branch

Member of Sports medical branch of Chinese medical association

Hua Feng

Advances in the scarring after spinal cord injury

Glial scar occurs after traumatic nerve injury, which is composed of astrocyte with its secreted extracellular matrix. Studies have shown that reactive scarring formation plays protective role in the early stage after injury. However, continuous astrocytic activation induces hard glial scar, which plays negative role in the neurological recovery. We investigated the temprospacial features of glial scar formation and quantified the thickness of scarring. We also revealed that CSPGs and Vim are the components of glial scar, as well as the underlying mechanism of their inhibition on the neuroregeneration. Furthermore, we found the optimal time window of intervening the scar and several strategies inhibiting the formation of scarring.

Kyoung-Suok Cho (South Korea)

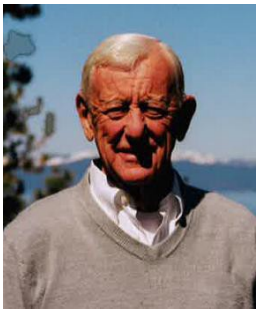
President of Asia-Oceania Neurotrauma Society

Professor & Chief of Spinal Surgery Dept.

of Neurosurgery Uijongbu St. Mary's Hospital

Hypothermia Treatment for Cervical Spinal Cord Injury in Rats

Introduction: Hypothermia has been employed during the past 30 years as a therapeutic modality for spinal cord injury (SCI) in animal models and in humans. We investigated that systemic hypothermia effect to spared tissue volumes, preserved neurons, and intact supraspinal axon projections as well as an enhanced rate of open field locomotor recovery and increased forelimb strength. Material & method: With our newly developed rat cervical model of contusive SCI, we investigated the therapeutic efficacy of transient systemic hypothermia (beginning 5 minutes post-injury for 4 hours, 33°C) with gradual rewarming (1°C per hour) for the preservation of tissue and the prevention of injury-induced functional loss. A moderate cervical displacement SCI was performed in female Fischer rats, and behavior was assessed for 8 weeks. Results: Histologically, the application of hypothermia after SCI resulted in significant increases in normal-appearing white matter (31% increase) and gray matter (38% increase) volumes, greater preservation (four-fold) of neurons immediately rostral and caudal to the injury epicenter, and enhanced sparing of axonal connections from retrogradely traced reticulospinal neurons (127% increase) compared with normothermic controls. Functionally, a faster rate of recovery in open field locomotor ability (BBB score, weeks 1–3) and improved forelimb strength, as measured by both weight-supported hanging (43% increase) and grip strength (25% increase), were obtained after hypothermia. Conclusion: The current study demonstrates that mild systemic hypothermia is effective for retarding tissue damage and reducing neurological deficits following a clinically relevant contusive cervical SCI.



Clinical Professor of Neurosurgery

University of California

Harry Sawyer Goldsmith (USA)

The Omentum in the Treatment of Acute and Chronic Spinal Cord Injuries in Animals and Man

Ramon Y. Cajal, the father of neuropathology, demonstrated over a century ago that the failure of neurological improvement following a spinal cord injury (SCI) is due to scar that develops at the site of the SCI which restricts axons from penetrating through the scar barrier. It will be shown that SCI scar that has been surgically excised can be followed by a successful reconstruction of the spinal cord that will lead to a return of function in animals and in a human.Information will be offered in five areas: The surgical method will demonstrate how a spinal cord scar can be surgically removed. The gap in spinal cord (SC) continuity can be corrected by using a collagen-omental bridge through which neurons advance at the rate of 1mm/per day down into the distal SC. Physiological reactions occurring at the omental-collagen bridge that connects the cut ends of the divided SC will be explained. Functional improvement following excision of SC scar has been successful in animals and in man. A video will be shown of a patient whose SC scar and 1.6" of her SC removed and will show the ability of the patient to walk 1½ years after surgery. Serial MRI's over a five-year period will show that the SC has the ability to heal.It will be shown how SC scar that routinely develops shortly after SCI can be completely prevented, and may be significant in the future treatment of SCI. This work strongly supports the concept of Ramon Y. Cajal who claimed that the removal of SC scar could lead to functional improvement.

Combined treatment: cellular therapy and rehabilitation for chronic SCI patients. Final results face I-II clinical trial.

Moviglia-Brandolino

Introduction: In June 2013, 8 chronic and complete SCI patients started combined treatment: Cellular therapy and intensive rehabilitation looking forward to restore the spinal cord interruption. After 18 months of treatment 7/8 showed electromyography recovery in muscles, previously denervated, distant more than 2 segments below the last full preserved one. Following this results, in June 2015, according to Simon's two step design, 6 more patients with the same conditions were added to the trial. Methods: Trial approvals, rehabilitation and cellular therapy were reported in ISCoS 2015. In June 2013, 8 Chronic ASIA A/Frankel A SCI patients (5 paraplegics and 3 quadriplegics) were accepted for the trial. Before starting treatment and every 6 months, electromyography of denervated muscles was performed. In June 2015 the same protocol was repeated on other 4 Chronic ASIA A/Frankel A SCI patients (2 quadriplegics and 2 paraplegics).Results: Electromyography recovery was observed in muscles previously denervated distant more than 2 segments below the last full preserved spinal segment on 7/8 patients from the first group and on 3/4 patients from the second group. These changes started after 18 months in the first group and after 10 months in the second group. Together with the electrophysiological changes, patients from both groups showed functional recovery. According to NIH-USA scale, no severe adverse events associated with the treatment were observed, Conclusions: Combined therapy applied to chronic and complete SCI patients is safe. Electrophysiological changes are objectives results to prove the efficacy of the treatment.

Electrophysiology Studies to Objectively Asses Efficacy and Efficiency after Regenerative interventions for Spinal Cord Injuries.

Dr.Albanese

Introduction: Modern regenerative therapies have defeat the long standing paradigm of impossibility of central nervous system repair. However, there is a lack of instruments to objectively evaluate the recovery achieved for these modern therapies. Clinical scales are based on the neural destruction but are poor tools to evaluate recovery. Instrumentalization of the spinal cord difficult the imagen based studies. Moreover, secondary atrophies of chronic denervated peripheral organs make the clinical evaluation even less accurate. To overcome these difficulties we have explored the utility of Sensitive Evoked Potentials as well as EMG registers after voluntary contraction as affordable and accurate methods to asses clinical recovery. Method: To achieve this task Different Series of untreated and treated, acute and chronic, partial and complete, Spinal Cord Injury Patients have been evaluated. Sensitive Limb Evoked Potentials as well as EMG analysis of the muscle groups affected by their lesions using Akonic® Model BIO PC with an Electro Stimulator 2000. Because the high degree of muscle atrophies a needle bipolar electrode was placed into the muscle parenchyma. They were localized using a sonogram machine (SonoSite® M turbo 500, with a linear Transducer of 5-10 MHz). After take a basal reading patient received an order to move the punched muscle. Registers were positive when a voltage and wavelength change during the execution of a voluntary movement. To evaluate abdominal muscle functions, superficial EMG was used.
Results: At the beginning, on acute patients, Electrophysiology studies showed differences that allow us re-stratify patients' ASIA condition to a better situation. On the chronic conditions the differences were observed after cellular interventions were the electrophysiological changes appear 6 to 12 months previous the clinical changes were detected. When appear registers improved with clinical improvements.
Conclusion: Electrophysiology studies seems to be necessary on the SCI patients to evaluate clinical condition, prognosis and treatment results.

Jianjun Li

China Rehabilitation Research Center

Capital Medical University

The Hong Kong Polytechnic University

Strategy of the brain motor control after spinal cord injury- A pilot study

Introduction: It is known that the brain motor system function is deficient after spinal cord injury (SCI). And motor output is not a static event but instead requires modulation during performance of most tasks. The effects of chronic SCI on modulation of brain motor function are preliminarily to be explored by this study. Methods: Eight chronic SCI with complete paralysis and ten healthy control participants were recruited with matched demographic characteristics. Functional brain MRI images were acquired when the participants were performing the visuo-motor imagery tasks of the upper or lower limbs. Event-related BOLD responses and the behavioral performances in terms of accuracy rate and response time were analyzed separately for the upper and lower limb conditions. Results: For the upper limb condition, in consistent with previous studies extensive sensori-motor brain areas were activated for the SCI, including the bilateral right precentral gyrus, the left postcentral gyrus, the right middle frontal gyrus, the bilateral superior temporal gyrus, the right superior and inferior parietal gyrus, the right external globus pallidus (GPe) and the thalamus. While, more activations in the frontal areas were revealed in the healthy control, such as the left middle frontal gyrus, the medial frontal gyrus, and the left anterior cingulate. For the lower limb condition, stronger BOLD responses elicited in the left lingual gyrus among the SCI than healthy control participants. No obvious significantly differences of the behavioral performances were revealed. Conclusions: The present study supported that brain neuroplasticity of motor control function occurred after injuries to the spinal cord due to the lacking of physical experience of the paralyzed limbs. It is meaningful to explore new rehabilitation strategy for SCI patients, also helpful to understand the mechanisms of brain motor control.

Maryam Shabany（Iran）

Brain and Spinal Cord Injury Research Center, Neuroscience Institute,
Tehran University of Medical Sciences,

Tehran, Iran

Perspectives of Health professionals on Barriers of empowerment for care of spinal cord injury patients: a Qualitative Study

Background: empowerment is promotion of self-care efforts purposely that individuals, families and communities perform for improving and maintaining their own health and others. According to the socio-cultural fields, empowerment in Iranian spinal cord injury patients has special barriers. In this qualitative study has been investigated health professionals' perspectives regarding these barriers. Materials &Methods: A qualitative content analysis method was used to analyze the gathered data. The study participants were 9 people of health professionals Who investigated in the field of spinal cord injury in Iran. They choosed by purposeful sampling. Data was gathered through semi structured interviews and face to face and it was conducted until data saturation. Data analysis and content analysis was conducted in desember 2015 to may 2016. The data analyzing was down with MAXQDA10 software. Results: Data analysis emerged four main categories as barriers to empowerment in the care of patients with spinal cord injury with categories 1. the lack of services in the acute phase (with subcategori of lack of acute care center), 2. the need for training (with three subcategories: information deficient patients, poor in proper education, poor care by the family of patients), 3. weakness in administrative matters (with four subcategories: lack of teamwork, lack of home care with low cost, lack of follow-up care by patients and health care systems, lack of professional competence), and 4. the lack of a suitable context for empowerment dimensions (with six subcategories: the lack of specialized centers, the lack of community facilities, lack of proper home environment, the lack of legislation, lack of good welfare, Not giving priority to rehabilitation of the disabled in the planning ministry). Conclusion: Achieving the above themes can increase the awareness of governments, communities, families and treatment authorities in care of patients with spinal cord injury and these barriers would empower authorities to develop more accurate planning and better support of patients.

Key words: Spinal cord injury, Empowerment, Health professionals, Content analysis, Iran

Yaping Feng

Standing Member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Indication and Operative Technique of Intramedullary Decompression After Spinal Cord Injury

Surgical Indications: Complete spinal cord injury （ ASIA-A ） ; High tension of spinal dura mater, fluctuation disappearance of spinal cord; CT or MR： intramedullary compression by bony fragments or foreign matter; severe contusion of spinal cord; intramedullary hematoma or softening region. Four Intraoperative Findings and Corresponding Interventions: Form Ⅰ: Arachnoid adhesion, fluctuation disappearance of spinal cord, obstruction of CSF, pale and swollen of spinal cord.ntervention: Release adhesion of the arachnoid, restoring the CSF flow and pulsation of the cord. Form Ⅱ: Intramedullary hematoma, bony fragments or foreign matter.Intervention: Remove hematoma, bony fragments or foreign matter and explore the cord. Form Ⅲ: The spinal cord was partly disrupted. Liquefied tissues might gush out as soon as the dura mater was opened.Intervention: Explore the injury site, removing the necrotic tissues and washing the region gently with NS. Form Ⅳ: Intramedullary softening region was found. Intervention: Making a 0.3~0.5cm longitudinal incision at the softening region, removing the softening tissue and washing the cavity gently with NS. Intramedullary decompression or not ? Our point of view: necessary, early stage.

Efficacy of Granulocyte-Colony Stimulating Factor Administration for Neurological Improvement in Incomplete Traumatic Spinal Cord Injuries: A Double Blind Randomized Clinical Trial

Nazi Derakhshan rad（Iran）

Introduction: Granulocyte-colony stimulating factor (G-CSF) is a major growth factor in the activation and differentiation of granulocytes. This cytokine has been widely and safely employed, in different conditions over many years. In this study we tried to administer the drug for spinal cord injury. Methods: Ninety patients with spinal cord injury of at least six month duration were included into the study. Patients were assessed by ASIA, SCIM III and IANR-SCIFRS just before intervention and at six month after subcutaneous administration of 5μg/Kg of Granulocyte-Colony Stimulating Factor in the case group and placebo in the control group. Randomization was perfumed with random block design, the patients and evaluators were blinded in regard to the treatment group. This RCT was conducted on 90 traumatic SCI patients. Forty five patients were studied in GCSF group and 45 patients in placebo group. The mean (SD) age of the patients in GCSF group was 31.6 and 33.8 years in the placebo group. About 80.0 % were male in GCSF group and 88.4% in the placebo group. The neurological level was 19 cervical, and 26 cases were thoracic lesions in the GCSF group. In the control group there were 23 cervical, and 22 thoracic cases. All the cases were incomplete post-rehabilitation spinal cord injuries. Results: After 6 months of intervention and follow up, ASIA Impairment Scale (AIS) in control group remained unchanged while in GCSF group 1 case improved from AIS B to C, and 4 AIS C patients improved to AIS D. The mean improvement in ASIA motor score in GCSF group was 5.8 scores that was significantly higher than control group (0.89 scores) ($P < 0.05$). The mean light touch and pin prick sensory scores increased by 6.67 and 10.2 scores in GCSF group and by 1.61 scores for light touch and 0.95 score for pin prick in the control group ($p = 0.003$). Evaluation of functional improvement by FRS instrument revealed significantly higher improvement in GCSF group (3.26 scores) compared to the control group (0.35 scores), ($P < 0.001$). Also significant difference in functional improvement between two groups observed by SCIM instrument (6.36 vs. 1.91, $P < 0.001$).Conclusion: Granulocyte-colony Stimulating Factor administration in incomplete spinal cordinjuries is associated with significant motor, sensory, and functional improvement. Multicenter
study would be the next step for treatment establishment.

Keywords: Spinal Cord Injury, Granulocyte-Colony Stimulating Factor, Neurological Restoration

Sang Ryong Jeon
(South Korea)

Department of Neurological Surgery,
Asan Medical Center, University of Ulsan College of Medicine

The strategy and present of stem cell therapy for human SCI

Mortality of spinal cord injury (SCI) was 95% before World War II, but decreased to 5% during WW II by treatment improvement. However, SCI is still intractable disorder and the survived patients suffer from disability. Until now, innovative treatment is not developed, and we are recently paying attention to the availability of stem cells to treat this disorder as stem cells have the characteristics of proliferation, differentiation, restoration of neuronal structures by several mechanisms including secretory factors. The strategiesare different depending on diseases to treat. In cerebral infarction or brain injury, stem cells should function as neuronal precursors replacing neurons to restore new neuronal circuits, and in degenerative CNS disorders such as Parkinson's disease, multiple system atrophy, or ALS, they should suppress neuronal apoptosis as well as replace neurons. On the other hand, they should function as enhancing axon regeneration in SCI. The authors performed clinical trials with mesenchymal stem cells (MSCs) for chronic SCI treatment as pilot study and following phase III. MSCs were autologous cells harvested from the patients' iliac bone and expanded by culturing for 4 weeks. Through these studies, we observed not only the evidence of MSCs effects in chronic SCI but also the weakness of pure MSC power regarding therapeutic effect which appeared only in upper extremity (not in lower extremity). Regarding these results, the future strategy should focus on the ways to enhance the effects of stem cells such as materials to be combined with stem cells , gene modification for secreting trophic factors or cytokines based on the safety of MSCs. When these developments progress continuously, successful treatment of SCI comes true.

Keung Nyun Kim (South Korea)

Department of Neurosurgery Spine and Spinal Cord
Institute Yonsei University College of Medicine

Clinical Trial of Neural Stem Cell Transplantation in Patients with Traumatic Cervical Spinal Cord Injury

In a phase I/IIa open-label and non-randomized controlled clinical trial, we sought to first test the safety, tolerability, and neurological status of 19 patients with traumatic sensorimotor complete (n = 17) or motor complete (n = 2) cervical SCI following transplantation of human neural stem/progenitor cells (hNSPCs) into the injured cord. Participants were 18-57 years of age with no concurrent peripheral nerve or nerve root injury. hNSPCs were derived from the fetal telencephalon at 13 weeks of gestation, grown, and maintained as neurospheres, and transplanted into the injured cord from 18 to 213 days after SCI. In the control group, who did not receive cell implantation, but were otherwise closely matched with the transplantation group, 15 patients with traumatic cervical SCI were included. Assessments included medical and neurological examinations using the American Spinal Injury Association Impairment Scale (AIS), electrophysiological monitoring, and magnetic resonance imaging. At 1 year after cell transplantation, there was no medical or surgical complication to indicate that the procedure was unsafe. There was no evidence of cord damage, syrinx or tumor formation, or deterioration in neurological status. AIS grade improved in 5 of 19 (26.3%) transplanted patients, 2 (A→C), 1 (A→B), and 2 (B→D), whereas no improvement was observed in the control group. In the early subacute treatment group, 30% (3/10) of patients with AIS-A improved to AIS-B or C. Improvements included increased motor scores, recovery of motor levels, and responses to somatosensory (35.3%) and motor evoked potentials (58.8%) in the transplantation group. These results demonstrate that the transplantation of allogeneic fetal hNSPCs into cervical SCI is safe and well-tolerated, and is of some neurological benefit up to 1 year post-implantation. Further long-term, larger-scale, and randomized clinical trials are required to establish evidence of safety and efficacy.

Key words: human neural stem/progenitor cells (hNSPCs); transplantation; cervical spinal cord injury

Advisory Board member of International Association of Neurorestoratology

Dajue Wang (UK)

Why does a little mean a lot when you have nothing? – a brief review of strategy of cell therapy for spinal cord injury

Without understanding of the functional recovery of the musculoskeletal system, the translation of knowledge about neuroscience from discovery in laboratory to bedside application is not complete. As improvements of neurological functions after cell transplant are minor and can be easily neglected, the article draws attention to the minimum improvements that are required to make a spinal cord patient or person walk. These minimia include (1) the power of the key muscle to make the trunk stable; (2) the power of the key muscle to make a paraplegic walk (3) the power of the key muscle to make a hand useful or functional.The grading of muscle power of the British Medical Research Council (MRC) is more sensitive and delicate than the ASIA Standards that only full range of movement is taken into account. The MRC system seems to be preferrable to the ASIA Standards in clinical trial of cell transplant where minute improvements of function may matter. The threshold of function is a power of grade 3 and more. Even if the power of all relevant muscles is of grade 3 only, the patient can be minimally functional and hence relatively independent. These muscles are latissimus dorsi, hip flexors, shoulder abductors and flexors, elbow flexors and extensors, and wrist extensors. They are innervated by C5-7 spinal cord segments.

黄红云

国际神经修复学会创始主席、 中国医师协会神经修复学专业委员会创始主委

《Journal of Neurorestoratology》主编 武警总医院特聘专家、主任医师、神经修复学研究所名誉所长

我国临床细胞治疗相关法律法规和现状

我国在 1991 年始制定了与细胞治疗相关的法规，随后一直试图制定或完善相关法规，但截至目前仍是依靠一纸通知（这是国务院三令五申强调要以法律法规取而代之的）来管理临床细胞治疗。原卫生部在 1991 年出台《中华人民共和国卫生部关于人流产胎儿制剂的研制和临床观察的意见》（1991 年卫科技发（006 号），其中具体规定：“人胎器官、组织的功能性移植，按临床常规进行。”我国不同省市区按此规定制定了一些实施细则，如 1999 年北京市卫生局和物价局对临床脑移植的收费做了具体规；这是我国从事临床细胞治疗一直遵循和执行的法律法规。2003 年 3 月 20 日国家食品药品监督管理总局发布《人体细胞治疗研究和制剂质量控制技术指导原则》，这是按药物管理思路制定法规，但仅是指导原则，后续没有发布具体实施细则。2009 年原卫生部公布了《首批允许临床应用的第三类医疗技术目录》（卫办医政发〔2009〕84 号），随后发布了《细胞移植治疗技术管理规范（干细胞除外）》、《自体免疫细胞（T 细胞、NK 细胞）治疗技术管理规范》、《脐带血造血干细胞治疗技术管理规范》、《造血干细胞（脐带血干细胞除外）治疗技术管理规范》、《组织工程化组织移植治疗技术管理规范》等于细胞相关的管理征求意见稿；这是按临床技术管理思路制定法规。2013 年卫生部、国家食品药品监督管理局发布《干细胞临床试验研究管理办法（试行）》、《干细胞临床试验研究基地管理办法（试行）》和《干细胞制剂质量控制和临床前研究指导原则（试行）》征求意见稿。2015 年卫计委和药监局联合发布《干细胞临床研究管理办法（试行）》。2015 年 7 月 2 号《国家卫生计生委关于取消第三类医疗技术临床应用准入审批有关工作的通知》发布，并附有《限制临床应用的医疗技术（2015 版）》，其中包括“造血干细胞（包括脐带血造血干细胞）移植治疗血液系统疾病技术”，但《细胞移植治疗技术管理规范（干细胞除外）》、《自体免疫细胞（T 细胞、NK 细胞）治疗技术管理规范》、《组织工程化组织移植治疗技术管理规范》未在列。而“未在上述名单内的《首批允许临床应用的第三类医疗技术目录》其他在列项目，按照临床研究的相关规定执行。”2016 年 5 月 4 号卫计委发布通知，重申未在《限制临床应用的医疗技术（2015 版）》名单内的《首批允许临床应用的第三类医疗技术目录》其他在列项目，按照临床研究的相关规定执行。”目前的现状是我国临床细胞治疗可以开展临床研究，不能收取细胞费用。

毛更生

国际神经修复学学会理事 中国医师协会神经修复学专业委员会副主任委员兼总干事 中国研究型医院学会脑血管病学专业委员会 副主任委员兼秘书长

血脑屏障临床研究进展

目的：复习血脑屏障（blood-brain barrier, BBB）研究进展，就 BBB 在脑慢病治疗中对神经修复作用的影响作初步探讨。方法：总结近 10 余年 BBB 相关研究的国内外文献以及武警总医院三年来典型脑慢病治疗病例分析。结果和结论：中枢神经系统(CNS) 与血液之间存在着复杂的 BBB，严格控制着血液与脑组织间的物质交换，虽有利于维持脑内环境稳定，但对中枢神经系统药物转运构成了巨大的挑战。目前对于 BBB 的临床研究取得了令人鼓舞的成果:BBB 结构特点，BBB 开放机制，BBB 转运系统、转运载体和细胞信号转导通路研究的越来越透彻，临床上多种脑慢病病例似乎有效，但如何设计更有效的开放 BBB 策略，实现药物等治疗因子安全、高效、可控地透过 BBB，在脑内发挥治疗作用，仍需要进一步的深入研究。

唐洲平

中国医师协会神经修复学专业委员会委员 中华医学会急诊医学分会心脑血管病学组副组长 中华医学会神经病学分会神经生化学组委员 中国医师协会神经病学分会疼痛与感觉障碍学组委员

脂肪干细胞在神经系统疾病中的临床应用进展

目的：总结脂肪干细胞临床应用的最新进展；方法：复习人脂肪干细胞移植治疗神经系统疾病近年来多篇文献，并结合本课题组的研究进展进行汇报总结。结果：人脂肪干细胞具有取材容易，体外增殖稳定，且多向分化潜能，是目前干细胞研究的热点。脂肪干细胞在自体移植方面已经进入了 II 期临床试验阶段，广泛应用于多个临床疾病。ADSC 应用于神经系统疾病的临床试验仍在进行阶段，相关实验室有望在 3 年内公布实验所得到的临床数据。目前 hADSC 的主要研究进展主要集中在多种神经系统疾病的动物模型中，包括自发性或继发性脑损伤、神经系统退行性变、脱髓鞘以及肌营养不良。移植方法可以是系统移植，如静脉或动脉移植，也可以是定向移植，如肌肉、侧脑室等。hADSC 的主要保护性机制主要通过分泌多种促存活的细胞因子以及多种抗炎因子，并且部分存活的 ADSC 能定向分化为神经细胞。脂肪干细胞的临床应用还有很长的路，其转化医学得到国家科技部的大力支持，未来还需干细胞的安全性、适用性、疗效性进行权威的评估。结论：脂肪干细胞是干细胞的新兴方向，仍有许多未知领域值得深入探索和研究。

肖娟

北京市虹天济神经科学研究院

临床细胞制备标准化探讨

目的:探讨临床细胞制备标准化操作流程。方法:通过探索制定取材、分离、扩增传代、分化、生 物学特征鉴定方法、细胞冻存及复苏、建立细胞质控体系及建立临床级标准细胞库等环节的标准操作 规程。结果:确定细胞扩增、传代能力及其生物学特征和变化规律，制定出临床细胞产品质量和质控评 估标准化流程。结论:本研究可为临床细胞治疗的细胞制备质控建立推广普及标准。
关键词:细胞制备 标准化 细胞治疗

高文勇

中国武装警察总医院神经修复研究所

北京市虹天济神经科学研究院

神经营养因子及其血脑屏障转运的研究进展

神经营养因子(Neurotrophin NT)是一类对神经系统的生长发育、保护修复、参与凋亡等起着重要作用的蛋白因子。根据其结构和受体等生物学特征可分为：神经生长因子（NGF）、脑源性生长因子(BDNF)、神经营养因子 3(NT-3)、神经营养因子 4(NT-4)等蛋白因子。但因其分子量以及血脑屏障(Blood Brain Barrier BBB)的特殊性等多种原因，导致其进入脑内的量极低。本文介绍通过以下三种分子生物技术方法可提高其生物学活性：（1）高纯度神经营养因子产出率；（2）优化编辑神经营养 因子的氨基酸序列，可靶向提高其对特种神经细胞的生物学效应；（3）添加连接特异性的生物载体，可以提高其在血脑屏障的通过率，达到临床有效的治疗浓度。
关键词：神经营养因子 血脑屏障 生物载

Committee member of Neurorestoratology Professional Committee of Chinese Medical Doctor Association

Mengzhou Xue

Matrix metalloproteinse-9 exacerbates brain injury following intracerebral hemorrhage in mice

Background: Matrix metalloproteinses (MMPs) have been implicated in inflammatory processes within the CNS and have received special attention as contributors to brain damage in intracerebral hemorrhage (ICH). Recent studies in ICH have shown that MMP-9 are implicated in pathogenesis in ICH. The modulation of MMP-9 may represent a potential therapeutic target for reducing brain injury and inflammation and improving clinical outcome. Methods: The human fetal neurons and the mouse ICH models induced by autologous blood were used to evaluate the neurotoxicity MMP-9 and the combination of MMP-9 with other proteases (thormbin and MMP-3). The area of brain damage, the extent of neuronal death and activation of microglia/macrophages as well as the neutrophil infiltration were investigated. Results: Human fetal neurons died when exposed to MMP-9 or thrombin, MMP-3 isolation but that their combination increased neurotoxicity in cell culture. Thrombin can activate proMMP-9 to active MMP-9. In ICH model in mice, MMP-9 is significantly increased following ICH; the area of brain damage, the extent of neuronal death and activation of microglia/macrophage were reduced in MMP-9 null mice compared to controls; moreover, the concordant antagonism of thrombin using hirudin alleviated further the injury found in MMP-9 null mice, emphasizing the collaborative role of MMP-9 and thrombin in inducing ICH injury. The brain damage and neuronal death induced by blood was reduced further in MMP-3/-9 double null mice treated with hirudin. Conclusions: These data suggest that in the acute phase of ICH, inhibition of MMP-9 activity represents a potentially effective target for ICH patients.

乔立艳

清华大学玉泉医院神经内科科主任 中国微循学会神经变性病专业委员会委员

脑卒中的临床修复学进展

脑卒中是世界上第二常见单因死亡原因，全球每年有 5 百万人因此死亡。到目前为止 常规治疗还不能有效地修复脑卒中所致的神经功能缺损。已有证据表明神经修复治疗对脑卒中的治疗有意义。干细胞是指一群有能力自我更新并分化为不同组织的细胞。在大量的动物试验中这些细胞及其分泌的因子能够改善功能恢复，降低梗死的面积。在脑卒中病人中的临床试验也证实某些种类细胞的安全性和可行性。细胞为基础的神经修复治疗可以被分为细胞内和细胞外治疗。细胞内治疗的目的是刺激已经在个体存在的干细胞或祖细胞。粒细胞集落刺激因子 G-CSF 是细胞内治疗的成功代表。细胞外治疗是指将细胞通过直接脑实质内、经鞘内或系统性（经静脉或动脉）等方式移植到体内。细胞为基础的神经修复治疗对脑卒中神经功能的修复，无论在急性还是慢性期治疗都可显示治疗价值，改善患者的生存质量，发展脑卒中细胞为基础的神经修复治疗是有依据可循的。本文基于脑卒中患者细胞为基础的神经修复治疗的现状，对各种潜在的方向进行了总结。

中国医师协会功能神经外科专业委员会全国委员
《中华神经创伤外科电子杂志》、《中华诊断学电子杂志》及《中国临床神经外科杂志》编委

王 翀

老年人三叉神经痛的神经修复策略

目的: 探讨老年人三叉神经痛手术治疗方式，方法: 针对老年人多发内科基础疾病的特点，介绍 5 例合并脑动脉瘤老年三叉神经痛的治疗方法选择。 结果: 4 例选择三叉神经血管减压+感觉根部分切断，1 例其它方法。结论: 老年人三叉神经痛存在神经变性可能，尤其合并动脉瘤者需个体评估MVD 可能性；手术需感觉根部分切断，防止复发及二次手术，提高生活质量。

血管内治疗对急性缺血性卒中患者神经功能修复的意义

杜世伟 目的：评估血管内治疗对急性缺血性卒中患者神经功能恢复的意义。方法：回顾性分析我科近五年实施血管内治疗的急性缺血性卒中患者临床资料，包括临床表现、发病时间、入院 NIHSS 评分、治疗方法、血管再通时间、血管再通程度、术后 24 小时 NIHSS 评分、术后 3 月 mRS 评分等数据，并进行统计学分析。结果：30 例急性缺血性卒中患者，术前均经影像学证实为急性颅内大动脉闭塞，通过对其实施动静脉联合溶栓或机械取栓，其中 90%（27/30）的患者可以实现 TICI2b-3 级血管再通，术后 3 月预后良好率 43.3%（13/30），血管内治疗可早期实现血管再通，恢复有效血流灌注，对神经功能修复具有重要意义，明显降低该类患者的致死率及致残率。结论：血管内治疗可有效实现急性缺血性卒中患者的早期血管再通，恢复有效血流灌注，对该类患者的神经修复具有重要的意义。

关键词：机械取栓；动静脉联合溶栓；缺血性卒中；神经修复

卒中后抑郁损害卒中后神经的自我修复：大鼠海马蛋白质组学研究

刘海朋

目的: 卒中后抑郁不但发病率高，而且能够阻碍卒中患者肢体和认知功能受损的恢复，增加卒中患者的死亡率。目前，卒中后抑郁的作用机制尚未完全阐明。方法: 左侧前额叶梗死的患者更容易出现卒中后抑郁，本实验采用左侧额叶运动皮层损毁法制作脑卒中模型，对卒中大鼠开展持续三周的慢性温 和刺激制作卒中后抑郁模型，使用糖水偏好实验、强迫游泳实验和水迷宫实验对大鼠模型做出评价。 分别获取三组（卒中后抑郁组、卒中组和假手术组）大鼠左侧海马，使用双向电泳对蛋白进行分离， 使用 PDQuest 软件寻找差异蛋白，最终使用质谱对差异蛋白进行鉴定。结果: 行为学结果提示卒中后抑 郁大鼠抑郁症状持续存在，卒中组大鼠在卒中后 1 周开始出现抑郁症状，但在第三周抑郁症状消失。与卒中组相比，卒中后抑郁大鼠空间记忆能力明显受损。蛋白质组学结果提示双向电泳共分离出 1475 个点，其中卒中组与对照组比较有 32 个差异蛋白，卒中后抑郁和对照组差异蛋白有 33 个。有 18 个差异蛋白在卒中组和卒中后抑郁组中反向调控：和对照组相比，其中有 18 个蛋白在卒中组表达升高，这些蛋白参与了神经发生、神经细胞迁移、抗氧化应激、抗凋亡和能量代谢，然而这些蛋白在卒中后 抑郁组明显降低。结论 卒中大鼠出现了短暂的抑郁，随后自然恢复。卒中后抑郁能够损害大鼠的空间记忆能力。卒中能够引发海马神经的自我修复。卒中后抑郁可能通过抑制海马神经发生和迁移，加重 能量代谢障碍，增加氧自由基损害，促进神经细胞凋亡等机理（Figure 1），损害了卒中引发的神经自我修复，阻碍了神经功能的恢复。

过表达 miR-34a 神经干细胞移植治疗 AD 模型小鼠的研究

王建勇

目的： 研究过表达 miR-34a 的神经干细胞移植对 AD 模型小鼠的治疗作用。方法： 使用第 2 代至第 5 代的神经干细胞进行转基因操作和移植实验。慢病毒浓缩时使用神经干细胞的增殖培养基进行病毒收集。细胞移植实验每侧海马齿状回区域植入细胞数量为 1×105 个，注射体积 2 μl，注射时间 5 分钟，留针 5 分钟。注射坐标参考小鼠脑图谱，植入坐标为：前囟后 2 mm，中线侧 1.75 mm，深度 1.75 mm。术后不同时间点进行动物行为学测试，包括物体识别实验、水迷宫实验和筑窝能力实验。术后 1 个月进行脑组织取材及免疫荧光染色观察。 结果：行为学实验证实：移植过表达 miR-34a 的神经干细胞减轻了 AD 模型小鼠的学习记忆等行为学损伤。免疫荧光和免疫印迹实验证实：和植入的未转 miR-34a 对照神经干细胞相比，植入过表达 miR-34a 神经干细胞分化为神经元的比例显著提高，海马区突触密度显著增加。结论：通过 miRNAs 来调控移植神经干细胞命运为治疗 AD 的研究提供一个新思路。

关键词：阿尔茨海默症；mciroRNA；神经干细胞；细胞移植

The study of Human umbilical cord mesenchymal cell stimulated by curcumin in the treatment of Alzheimer's disease

Yunliang Wang

Objective: The aim of the study is to evaluate the effect of mesenchymal cells derived from human umbilical cord (hUC-MSC) and hUC-MSC induced by curcumin (hUC-MSC-CUR) in treatment of Alzheimer's disease (AD), and to explore its related mechanism. Methods: The hUC-MSC were isolated by tissue explant technique, and the molecular surface markers (CD29, CD44 , CD105, CD31, CD45 and HLA-DR) were detected by flow cytometry. Bacteria, fungus, hepatitis B virus, HIV virus and endotoxin in hUC-MSC were detected by culture medium, Enzyme Linked Immunosorbent Assay(ELISA) and limulus reagent. The hUC-MSC were stimulated by curcumin (CUR) of different concentrations, and the cell proliferation was detected using CCK-8 assay. The ultrastructure of hUC-MSC-CUR were observed under the transmission electron microscopy, and ELISA was used to detect the level of a variety of cytokines and growth factors. The cell supernatant of hUC-MSC-CUR concentrated into conditioned medium CM-CUR, hUC-MSC cell supernatant on the same terms made into conditioned medium of CM-MSC. The PC12 AD cells model was made by amyloid β-peptides1-42 (Aβ1-42), CCK-8 and the Flow cytometry were used to detect the PC12 cell proliferation after adding the same concentration of CM-CUR and CM-MSC; Immunocytochemistry was used to detect the expression of specific protein microtubule associated protein 2 (MAP2). The hUC-MSC and hUC-MSC-CUR were injected to 5 months age AβPP/PS1 double-transgenic mice through tail intravenous, once a week, 107 /kg, continuous treatment for 8 weeks. the behavioral changes of mice were detected by Morris water maze, The Open Field and The step-down test. The brain tissue pathology change of AβPP/PS1 double- transgenic mice Mouse were observed under microscope, and the ultrastructure of the hippocampus and cortex tissue was observed under the transmission electron microscopy. Immunohistochemistry and western blot test were used to detect the Aβ and MAP2 in the cortex and hippocampus of AβPP/PS1 double-transgenic mice; the neprilysin (NEP) and insulin degradation enzyme (IDE) were measured by Western blot test. ELISA method was to detect the changes of tau protein in cerebrospinal fluid (CSF) of AβPP/PS1 double-transgenic mice after hUC-MSC-CUR transplantation. And some kinds of cytokines and growth factors in cortex and hippocampus of mice were detected by RT-PCR test. All the experimental data were analysed by the statistical software SPSS10. 0, the data between the two groups using T test, multiple samples were compared using single factor analysis of variance. Results: The results show that hUC-MSC can be successfully isolated from human umbilical cord by tissue block method, which expresses. CD29, CD44 and CD105, the known surface markers for mesenchymal cells; but not hematopoietic cell marker CD45, epithelia cell marker CD31, Lymphocyte HLA-DR. The hUC-MSC did not polluted with bacteria, fungi, hepatitis B virus, HIV virus and endotoxin, so the hUC-MSC can be used for the next experiment. According to the results of CCK-8, 5 μmol/L was selected as the experimental concentration of curcumin stimulation hUC-MSC; The number of the endolysosome and rough endoplasmic reticulum obviously increased in hUC-MSC-CUR than that of the hUC-MSC without any treatment. The levels of Interleukin-4 (IL-4), Interleukin-10 (IL-10), Nerve Growth Factor (NGF) were significantly up-regulated in the hUC-MSC treated with CUR. The Aβ1-42 could induced PC12 cell to apoptosis, AD cell model, while CM-CUR and CM-MSC could promote the PC12 AD cell proliferation, differentiation, and stimulate the expression of MAP2. After hUC-MSC and hUC-MSC-CUR treatment, regardless of the behavior, the CSF level in the CSF, histopathology and ultrastructure, all results showed that the effects of hUC-MSC-CUR on AD mice was obviously higher than that of hUC-MSC. To further explore the mechanism, found that hUC-MSC-CUR transplantation can obviously up-regulate the expression of Aβ degradation enzyme NEP and IDE, so as to accelerate the degradation of Aβ in the AβPP/PS1 double-transgenic mice brain, and inhibit the apoptosis of the neurons in hippocampus and cortex, and promote the expression of MAP2; RT-PCR results suggest hUC-MSC-CUR transplantation can increase the level of IL- 4, IL-10 and NGF, and down-regulate the expression of IL-1and TNF-α. Statistical analyses were performed using SPSS10. 0 software. Data presented as means± SEM were subjected to one or two-way ANOVA, followed by either Newman-Keuls or Bonferroni's multiple-comparisons test (as a post hoc test). $P < 0.05$ was considered to indicate statistical significance. The results of the immunocytochemistry and western blot were analyzed by Image-Pro Plus 5.0 image analyzer (Media Cybernetics, USA). The integrated optical density (IOD) and gray values were assayed by statistical analysis. Conclusion: ① the hUC-MSC can be successfully isolated from

human umbilical cord by tissue block method. ② The supernatant of hUC-MSC-CUR can promote the cells proliferation, and neurons differentiation in the AD cell model. ③In the AβPP/PS1 double-transgenic AD mice, behavioral test, immunohistochemistry and Western Blot results showed that the effect of hUC-MSC-CUR was better than that of hUC-MSC for treatment of AD. ④ hUC-MSC-CUR can up-regulate the level of IDE and NEP, so as to accelerate degradation of Aβ in the AβPP/PS1 double-transgenic mice brain. ⑤hUC-MSC-CUR can inhibit the apoptosis of the neurons in hippocampus and cortex, and promote the expression of MAP2. ⑥ The ELISA result showed that the expression of IL-4, IL-10, and NGF in the supernatant hUC-MSC-CUR enhanced compared with that in the hUC-MSC. ⑦ The phagocytosis and secretion ability of hUC-MSC become stronger after induced by CUR, and the level of IL-4, IL-10 and NGF in the supernatant increases, playing very important role on the regeneration of AβPP/PS1 double-transgenic mice.

keyword: Alzheimer's disease, Curcumin, Human umbilical cord mesenchymal cells, AβPP/PS1 double-transgenic mice, Amyloid β-peptides, Tau protein, Microtubule associated protein-2, Cytokine, Neprilysin, Insulin degradation enzyme, Nerve growth factor, Nerve regeneration

鞘氨醇激酶-1 基因转染的脐带间充质干细胞对 EAE 小鼠的疗效及机制研究

许春阳

多发性硬化症(multiple sclerosis，MS)作为一种中枢神经系统的退行性疾病，是青少年致残的常见病因。目前传统的治疗方法效果并不理想，并且存在严重的副作用。间充质干细胞（mesenchymal stem cells，MSCs）以其潜在的神经再生和免疫调控能力，为多发性硬化的治疗提供了新的思路。鞘氨醇激酶-1（spk1）是生成鞘氨醇-1-磷酸（S1P）的关键酶，而 S1P 的类似物芬戈莫德（FTY720），已在临床上广泛用于 MS 的治疗。在实验中我们发现，经 spk1 基因转染的脐带间充质干细胞（UCMSC）移植显著降低实验性自身免疫性脑脊髓炎（EAE）小鼠神经功能损害的程度，同时抑制了髓鞘的脱失和星形胶质细胞增生。因 此，转染 SPK1 基因的 MSCs 移植可能为 MS 的治疗带来更好的疗效。

关键词：多发性硬化；间充质干细胞；鞘氨醇激酶 1；鞘氨醇-1-磷酸；芬戈莫德。

中国医师协会神经修复学专业委员会委员 中华医学会神经外科分会 小儿神经外科学组创始组长 亚澳小儿神经外科学会（AASPN）常委及发起人

儿童难治性癫痫的 VNS 治疗进展

目的：评价迷走神经刺激术（VNS）治疗儿童难治性癫痫的手术效果。方法：回顾性分析上海新华医院小儿神经外科近 3 年内接受过 VNS 手术治疗的 18 例难治性癫痫的随访情况。随访数据中，我们利用 Engel 癫痫疗效分级评估了儿童的恢复情况。结果:18 个病人均接受了上述评估。10 男 8 女，平均手术年龄 6.3 岁（0.5-12 岁），平均术后随访时间 17.5 月（6 月-30 月）。VNS 术后明显降低癫痫发作频率，平均降低 57.7%，P<0.05。61.1%的患者癫痫发作频率降低 50%以上，33.3%的患者癫痫发作频率降低 70%以上。并发症包括 1 例感染和 1 例颈部疼痛。结论:VNS 是安全有效的治疗癫痫的方法，能够明显降低癫痫发作频率，改善患者认知和神经发育功能，患儿早期实施 VNS 手术，能从手术中获益。

Feasibility of Paracrine factors from Human adipose stem cells for Infants with moderate/severe Hypoxic-Ischemic Encephalopath

Feng Wang

Objective: To assess feasibility and safety of intraspinal injections of paracrine factors from human adipose stem cells (hASCs) to neonates with Hypoxic-Ischemic Encephalopathy (HIE). Methods: We conducted an open-label, multi-center randomized clinical trial at seven Intensive Care Nursery. Neonates diagnosed with HIE were randomly assigned to either standard therapy or intrathecal injections of paracrine factors from hASCs in 12, 24, 48 hours after birth. The paracrine factors were obtained from cultured human adipose stem cell .We recorded all patients'baseline characteristics，pre-and post-infusion vital signs, neural manifestations and adverse events such as fever, infection, seizures coursed by intervention. Neurobehavioral testing were undertaken before participants were enrolled and reexamined at the 12 and 18 month of age. Neonatal behavioral neurological assessment （NBNA）at 14 and 28d were recorded as hospital outcomes. At the 12 and 18 month after birth, we compared survivals'Bayley Scores, Peabody Development Measure Scales， and Gross Motor Function Measure Scale for children diagnosed Cerebral Palsy for statistical analysis. Results: 72 patients were enrolled and all received standard therapy .44 Infants were assigned to experiment group.Clinical characteristics were similar between groups.Vital signs including heartbeats and oxygen saturation were similar before and after intrathecal injections. NBNA score of 14d after birth group was similar in experiment group （ 33.76 ± 4.652 ） and control group （ 32.25±2.364 ） ， (95%CI:0.539-3.566， P=0.146).28d NBNA score of hASCs paracrine factors recipients （37.1±2.178）significantly higher than the conventional group（35.71±2.758）(95% CI:0.136-2.658，P=0.03).In the experiment group， death rates were 6 of 44 neonates (13.6%);11 patients (5 moderate and 6 severe)didn't reach the time of 12-18month follow up, statistical analysis of long-term outcomes were from 33 patiens;4/33(12.1%) patients had development delay,and 3/33(9%) had psychomotor retardation or cerebral palsy.No clinically important complications occurred in the experiment groups except:5/44(11.3%) patients had low –grade fever or irritability in the first 24h after intrathecal injection.Most data of control group for long-term outcome are being collected. Conclusions In an analysis of these partial data， paracrine factors derived from hASCs was associated with a reduced risk of brain injury. More following data for neurobehavioral outcomes are being collected and analyzed.

Keywords: paracrine factor, human adipose stem cells, Hypoxic-Ischemic, Encephalopathy， newborn

杨明亮

中国医师协会神经修复学专业委员会委员 北京脑重大疾病研究院神经损伤修复所副所长 中国医师协会脊髓损伤康复专业常务委员 中国残疾人康复协会脊髓损伤康复专业常务委员

基于神经解剖结构的脊髓栓系综合征分类研究

研究背景：脊柱生长持续牵拉粘连的神经结构，是腰、骶髓拴系综合征(tethered cord syndrome，TCS)最重要的病理生理过程。外科手术很难松解粘连神经结构。近年来，通过缩短腰段脊柱来缓解脊髓牵拉，缓解栓系症状，受到临床医师的关注。 研究目的：基于脊髓栓系的神经解剖结构特点，探讨缩短腰段脊柱手术治疗栓系综合征的适应征。研究方法：通过分析拴系综合征患者的临床及MRI特点，复制动物模型的MRI特点，总结栓系综合征的临床及病理特点。 结果：根据栓系患者异常神经解剖结构及病理进展特点，将栓系分为：I型(终丝型)：患者存在拴系并出现神经症状，拴系结构为终丝或其他带、索状非神经结构，神经损伤主要由拴系部颅侧保持高张力所致。Ⅱ型(神经根型)：患者存在拴系并出现神经症状，拴系结构为神经根。其中又分为3个亚型：(1)前根型：拴系结构主要为前根，拴系部远侧保持较高张力，早期以运动神经损伤为主；(2)后根型：拴系结构主要为后根，拴系部颅侧保持较高张力，早期以感觉神经损伤为主；(3)混合型：拴系结构包含前、后根，出现感觉、运动障碍。Ⅲ型(脊髓型)：患者存在拴系并出现神经症状，神经损伤主要由拴系区对腰、骶髓的直接机械压迫、侵袭等所致(多见于椎管内肿瘤、脊髓纵裂等)。结论： I型、Ⅱ型，较适合于脊柱缩短手术。

中国医师协会神经修复学会委员会委员
中华医学会会员 泰安市物理医学与康
复学会委员

匡乃峰

嗅鞘细胞移植治疗痉挛型脑瘫初步临床报告

目的：分析和总结应用嗅鞘细胞对脑瘫患儿的运动功能疗效。方法：随机筛选适合进行嗅鞘细胞移植 的痉挛型脑瘫患儿 15 例作为移植组，同时选择年龄、性别及疾病严重程度相仿的 15 例痉挛型脑瘫患儿为 对照组。移植组采用微创定向手术进行直接颅脑穿刺注射，注射靶点为双额放射冠，辅助物理康复治疗， 对照组仅采用物理康复治疗。应用小儿脑瘫粗大运动评价量表(GMFM-88) ，改良 Ashworth 法(肌张力评定) 对术后 1 个月、3 个月、12 个月患儿的粗大运动功能、肌张力进行病例对照分析，t 检验。结果：移植组治 疗后 1 个月、3 个月、12 个月的 GMFM 较 GMFM0 上升率均明显高于对照组(P = 0． 025) ; 移植组治疗后 3 个月 GMFM3 较 1 个月 GMFM1 的上升率比较有明显差异，而治疗后 12 个月 GMFM12 较 3 个月 GMFM3 的 上升率比较无统计学差异。对照组 GMFM 上升率无此特征;肌张力评定结果显示，治疗 1 个月、3 个月、12 个月移植组患儿肱二头肌肌张力评定低于对照组(t = 2． 376，P = 0． 004 ； t = 3． 376，P = 0． 002； t = 3． 355，P = 0． 002)。结论：嗅鞘细胞移植可不同程度改善痉挛型脑瘫患儿的运动功能。 关键词：嗅鞘细胞; 痉挛型脑瘫;移植

陈光福

中国医师协会神经修复学会委员会委员 中国康复医学会儿童康复专业委员会委员 广东省医学会儿科学分会委员、神经学组委员

早产儿血清 EPO 水平与脑损伤的关系

目的：探讨早产儿血清促红细胞生成素（EPO）水平与脑损伤的关系。方法：选取 2014 年 10 月至 2015 年 9 月出生胎龄在 28~34 周的早产儿作为研究对象。采用颅脑 B 超和 MRI 检查诊断脑损伤，ELISA 法检测血清 EPO、S100 蛋白、神经元特异性烯醇化酶（NSE）和髓鞘碱性蛋白（MBP）水平，比较脑损伤组和无脑损伤组 EPO、S100、NSE 和 MBP 的差异，不同血清 EPO 水平早产儿脑损伤的发生率，分析血清 EPO 水平与各指标的相关性；采用多因素 Logistic 回归分析血清 EPO 水平与脑损伤的关系。 结果：共纳入 304 例早产儿，其中男 172 例，女 132 例；胎龄 28~34 周，平均 30.9 ±1.3 周，胎龄<32 周 149 例，32~34 周 155 例；出生体重 800~2740g，平均 1656±389g，<1 500g 110 例，>1 500g194 例；多胎妊娠 25 例；围产期窒息 33 例；自然分娩 101 例，剖宫产 203 例；孕母妊娠期并发症 46 例（感染 10 例，糖尿病 11 例，高血压 25 例），胎盘早剥 16 例，前置胎盘 24 例；胎膜早破 63 例；NRDS 87 例；使用 nCPAP/机械通气 131 例；感染（新生儿肺炎、败血症、NEC）96 例；低血糖 23 例；贫血 75 例。304 例早产儿中，诊断早产儿脑损伤 125 例，总发生率 41.12%。其中出血性脑损伤 54 例（17.76%）， 缺血性脑损伤 59 例（19.41%），出血+缺血性脑损伤 12 例（3.95%）。出生胎龄越小，脑损伤发生率越高，<32 周胎龄早产儿脑损伤发生率高于>32 周早产儿（P<0.05）； 出生体质量与脑损伤发生率无关（P>0.05）。将血清 EPO 分为低水平（<5.0mIU/ml）、中水平（5.0~10.0 mIU/ml）和高水平（>10.0 mIU/ml）三组，比较各组脑损伤的发生率，低水平 EPO 组缺血性脑损伤发生率明显高于中高水平 EPO 组（P<0.001）。无脑损伤组血清 EPO 水平明显高于各种脑损伤组（P<0.05），而脑损伤组间无差异（P>0.05）；3 组脑损伤早产儿血清 S100 蛋白、NSE、MBP 水平均高于无脑损伤组（P<0.001），其中出血+缺血性脑损伤组血清 S100 蛋白、NSE 水平高于出血性脑损伤和缺血性脑损伤组（P<0.001），出血性脑损伤组血清 MBP 水平低于缺血性脑损伤组和出血+缺血性脑损伤组（P<0.05）。血清 EPO 水平与 S100 蛋白浓度（r=-0.326，P=0.000）、NSE 水平（r=-0.143，P=0.012）均呈负相关，与 MBP 浓度无明显相关（r=-0.026，P=0.646）。Logistic 回归分析显示低胎龄（OR=0.733，P<0.05）、低出生体重（OR=1.001，P<0.05）、窒息复苏（OR=3.900，P<0.01）、机械通气 时间（OR=2.618，P<0.01）、贫血（OR= 2.201，P<0.05）和 EPO 水平（OR=0.702，P<0.01）均是脑损伤发 病的独立危险因素。结论：血清 EPO 低水平的早产儿脑损伤发生率高，血清 EPO 水平与早产儿脑损伤密切 相关。

关键词：促红细胞生成素；脑损伤；危险因素；早产儿

孤独症神经修复学研究治疗进展

李　彤

孤独症谱系障碍（Autism spectrum disorders）是一种神经发育障碍性疾病，其中以孤独症（Autism）最为常见，主要表现为不同程度的言语发育障碍、人际交往障碍、兴趣狭窄和行为方式刻板，且多数患者 还存在智力障碍。近年来 ASD 的发病率明显增加，美国的一项调查显示从 2007 年到 2012 年，美国学龄期的儿童 ASD 的发病率已从 1.16%上升至 2.00%[1]。虽然经过大量研究，ASD 的病因仍不清楚，多数认为是遗传和环境的共同作用。由于 ASD 病因不明，ASD 的治疗无论是药物还是行为康复训练，效果均有限，不能改变疾病病理基础。细胞疗法具有刺激细胞再生，促进神经修复的作用，可能为 ASD 的治疗开辟了一条新的路径。通过检索，目前关于细胞疗法治疗孤独症的系统评价、meta 分析、大型双盲随机对照临床试验尚未见报道。但已有大量的国际临床试验及动物实验的研究报道。间充质基质细胞：临床实验：2013 年中国的一项非随机开放性单中心的 I/II 期临床实验：37 位患者分为 3 组[2]。1 组：人脐带血单核细胞+康复治疗；2 组：人脐带血单核细胞联合人脐带来源的间充质基质细胞+康复治疗；3 组： 康复治疗。随访时间为 24 周。安全性评估：通过静脉及鞘内注射，患者均可以耐受，并且在注射时期及随访期间，虽然有少数患者发生低热，但都不需要特殊的治疗，并且并未发现过敏、免疫反应等严重的不良反应发生。有效性评 估：通过儿童自闭症评定量表(CARS)、 异常行为量表（ABC） 、全球临床印象量表（CGI)）评估后发现，与对照组相比，1 组和 2 组患儿的注意力（自发的眼神交流），情感、智能反应，适应变化能力，恐惧及神经质，刻板行为，异常活动等方面的改善明显优于对照组，且以 2 组效果最佳（人脐带血单核细胞与脐带来源的间充质细胞有协同作用）。中国的另一项非随机开放性单中心的 I/II 期临床实验[3]：20 位自闭症患者，采用脐带单核细胞联合脐带间充质细胞移植，发现移植后，患者脑脊液中的肝细胞生长因子、神 经生长因子、脑源性神经营养因子明显升高。（神经生长因子及脑源性神经营养因子对神经的存活、分化 及髓鞘形成有重要作用）。此外国外尚有关于自体骨髓单核细胞 （BMMNCs）移植治疗 ASD 的临床个案 报道及临床 I 期试验[4、5]，移植后，在随访期间发现患儿的孤独症行为（包括自发的眼神交流，注意力，社会交往能力）均得到了改善。其中，动物实验[6]：通过向 ASD 模型小鼠移植人间充质干细胞后发现。行为学观察：同时改变刻板行为、认知功能、社会功能（目前未见单一药物可以达到全面改善症状的效果）。组 织学观察：移植后的小鼠与未移植小鼠相比，海马区的脑源性神经营养因子水平明显提高。研究表明脑源 性神经营养因子对完整的社会认知功能有重要的作用。并且有研究认为脑源性神经营养因子会促进神经的 再次发育。此外，在移植后小鼠的海马齿状回区观察到了 Ki67 阳性细胞和具有肾上腺皮质激素活性的神经 元的增长，说明了移植后的间充质干细胞发挥了神经再生作用，研究表明神经发育异常是 ASD 病理生理基 础，这可能解释了间充质干细胞治疗 ASD 的机制。 因此间充质干细胞移植治疗儿童孤独症具有安全性和有效性[7]。其机制应为纠正免疫紊乱，定植到受损的神经区域，促进神经修复，促进血管新生改善脑内低灌 注。胎儿干细胞：胎儿干细胞更为原始，有更好的增殖及扩增潜能，具有强有力的免疫调节功能，且与胚胎干细胞相比，胎儿干细胞是利用废弃的组织获取，因此有更少的伦理争议。此外胎儿干细胞是强大的、 可植入的“生物药厂”，可为宿主提供营养支持，进而影响大脑的发育。这些特性使胎儿干细胞具有更强的临床利用潜能。临床试验：45 名孤独症患儿通过静脉及皮下注射两个剂量的 FSCs，随访 1 年时间，在移植前，移植后 6 个月，移植后 12 个月分别进行安全性及有效性评估。安全性评估：实验室检查和不良反应的临床评估发现胎儿干细胞移植治疗具有安全性及耐受性，没有观察到直接及长期随访期间（1 年）的不良反应，且无感染及免疫并发症发生。有效性评估：发现经 FSCs 疗法后，78%的患儿有明显的症状改善: 26%患儿更加安静，9%的患儿在自发眼神交流方面有所改善，29%的患儿有更好的食欲，23%的患儿在情感反 应反面有所改善。通过孤独症治疗评估检查表（ATEC）及异常行为量表（ABC）的评估发现，与移植 FSCs 相比，移植后 6 个月及 12 个月后，患儿的言语功能，社会交往能力，认知功能，总体健康及行为、总体评分方面都有所改善，且差异具有统计学意义。本临床试验初步证明了 FSCs 治疗孤独症是具有安全性及有效性的。无论 ASD 的严重程度，FSCs 疗法均会影响患儿的发育及免疫标记物。FSCs 疗法治疗 ASD 的潜在机制可能是旁分泌及免疫调节功能和促进神经再生及神经修复能力。造血干细胞：同样也有研究[8]指出造血干细胞可以分泌大量的生物活性因子，这些生物活性因子可以抑制 ASD 患儿异常的免疫，如下调促炎因子 TNF-α, IFN-γ, IL-1，上调抗炎因子 IL-10（这些因子在 ASD 神经免疫过程中有重要作用）。此外这些生物活性因子还可以促进干细胞的趋向、定值、激活进而起到神经修复的作用。因此造血干细胞也是一种治疗 ASD 的有吸引力的选择。

郁海涛

国际神经修复学会理事 中国医师协会神
经修复学专业委员会委员
《中华临床医师杂志（电子版）》编委

细胞神经修复治疗抽动秽语综合征病例报告

抽动秽语综合征是一组以多发性不自主的肢体抽动，语言或精神行为障碍为特征的综合征。对于本病 的治疗多以药物控制临床症状为主，但药物因需较长时间服用，不良反应明显，且依从性较差。在细胞神 经修复治疗治疗中枢神经系统疾病获得一定临床疗效基础，尝试细胞治疗抽动秽语综合征。报告 1 位 24 岁的中国男性病人，因肢体多动，注意力难以集中 18 年入院，伴有眨眼、摇头和扭动颈部等症状。分别于 2011 年 4 月、7 月，2012 年 6 月，2013 年 1 月四次行细胞神经修复治疗，每次治疗后患者多动、精神症状均有改善。本病例报告表明，细胞治疗可作为抽动秽语综合征治疗的新选择，多次治疗症状进一步改善。

关键词：抽动秽语综合征 多动 细胞移植 细胞神经修复治疗

中国医师协会神经修复学会委员会副主任委员
国际神经修复学会理事 中国残疾人康复创伤
协会理事

郑遵成

脊髓损伤嗅鞘细胞移植的有效性及安全性——40 例患者长期随访

目的：在证实嗅鞘细胞移植治疗晚期脊髓损伤的近期安全性和有效性的基础上进一步评价其长期的安全性。方法：随机选择 2006.07—2008.07 泰安市中心医院神经修复中心收治的晚期脊髓损伤患者 40 例，男性 29 例，女性 11 例，年龄 20—54 岁，平均年龄 35 岁，病程 1—14 年，平均 5.2 年；受伤原因包括车祸、摔伤、医源性损伤等。所有患者均接受嗅鞘细胞移植治疗。手术前后行感觉、运动及植物神经功能 ASIA 评分，然后将手术前、手术后 3 个月、手术后 7 年 ASIA 评分进行对比分析；观察 40 例患者手术前 MRI 及术后 7 年的 MRI 变化；统计手术后 7 年内脊髓损伤患者新增的并发症，探讨并发症与细胞移植本身的关系。结果：晚期脊髓损伤手术后 1 年感觉、运动及植物神经功能均较术前有明显改善（P＜0.01）；手术后 3年感觉、运动及植物神经功能均较术前有明显改善（P＜0.01）；手术后 3 年较手术后 1 年仅有运动神经功能变化有统计学意义（P＜0.01），植物及感觉神经功能变化无统计学意义（P＞0.01）；40 例患者 MRI 无明显结构性改变，无占位、瘤变、囊变等变化，MRI 证实嗅鞘细胞移植治疗脊髓损伤是安全的；并发症随访结果：肾功能不全 1 例、压疮 2 例、坠积性肺炎 1 例、异位骨化 1 例，关节僵直 1 例，经论证随访的相关并发症与细胞移植手术本身无关联。结论：采用嗅鞘细胞移植治疗脊髓损伤 3 年随访结果是有效的、安全的。

关键词：嗅鞘细胞；晚期脊髓损伤；有效性；安全性；

卢明

中国医师协会神经修复学会委员会常委 全军神经外科专业委员会脊柱脊髓学组委员 中国医师学会湖南省分会理事

自体嗅黏膜间充质细胞生物学特性与移植治疗脊髓损伤的临床观察

目的：研究嗅黏膜间充质细胞生物学特性，使其成为促进中枢神经再生的理想候选细胞之一，同时进行脊髓损伤移植治疗的临床观察。方法 分离、培养、鉴定人嗅黏膜间充质干细胞，透射电子显微、扫描电子显微镜下观察其超微结构，并在体外诱导其向脂细胞、骨细胞、神经干细胞球和神经元分化。经医学伦理委员会同意，对 8 例晚期脊髓损伤的病人（自愿者）进行临床移植观察治疗。结果：人嗅黏膜间充质干细胞生长以梭形细胞为主，呈放射状排列的细胞集落，高表达表面标志 CD73、CD90,不表达 CD34、CD45；在扫描电子显微镜下可见细胞表面有短而粗的微绒毛突起，透射电子显微镜下可见到两种不同的细胞形态；具有成脂、成骨、成神经元干细胞球和成神经元分化的能力。OM-MSCs 不仅具有间充质干细胞的一般特性，还具有其他优点：1、具有更高的增殖效率和更短的传代时间；2、可自体移植，无免疫排斥反应；3、广泛位于鼻腔各部位中，易于取材；4、来源稳定，嗅粘膜终生可更新；5、安全性高，染色体核组分析和肿瘤基因分析显示体外无限传代后没有基因变异。临床治疗均有效，取得满意的治疗结果。结论：人嗅黏膜间充质干细胞具有间充质干细胞的一般生物学特性，经诱导培养后具有多向分化潜能，可作为组织工程修复的理想种子细胞。临床移植治疗观察能达到理想的治疗结果。

封亚平

中国医师协会神经修复学专业委员会常委
兼神经创伤修复组组长 中国医师协会神
经外科医师分会 脊柱脊髓专家委员会委
员

雪旺细胞移植治疗慢性脊髓损伤手术移植方法探讨

目的：探讨自然流产的人胚雪旺细胞(SC)移植治疗慢性脊髓损伤（SCI）的手术方法及疗效。方法：入选标准：临床确诊的 SCI； MRI 或 CT 确定的 SCI；受伤 3 个月以上的患者；评分为 ASIA A 的患者；MRI 证实脊髓损伤后有形态学改变的患者。排除标准：严重颅脑损伤或者合并心肺及其它系统疾病，不能耐受麻醉及手术风险者；存在急性感染需要积极治疗者；任何其它的潜在实验治疗者；排除脊髓脱髓鞘疾病和肿瘤者；医疗及精神状态不稳定者；孕妇；任何证据表明患者对治疗不依从者。本组完全性脊髓损伤（ASIA A）患者 53 例。采用脊髓空洞内移植法：全麻下行脊髓损伤节段后方入路，咬除棘突、椎板仍存在者，悬吊切开硬脊膜，显露出脊髓。显微镜下松解粘连，切除增厚的蛛网膜及疤痕组织，合并脊髓囊肿或空洞者，于脊髓受损处，选择最薄、呈透明的部位纵形切开空洞，彻底清除囊腔内液化、坏死组织，用神经剥离子轻柔的搔刮囊壁，生理盐水反复冲洗囊腔，直至冲洗液清亮。将附有 SC 的薇乔 3-0 紫色可吸收线按囊腔的长度剪好，置于囊腔内，滴入 SC 悬液 1ml（4~6×106 个 SC），再用附有 SC 的薇乔网覆盖，几丁糖（chitosan）封闭创面，缝合硬脊膜，置多侧孔引流管，逐层缝合切口。术后配合康复训练，采用 ASIA 评分、MRI、肌电图、诱发电位、昆明运动分级进行疗效评定。结果：对接受 SC 移植的 53 例完全性脊髓损伤（ASIA A）患者随访 6 个月，脊髓功能均有部分恢复，其中运动功能评分由术前 41.49±15.83 提高到 44.62±15.39，浅感觉评分由术前 57.89±22.87 提高到 63.94±23.67，深感觉评分由术前 55.96±20.99 提高到 59.68±20.57。运动功能、浅感觉以及深感觉较术前均有明显的改善（$P<0.05$）。患者术后无脊髓感染、功能损伤加重等并发症。受试者术后无一例死亡，也无损伤平面上移、症状加重及胶质瘤样增生、囊腔扩大等并发症出现。结论：临床研究证实 SC 移植治疗脊髓损伤安全可行，对晚期脊髓损伤患者的脊髓功能恢复有一定效果,虽然不能完全恢复脊髓功能，但在尚无有效治疗对策的脊髓损伤领域做了一些开创性工作，推动了脊髓损伤的基础和临床研究。

武亮

中国医师协会神经修复学专业委员会委员 中国康复医疗机构联盟副主席 中国医师协会康复医师分会常务委员

脊髓损伤心肺功能康复

目的：阐述脊髓损伤领域心肺康复工作开展的必要性、现状、工作流程、工作重点及难点。方法：使用“心肺+脊髓损伤”、“心肺功能+脊髓损伤”、“心肺康复+脊髓损伤”等关键词，通过万方网、中知网及 Pubmed 查阅近 15 年的关于脊髓损伤心肺康复领域的国内外研究报道，结合我院目前心肺康复工作开展情况，进行总结及展望。结果：目前国内外尚缺乏针对脊髓损伤患者心肺康复的专门报道，仅存在少量康复过程中使用最大摄氧量（VO_{2max}）、用力肺活量（FEV）、第一秒用力肺活量（FEV1）及 FEV1/FEV 等指标进行评估功能改善，但缺乏完备的心肺康复评定体系及心肺康复治疗体系。我院在总结前人工作基础上，组建了包括包括心内科医生、康复医生、心肺评定治疗师、PT 治疗师、OT 治疗师、理疗治疗师、水疗治疗师等在内的心肺康复团队，设立了包括临床评估、心肺功能及危险度量表评定、心肺静态及运动功能测试、心理及睡眠评定、活动参与能力评定等在内的评估体系，制定了囊括住院卧床期间、住院离床期间、出院后的康复训练计划体系及跟踪随访制度。结论：心肺康复起步较早，但发展相对缓慢，近 5 年在我院发展较快。但尚有很多工作需要完善。

中国医师协会神经修复学专业委员会委员

邹清雁

脐血单个核细胞治疗持续植物状态的回顾性分析研究倪

目的：回顾性分析脐血单个核细胞治疗持续植物状态（PVS）的安全性及临床疗效。方法：选取广东三九脑科医院康复科 2013 年 4 月至 2015 年 4 月收治的 69 例持续植物状态患者。将进行过脐血单个核细胞移植患者纳入治疗组（共 39 例），常规治疗患者纳入对照组（共 30 例）。记录患者的病因、病程、病变定位、并发症状及症状改善情况。以 PVS 量表评分为主要指标，比较两组的疗效、不良反应。结果：1.在整体有效率方面，治疗组和对照组的整体有效率分别为 47.37%和 31.58% (n=?, P＜0.05)。2.在症状改善方面，肢体运动治疗组和对照组的改善比例分别为 58.97%和 30.00%（P＜0.05）；眼球运动治疗组和对照组的改善比例分别为（35.90%，23.33%；P＜0.05）；情感改善治疗组和对照组的改善比例分别为（25.64%，13.33%； P＜0.05）。3.在年龄细分分析中，年龄组以 15-29 岁的治疗效果最佳；而在针对病因的细分分析中，由脑外伤和脑卒中引起的持续植物状态效果最佳。其中脑外伤治疗组和对照组的有效率分别为（55.56%，45.45%；P＜0.05）；脑卒中治疗组和对照组的有效率分别为（66.67%，33.33%；P＜0.05）。4.不良反应，治疗组仅有 1 例在细胞移植 1 天后出现低烧情况，对症处理后痊愈。结论：脐血单个核细胞对改善持续植物状态意识，情感及运动功能障碍有积极作用，且副作用少。

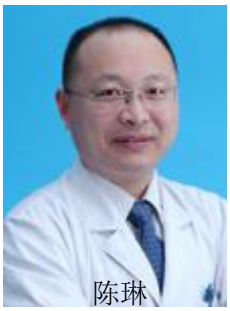
关键词：持续植物状态；脐血单个核细胞；PVS 量表；神经康复

中国医师协会神经修复学专业委员会委员
中华医学会北京骨科专业委员会委员
北京市医师学会骨科专家委员会委员
北京卫生局骨科专业职称评审专家

洪毅

高位颈髓损伤患者呼吸功能的基础与经皮电刺激治疗的研究

高位颈脊髓损伤后（颈 4 及颈 4 以上水平的损伤）早期首要问题是神经源性呼吸功能障碍，也是颈脊髓损伤患者早期死亡的首要原因，占全部死亡患者的 80.2%。目前高位颈脊髓损伤后早期赖以生存是依赖呼吸机存活。高位颈髓损伤呼吸功能的基础与经皮电刺激治疗的研究是本研究的重点。本研究分两个部 分，一是通过临床病历观察:高位颈脊髓损伤 4 例，均为男性，年龄 32-44 岁。两侧膈肌均瘫痪 1 例，颈 2 脊髓完全损伤（ASIA 评分 A 级）， 颈 5/6 骨折脱位。一侧膈肌瘫痪一侧基本正常 2 例，颈 3 完全损伤；一侧膈肌活动微弱存在，另一侧大致正常 1 例，为颈 4 完全损伤。所有患者每月 X 线透视复查 1 次膈肌功能，每 2 月检查一次肺功能，3 个月复查颈椎 MRI。观察膈肌功能恢复时间及肺活量变化结果，提示人颈髓损伤后也存在自发性恢复现象。二是膈神经刺激器应用于颈脊髓损伤呼吸功能障碍的实验研究，将 30 只新西兰大白兔随机分为对照组（A 组）和实验组（B 组，建立兔颈脊髓半切损伤模型，实验组每日施加电刺激干预，对照组不施加电刺激干预。两组分别于颈髓损伤前、术后 1、2、4 周行血气分析检测，进行统计学分析；术后 1、2、4 周进行膈肌、膈神经、膈肌运动终板的形态学观察，其中膈肌肌纤维细胞横截面面积、膈肌运动终板数量，进行统计学分析；形态学观察、免疫组化检测。结果 2 周以上刺激的结果显示电刺激作用可延缓颈髓损伤后膈肌萎缩、膈神经变性。Porter 在犬和兔的动物实验中，先是将膈神经运动神经核的头侧半切（C2 脊髓水平半切），同侧膈肌随即瘫痪；随后将半切对侧的膈神经切断，造成对侧膈肌瘫痪，随后发现半切侧原本瘫痪的膈肌却恢复了运动功能，这一现象后来被称为膈神经交叉现象（crossed phrenic phenomenon, CPP）。上世纪 90 年代发现，雌性 S-D 大鼠 C2 半切后如果不作对侧膈神经切断，4 周后患侧膈肌会自发性恢复运动功能，即 CPP 会自发性产生，称之为自发性膈神经交叉现象（spontaneous CPP,sCPP）。人高位脊髓损伤后由于呼吸肌瘫痪得以生存一方面依靠呼吸机维持生命，部分患者能够在伤后 2 年内最终能够脱离呼吸机，说明人的这种自发性恢复的存在，确切机理还不清楚，但有学者认为这与大鼠脊髓损伤 sCPP 类似。应用针对于高位颈髓损伤所致的呼吸功能障碍在自发性恢复的基础上膈神经刺激，并以兔为实验对象的测试，刺激器作用 2 周以上可延缓颈髓损伤后膈肌萎缩、膈神经变性，为进一步临床研究奠定基础。



陈琳

中国医师协会神经修复学专业委员会常务委员 国际神经修复学会第一、二、三届秘书长 中华中青年神经外科交流协会委员 中国康复医学会脊柱脊髓专业委员会基础研究学 组委员、中国康复医学会神经再生专业委员会委员

眼睑痉挛（梅杰综合征）功能神经外科手术的初步报告

目的：Meige 综合征（眼睑痉挛），又称眼睑痉挛一口下颌部肌张力障碍，是一种局限性肌张力障碍性疾病，主要表现为双眼睑痉挛、面部肌张力障碍样不自主运动。发病机制可能与脑基底节部损害，黑质-纹状体γ-氨基丁酸能神经元功能低下导致多巴胺能受体超敏或多巴胺递质失衡，胆碱能作用失衡有关。精神科（按照强迫症，给予舍曲林及氯硝西泮口服）和神经内科（按照锥体外系病，给予硫必利及苯海索）药物治疗均有一定疗效，但仍有大约 2/3 的病人效果差。手术为本病提供了治疗选择，但各种术式的风险和收益需要探讨。方法：我科 2016 年 1 月-2016 年 6 月共手术 12 例，其中男 3，女 9，男：女为 1:3；年龄 36~58 岁，平均 51 岁；病史 1~11 年，平均 8.2 年。我们采用 3 种术式：单侧立体定向苍白球内侧部毁损术（n=10 例），双侧苍白球内侧部脑深部电刺激术（Deep Brain Stimulation，DBS）（n=1 例），面神经、三叉神经梳理术（单侧）（n=1 例）。结果：本组无死亡患者，所有病例术后第 2 天开始症状均明显好转。手术并发症 2 例，其中穿刺道出血（4ml）1 例，为苍白球内侧部核团毁损患者，自行吸收，无任何神经系统后遗症；同向偏盲视野缺损 1 例，给予神经营养修复治疗后痊愈。随访 1~5 个月，平均 3 个月，10 例（10/12，83.3%）患者疗效满意，其中 DBS 患者术后调控 4 次，随访 4 个月效果良好。2 例复发，给予复方樟柳碱组方药物颞浅动脉旁注射+眼睑肌多点注射，症状缓解。结论：对于内科治疗无效的眼睑痉挛（梅杰综合征）患者，手术是一种安全有效的手段。但手术的风险/效益比以及长期对认知等方面的影响，值得大样本 RCT 试验深入研究。

关键词：眼睑痉挛，梅杰综合征，功能神经外科，手术，立体定向

Olfactory ensheathing cell transplantation for a patient with chronic sciatic nerve injury

Feng zhang

Objective： To observe the effect of olfactory ensheathing cell transplantation for a patient with chronic sciatic nerve injury. Case report: a patient male 53 year old with left chronic (one year) sciatic nerve injury got olfactory ensheathing cell (OEC) transplantation in lesion area. Follow-up each three months after OEC therapy, he has been increasing his muscle strength of left lower limb, recovering limp walking gait, and lowering numbness. By ASIA scale evaluation neurological functions of post-treatment is better than pre-treatment. There are no side effects. Conclusion Olfactory ensheathing cell transplantation is optional for chronic peripheral (sciatic) nerve injury is an effective method.

Keywords：olfactory ensheathing cell transplantation; sciatic nerve injury; function improvement

李德志

中华中青年神经外科交流协会委员会常委

预变性自体神经桥接舌下神经-面神经吻合术治疗 CPA 术后周围性面瘫的疗效分析

目的：分析预变性自体腓肠神经移植桥接舌下神经-面神经“侧”-侧吻合术治疗桥脑小脑区（CPA）肿 瘤切除术后周围性面瘫的疗效。方法：回顾性分析2013年6月至2016年6月北京天坛医院神经外科行腓肠神 经预变性桥接舌下神经-面神经“侧”-侧吻合术患者48例，患者CPA肿瘤切除术后重度周围性面瘫（H-B V-VI级），吻合术前术后分别行House-Brachmann评级和电生理检测并随访。结果：随访时间2~36个月，平均 12个月，H-B评级明显改善24例、改善21例，电生理F和M波潜伏期明显缩短分别为25例和22例，差异有统计 学意义（P<0.05）。结论：预变性自体神经移植桥接面舌神经“侧”-侧吻合术能有效治疗CPA区肿瘤切除 术后周围性面瘫，CPA术后6个月左右行吻合术效果最佳，吻合术后6个月左右面神经功能明显改善。

关键词：预变性神经；神经吻合术；CPA 肿瘤；周围性面瘫

In vivo evaluation of Bombyx mori and tussah silk fibroin tube for peripheral nerve regeneration

Zhihai Fan

Objective: The object of this study was to provide experimental evidence for silk fibroin (SF) clinical use in nerve regeneration. In addition, Bombyx mori silk fibroin (BSF) and tussah silk fibroin (TSF) were compared to provide a superior material for nerve regeneration. Methods: Silk fibroin were electrospun into nanofiber tubes for repairing peripheral nerve defects. The SF nanofiber tubes was implanted in Sprague-Dawley (SD) rats to bridge a peripheral nerve defect that was 10 mm long. Two and four months after implantation, a comprehensive evaluation of morphological and functional investigations included electrophysiological assessment, the percentage of wet weight loss of tibialis anterior muscle and histological investigation.Results: There were no distinct regional inflammation response and scar formation in the rats in the SF graft group over a 4-month period after implantation, similar changes were observed in the autograft group. At 4 months after implantation, the SF graft had disappeared due to degradation, and the original 10-mm long nerve defect was replaced with a tissue that had a nerve-like appearance between both stumps. CMAPs amplitude has decreased significantly when compared with the normal CMAPs amplitude value recorded at the contralateral unoperated side, there was no significant difference between the SF graft and autograft groups. Conclusion: Electrospun SF grafts could promote nerve regeneration following peripheral nerve injury and become a potential possibility of newly developed nerve grafts as an alternative of autografts to peripheral nerve regeneration. More importantly, tussah silk fibroin tube showed superior results compared to Bombyx mori silk fibroin tube, implying that tussah silk fibroin tube is preferable choice for peripheral nerve regeneration.

Key words: peripheral nerve defect; tussah silk fibroin, Bombyx mori silk, nanofiber tube

Qiang Ao

Committee ember of Neurorestoratology Professional Committee of
Chinese Medical Doctor Association
Director of tissue engineering laboratory of medical center
at China Medical University

MicroRNA-338 and microRNA-21 co-transfection for the treatment of rat sciatic nerve injury

Objective To find if cotransfecting microRNA-338 and microRNA-21 into the neurons in the spinal cord can promote functional recovery after peripheral nerve injury in rats. Methods Animals were divided into three groups: 20 animals in the GFP control vector group (group A), 20 animals in the GFP experimental vector group (group B) and ten animals in the normal control group. Right sciatic nerves of animals in groups A and B were transected and were bridged with collagen nerve conduits with 10 mm distance between the stumps. 3 ul GFP control vector or 3 ul lentiviral vectors encoding the sequence of microRNA-338 and microRNA-21 were injected in the conduit. Results 8 weeks after the surgery, the treatment effect was evaluated by functional analysis, electrophysiological analysis, immunohistochemical analysis as well as transmitting electronic microscope observations in all the rats. Animals treated with microRNA-338 and microRNA-21 showed significantly better recovery than GFP control group animals by means of functional analysis (Sciatic nerve index -47.7 ± 2.5 vs -59.4 ± 3.7), electrophysiological analysis (Conduction velocity 20.5 ± 2.8 vs 10.5 ± 1.4 m/s), ratio of wet weight of the gastrocnemius muscles (0.83 ± 0.03 vs 0.55 ± 0.06), axon diameter (5.0 ± 1.8 lm vs 4.0 ± 2.2), myelin sheath thickness (1.4 ± 0.43 vs 0.80 ± 0.31 lm) and G-ratio (0.80 ± 0.06 vs 0.75 ± 0.04). Conclusions Lentiviral vectors encoding microRNA-338 and microRNA-21 might be explored in the future as potential therapeutic intervention to promote nerve regeneration.

Keywords: Peripheral nerve injury;Sciatic nerve; Nerve conduit; MicroRNA

EphA4 通过 FGF 信号通路调节神经干细胞的增殖和分化

陈清法 在小鼠大脑皮层发育过程中，神经元是通过放射性胶质细胞或其中间产物-神经前体祖细胞分化形成的。神经元前体细胞的自我增生及细胞命运规范间的平衡对于大脑皮层正常的发育非常关键，但是调控这一过程的信号机理还不是很清楚。EphA4 作为受体酪氨酸激酶超家族的一员，在胚胎发育过程中的放射性胶质细胞中表达。为了阐明 EphA4 在大脑皮层发育早期放射性胶质细胞命运决定中的功能，我们分别在孕龄 11.5 天及 13.5 天皮层细胞中敲掉 EphA4。在两个时期敲掉 EphA4 小鼠中放射性胶质细胞都提前向神经元分化。两种突变小鼠的孕龄 14.5 天或 15.5 天分离的皮层细胞中成神经球能力都减弱，而神经元分化能力都增强。同时，这些分离的皮层细胞经 FGF 作用时 ERK 及 FRS2α 磷酸化减弱。孕龄 11.5 天敲掉 EphA4 新生小鼠大脑皮层比正常新生小鼠的要小，而孕龄 13.5 天敲掉 EphA4 新生小鼠大脑皮层与正常新生小鼠相似，两种突变小鼠大脑皮层层状结构都未见异常。在皮层发育末期 Pax6 阳性的放射性胶质细胞数目仅在孕龄 11.5 天敲掉 EphA4 小鼠中减少。结果表明 EphA4 在大脑皮层发育早期的作用尤其关键且不可补偿，使得孕龄 11.5 天敲掉 EphA4 小鼠新生大脑皮层尺寸减小。 EphA4 通过 FGF 信号通路促使放射性胶质细胞维持其自我增生并抑制其向神经元方向分化。这些发现为临床治疗脊髓损伤及阿尔茨海默症等神经疾病提供了理论依据。

关键词：大脑皮层发育；神经干细胞/神经前体祖细胞；EphA4；FGF 信号；Cre-loxp

小鼠海马神经元细胞 HT22 诱导骨髓间充质干细胞神经分化的作用

侯伟健

目的：探讨小鼠海马神经元细胞系 HT22 细胞培养上清液对骨髓间充质干细胞（BMSCs）向神经元方向分化的定向诱导作用及其分化过程中细胞内的分子变化。方法：分别培养 GFP 转基因荧光标记小鼠的骨髓间充质干细胞和小鼠海马神经元细胞系 HT22 细胞，流式细胞术检测 BMSCs 的特定表面抗原组合以确定干细胞性质；采用两种细胞共培养及利用 HT22 细胞培养液诱导 BMSCs 两种方法，培养一定时间后，于荧光显微镜下观察干细胞形态变化并拍照；收集 HT22 培养液诱导分化的 BMSCs 细胞，进行 Western blot 实验，分析 BMSCs 中的神经元特异的抗原（GFAP，NSE 及 NeuN 等）的表达；利用 GFAP 和 NSE 抗体进行免疫荧光检查，观察 BMSCs 细胞内与神经元相关的抗元表达变化；结果：流式细胞术检测培养骨髓贴壁细胞 CD11b、CD45 阴性，CD90 阳性；荧光显微镜下见与 HT22 细胞共培养及 HT22 细胞培养液诱导的 BMSCs 形态发生较大变化，细胞长轴增长，轴突样突起明显；Western Blot 可见经 HT22 细胞培养液诱导的 BMSCs 内 GFAP、NSE 和 NeuN 表达逐渐增强；免疫荧光实验结果显示经诱导的 BMSCs 表达 GFAP 和 NSE。结论：BMSCs 在小鼠海马神经元细胞系 HT22 细胞培养的微环境下，可被诱导向神经元样细胞方向分化。说明骨髓间充质干细胞的分化方向可明显受到其所在微环境的诱导并向其微环境来源的方向分化。

关键词：HT22 细胞；骨髓间充质干细胞；神经分化；微环境

张志文

中国医师协会神经修复学专业委员会委员

中国神经科学学会神经损伤与修复分会常委

国家突发公共卫生事件专家咨询委员会委员

北京市神经外科学会委员

CIRP: 一种具有神经保护功能的 RNA 结合蛋白

目的：本研究旨在明确冷诱导 RNA 结合蛋白（CIRP）是否具有通过抑制神经元凋亡而发挥神经保护作用及其可能的分子机理以方法：采取体外分离并接种培养大鼠皮层神经元的方式，应用 Annexin V-FITC/PI 标记法对神经元进行流式凋亡检测、透射电镜扫描观察神经元超微结构、慢病毒包装 CIRP 过表达和 shRNA 干扰载体并侵染神经元、实时定量 PCR 检测信号传导通路的基因表达分布（RT2 Profiler PCR Arrays Pathway Analysis）及 Western blotting 等方法分别从细胞、基因和蛋白水平上研究亚低温下 CIRP 介导抗凋亡信号转导通路上相关因子的变化情况。利用原核表达系统大量表达 CIRP 并纯化提取 CIRP 蛋白、观察纯化的外源性重组人冷诱导 RNA 结合蛋白（rh-CIRP）对小鼠 neuro2a 细胞凋亡的影响。结果：通过流式凋亡检测和透射电镜扫描后观察到 CIRP 过表达和亚低温处理后神经元凋亡的数量明显下降，神经元胞内超微结构相对保持完整。实时定量 PCR 检测信号传导通路的基因表达分布结果显示：过表达 CIRP 与亚低温 32℃12h 处理后存在着一些共同变化的因子，其中部分因子在进行 RNA 干扰 CIRP 表达并 32℃12h 处理之后未发生相应变化。对这部分因子仔细分析后，发现它们的分布和变化情况基本符合线粒体凋亡途径，相关 因子包括 Bcl-2、Bax、Bad、Bak，Cycs、Apaf-1、Caspase-9、Caspase-3 等。Western blotting 检测结果发现各因子蛋白水平的变化情况与基因水平变化基本一致：对神经元进行亚低温 32℃12h 处理后目的蛋白 CIRP 和 RBM3 表达均显著升高，Bcl-2、Akt、p-ERK1/2 表达水平也随之升高，而 Bax、Bad、Bak，Cycs、Apaf-1、Caspase-9、Caspase-3、TRX 等表达均有不同程度的降低；过表达 CIRP 处理后各因子的变化趋势与亚低温 32℃12h 处理后基本一致。外源性 rh-CIRP 预处理后细胞凋亡数目明显下降；p-Akt、pErk 等信号通路因子表达水平相比对照组显著增高，与内源性 CIRP 过表达结果一致。结论：亚低温下冷休克蛋白表达增高，之后激活并启动 CIRP 介导的抗凋亡信号转导通路，主要通过阻断线粒体凋亡途径，抑制神经元凋亡，从而起到神经保护作用。由此认为冷休克蛋白可能参与亚低温或常温环境下的神经保护功能，并在其 分子调控方面起着重要的作用，同时为亚低温治疗脑损伤的作用机理提供了一定依据。一定剂量的外源性 rh-CIRP 蛋白具有常温环境下抗神经元凋亡作用。

高温预处理嗅鞘细胞后诱导神经干细胞向神经元分化的研究

段答 目的：研究高温预处理嗅鞘细胞（OECs）后其上清液诱导小鼠神经干细胞系 C17.2 向神经元分化的效率，并初步探讨其机制。方法：采用新生 3 天昆明小鼠嗅球，分离培养 OECs，经 40℃高温预处理 6 小时，37℃培养 42 小时后，收集上清液诱导 C17.2 分化。实验以未做预处理的 OECs 上清液诱导组及加入了 HIF-1a 的诱导组为对照组，利用细胞免疫荧光、Western blot 检测 tuj-1 的表达，利用 Western blot 检测 HIF-1a 的表达，利用 RT-PCR 检测 HIF-1a 及其下游基因 EPO、VEGF 的表达。结果：高温预处理 OECs 后诱导 C17.2 分化，实验组 tuj-1、HIF-1a、EPO、VEGF 均较对照组表达增高。结论：高温预处理 OECs 后其上清液能更加有效的诱导 C17.2 向神经元分化，其机制为 HIF-1a 的上调从而引起其下游基因 EPO 及 VEGF 的上调。

关键词：高温预处理，嗅鞘细胞，神经干细胞，分化

Effects of IGFBP-2 on proliferation and differentiation of neural stem cell line C17.2

Xiaohua Teng

Objective: Recent studies provide solid evidence for the importance to delineate the transplantation of olfactory ensheathing cells (OECs) for the repair of central nervous system injury. Previously, we reported that olfactory ensheathing cells-conditioned medium (OEC-CM) facilitates the differentiation of multipotent cerebellar stem cell line (C17.2) into neurons. Moreover, Insulin-like growth factor binding protein-2 (IGFBP-2) showed significantly high expression in OEC-CM. This study is designed to investigate the roles of IGFBP-2 for the regulation of C17.2 differentiation and proliferation.Methods: Changes in cell morphology were imaged under a light microscope, and proliferating cells were counted. Cell viability was determined by MTT. Additionally, western blot and flow cytometry analysis were performed to detect protein expression levels. Results: Results of the present study demonstrated that IGFBP-2 increased the expression of proliferation-related antigen (Ki67), proliferating cell nuclear antigen (PCNA), neuroectodermal stem cell marker (Nestin). Cell cycle assay showed that IGFBP-2 promoted the S- and G2/M-phase in C17.2 cells. However, western blot analysis shown that there is no difference in neuron specific Class III beta Tubulin (TUJ-1) and glial fibrillary acidic protein (GFAP) between experimental group and control group.Conclusions: Our results show an important role of IGFBP-2 in regulation of C17.2 proliferation, however, IGFBP-2 have little effect on differentiation of C17.2.

Key words: insulin-like growth factor binding protein-2, neural stem cells, proliferation, differentiation

视神经脊髓炎谱系疾病合并妊娠 21 例临床分析

石冰心 目的：评估视神经脊髓炎谱系疾病（neuromyelitis optica spectrum disorder ,NMOSD）合并妊娠患者的母婴结局，分析妊娠与 NMOSD 之间的相互影响。方法：回顾性分析 2004 年 6 月~2015 年 6 月清华大学玉泉医院神经内科收治的有妊娠史的 NMOSD 患者。对这些患者进行电话随访，记录患者不同孕期及产后的年复发率、EDSS 评分、药物治疗、麻醉方式、生产方式、喂养方式、子代生长发育情况等，并进行统计学分析，比较不同时期复发率、EDSS 评分等。结果：21 例 NMOSD 女性患者共 25 次妊娠（20 次成功分娩，5 次流产），妊娠前确诊且成功分娩的 12 例患者孕早期（孕 1-3 月）、孕中期（孕 4-6 月）、孕晚期（孕 7-9 月）、产后 3 个月、产后 4-6 个月年复发率（annual relapse rate，ARR）与其孕前相比未见统计学差异，且各个时期 ARR 间未见相关性。12 例患者产后半年 EDSS 评分较妊娠前升高(p=0.006)。生产方式(p=0.642)、麻醉方式（p=0.622)、喂养方式(p=1.000)对产后复发无影响。全部的 25 次妊娠中，5 次妊娠失败，剩余 20 次均为活产，除 1 例为低出生体重儿（2000g）、1 例 36 周胎膜早破早产外，无发育生长异常。结论：妊娠对 NMOSD 复发率无明显影响。缓解期妊娠，妊娠期及产后有效的维持治疗有助于控制病情波动。生产方式、麻醉方式、哺乳对 NMOSD 复发无影响。NMOSD 不会增加流产、早产、死产、先兆子痫、畸形儿、新生儿免疫力功能低下等风险。

A Single-centre Cohort Study on The Association of Severity of Hemoglobin A1c and Leukoaraiosis

Liyan Hu

Obejects:HemoglobinA1c (HbA1c) is a biomarker of longterm glycemic control which may play an important role in the development and severity of leukoaraiosis. We sought to determine the relations hip between HbA1c level and leukoaraiosis on magnetic resonance imaging.Methods : We included 215 patients who were diagnosed as having symptomatic lacunar infarcts or small artery occlusion, or transient ischemic stroke, neurologically normal individuals with headache or dizzness, underwent Serial MRI scans, and white matter hyperintensity were subsequently evaluated according to Fazekas score . HbA1c concentrations were measured and divided into two groups (HbA1c≤6.4% , N=136; Hb A1c≥6.5%, N=79). Other risk factors and laboratory parameters were assessed. Univariate and multiple logistic regression analyses were performed.Results: The higher HbA1c group showed higher incidence of hypertension (65.4% vs 77.2%, p= 0.047), diabetes mellitus (11.0% vs 70.9%, p<0.001) and prior stroke (19.1% vs 45.6%, p<0.001). There are significant differences of admission glucose (5.1±0.9 vs 7.7±3.4; p<0.001), Vitamin B12(335.4±274.6 vs 440.8±332.2; p=0.034) and Fazekas score (1.7±0.9 vs 2.6±0.8; p<0.001). In univariate analysis, hypertension, 2-DM, prior stoke, age, creatinine , Vitamin B12 and HbA1c were associated with the severity of WMH (leukoaraiosis). In multiple regression analysis HbA1c (coefficient of partial regression 0.233, p<0.002) remained independently associated with the level of WMH (leukoaraiosis).Conclusion: The association between HbA1c and WMH reveals that chronic high glucose may play an important role in the pathophysiology of leukoaraiosis.

Key words: leukoaraiosis; hemoglobinA1c; Diabetes Mellitus; creatinine; hypertension

仿生矿化胶原材料改善椎体成形术 PMMA 骨水泥的研究

仇志烨

聚甲基丙烯酸甲酯（polymethyl methacrylate, PMMA）是经皮椎体成形术（percutaneous vertebroplasty, PVP）治疗椎体压缩性骨折（osteoporotic vertebral compression fractures, VCFs）的手术中普遍应用的一种骨水泥材料，其安全性、有效性和即刻效果已经获得大量理论研究证明和临床实践 证实。然而，近年来陆续有报道称 PMMA 骨水泥在椎体成形手术后会引起一些并发症，如相邻椎体再骨折、骨水泥固化体松动甚至在椎体中脱落等。这一方面是由于 PMMA 弹性模量太高，对椎体的上下终板产生磨损，进而造成相邻椎体的再骨折；另一方面，生物惰性的 PMMA 与自体骨组织无法形成骨性结合，因此骨水泥固化体较易在椎体内产生松动乃至滑脱，严重的对脊髓产生压迫，造成严重后 果。矿化胶原 PMMA 改性骨粉是通过体外仿生矿化技术制备的一种具有天然骨成分和结构的仿生材料。研究表明，将矿化胶原 PMMA 改性骨粉与目前市面上广泛使用的椎体成形 PMMA 骨水泥混合使用，能够在保持骨水泥固化体强度的同时，显著下调其弹性模量，从而减轻对上下终板的压迫，降低 相邻椎体再骨折的风险；另一方面，骨水泥表面的矿化胶原微粒能够在体内被降解吸收的同时引导骨 组织向骨水泥表面长入，使得原本惰性的 PMMA 骨水泥材料具备良好的生物活性，骨水泥能够与植入 部位骨组织形成骨整合，避免了骨水泥在植入部位产生松动和位移，极大地提高了 PMMA 骨水泥的生物安全性，降低了术后并发症的风险。目前，矿化胶原 PMMA 改性骨粉已发展出多种成熟产品，可配合德国 Heraeus 公司 Osteopal® V、意大利 Tecres 公司 Mendec® Spine 和美国 Stryker 公司 SpinePlex®等三种临床常用椎体成形骨水泥的使用，具有显著的临床效果。

The Underlying Molecular Mechanisms of How Co-Grafting of BMSCs and SCs Promote Nerve Repair after Spinal Cord Injury

Yao Wang
Tianjin Medical University General Hospital

Spinal cord injury is characterized by acute secondary injury involving several inflammatory responses and pathological tissue remodelling. Our previous experiments have found that co-grafting of BMSCs and SCs can, to a large extent, improve functional recovery after spinal cord injury in rats compared with the single cells transplantation. However, the biological mediators underlying these processes are still largely unknown. Here we apply an innovative proteomics approach (iTraq) targeting the all different proteome between treatment groups with co-grafting cells and control groups with DMEM. Proteomics revealed multiple matrix proteins not associated with injured spinal tissue, including small proteoglycans involved in cell-matrix adhesion and collagen fibrillogenesis. In mechanistic experiments, MCP-1 (CCL2), KC (CXCL1), MIP-2 (CXCL2) are macrophages or neutrophil chemokines, ICM-1, VCAM-1, leukocyte 2 integrin complex are the related proteins of leukocyte infiltration. Network analysis of transcriptomics and proteomics datasets covered persistent low expression of these chemokines and cytokines that can trigger inflammation、 cell apoptosis and axonal damage. These data reveal the change of environmental promotes tissue pathology and impedes neuronal repair after spinal cord injury

Angiopoietin-2 Promotes Neuronal Differentiation of Neural Stem Cells through PI3K-Akt-mTOR Signaling

Lu Lu
Tianjin Medical University General Hospital

Objective With the capabilities in compensating neuronal damages and reconstructing disrupted neuronal connections, multipotent neural stem cell (NSC) is considered to be the most potential candidate for the regeneration of central nervous system (CNS) injury, especially spinal cord injury (SCI). However, it is still in urgent need to regulate the differentiation of NSCs into neurons to guarantee efficient regenerative effects. Recent studies indicate that angiopoietin2 (Ang2) plays an important role not only in angiogenesis, but also in neurogenesis during the development and regeneration of central nervous system (CNS). Nevertheless, the underlying mechanism is still unknown. This study investigated the effects of Ang2 on the PI3K-AKT-mTOR signaling pathway in regulating the differentiation of NSCs. NSCs were isolated from the cortex of C57BL/6J mice on embryonic day 12.5 and cultured with recombinant human Ang2. Furthermore, specific inhibitors of PI3K-AKT-mTOR signaling pathway were used to study the molecular mechanism and its key factor of Ang2 in the regulation of NSCs differentiation. Methods NSCs were isolated from the cortex of C57BL/6J mice on embryonic day 12.5. The purified NSCs were observed under light microscope, identified by Nestin, and immuostained with specific markers of neurons, astocytes and oligodendrocytes to demonstrate its capacity in multilinage differentiation. Besides, we improved the cryopreservation and recovery protocol of NSCs, and the thawed NSCs after different freezing duration (0, 1, 6, and 12 months) were assayed by morphorlogy observation, Nestin identification, and differentiation capacity evaluation. After in vitro cultue in serum-free medium for two passages, the purified NSCs were cultured in plates coated with Poly-L-lysine (PLL) and cultured with recombinant human Ang2. By using Reverse-transcription polymerase chain reaction (RT-PCR), immunocytochemistry and Western blot, this study evaluated the differentiaton diection and efficiency of cultured NSCs with Image-Pro Plus 6.0 and Quantity One. By blocking PI3K-AKT-mTOR signaling pathway using specific inhibitors, LY294002 and rapamycin, we evaluated the alered differentiation effects and efficiency of Ang2-induced NSCs with immunocytochemistry, Western blot, and Flow cytometry. Results NSCs were successfully isolated from the cortex of C57BL/6J mice on embryonic day 12.5, and identified with morphology observation, Nestin immuostaining, and specific markers immuostaining of neurons, astocytes and oligodendrocytes. The improved cryopreservation and recovery protocol was demonstrated feasible for NSCs by morphology observation, Nestin identification, and differentiation capacity evaluation. No significant changes were found in the differentiation capacity of thawed NSCs after different freezing duration (0, 1, 6, and 12 months) ($p > 0.05$). Immunofluorescent staining showed a significant increase in the percentages of βIII-tubulin-positive cells and microtubule associated protein2 (MAP2)-positive cells ($p < 0.001$; $p < 0.001$), both of which are markers of neurons, while no significant alteration were observed in the percentage of glial fibrillary acidic protein (GFAP)-positive cells, a marker of astrocytes, nor the percentage of cyclic nucleotide 3'phosphohydrolase (CNPase)-positive cells ($p > 0.05$； $p > 0.05$), a marker of oligodendrocytes. It was further confirmed by Western blot results showing the significant improvement in the βIII-tubulin and MAP2 expression ($p < 0.001$； $p < 0.001$) without obvious disruption in the GFAP or CNPase expression ($p > 0.05$； $p > 0.05$). The phosphorylation of mTOR was demonstrated to be up-regulated through the PI3K–AKT signaling pathway during the neuronal induction of NSCs. The blockage of PI3K-AKT-mTOR signaling pathway using specific inhibitors, LY294002 and rapamycin, abrogated the promoted neuronal differentiation of NSCs by Ang2. Conclusion This study revealed the novel effects of Ang2 on the neuronal differentiation of NSCs, which were isolated from the cortex of C57BL/6J mice on embryonic day 12.5. It further uncovered the critical roles of mTOR in a PI3K-AKT dependent manner during the regulation of Ang2 in NSC differentiation. This study provides the experimental evidences of Ang2 on the aspect of neurogenesis and neural regerneration, and reveals the potential of Ang2 in promoting the regenerative effects of NSCs for central nervous system injury, especially SCI.

ShRNA against NgR Promotes Neurite Outgrowth of Cortical Neurons and Functional Recovery in Spinal Cord Contusion Rats

Jun Liu
Tianjin Medical University General Hospital

Axon growth and neurological recovery after spinal cord injury (SCI) are thought to be limited in part by myelin-associated inhibitory factors (MAIFs) that bind with high affinity to the GPI-anchored Nogo-66 receptor (NgR) protein found on neuronal or axonal membranes. NgR is therefore an attractive therapeutic target in SCI. Lentivirus-mediated short hairpin RNA (shRNA) stably inhibits target genes and can efficiently transduce most cells. In this study, the therapeutic effect of lentivirus-mediated shRNA targeting NgR was investigated both in vitro and in vivo. We demonstrated that lentivirus-mediated shRNA targeted to the NgR gene efficiently reduced its expression in cortical neurons and spinal cord tissues at both the mRNA and protein levels. An in vitro neurite outgrowth assay revealed that the knockdown of NgR significantly promoted the neurite outgrowth of cortical neurons and enhanced axons crossing onto inhibitory Nogo-66. Furthermore, the injection of lentivirus-mediated NgR shRNA to the injured site improved functional recovery in rats after SCI. The ability of lentivirus-mediated NgR shRNA to promote neurite outgrowth and functional recovery demonstrates the therapeutic potential for NgR knockdown by RNA interference (RNAi) technology in SCI.

Proteomic and Bioinformatic Analyses of Spinal Cord Injury Induced Skeletal Muscle Atrophy in Rats

Zhi-jian Wei
Tianjin Medical University General Hospital

Spinal cord injury (SCI) may result in skeletal muscle atrophy. Identifying diagnostic biomarkers and effective targets for treatment is an important challenge in clinical work. The aim of the present study is to elucidate potential biomarkers and therapeutic targets for SCI induced muscle atrophy (SIMA) using proteomic and bioinformatic analyses. The protein samples from rat soleus muscle were collected at different time points following SCI injury and separated by two dimensional gel electrophoresis and compared with the sham group. The identities of these protein spots were analyzed by mass spectrometry (MS). MS demonstrated that 20 proteins associated with muscle atrophy were differentially expressed. Bioinformatic analyses indicated that SIMA changed the expression of proteins associated with cellular, developmental, immune system and metabolic processes, biological adhesion and localization. The results of the present study may be beneficial in understanding the molecular mechanisms of SIMA and elucidating potential biomarkers and targets for the treatment of muscle atrophy.

Angiotensin II Receptor Blockade Attenuates Skeletal Muscle Atrophy in Spinal Cord Injured Rats

Ang Li
Tianjin Medical University General Hospital

Skeletal muscle atrophy is a common symptom caused by spinal cord injury. Losartan, which is an angiotensin II type I (AT1) receptor blocker and has been widely used in the treatment of hypertension, is proved to have protective effects against disuse muscle atrophy through activation of Insulin-like growth factor I (IGF1)/Akt/mTOR (mammalian target of rapamycin) signaling pathways. In the present study, we evaluated the hypothesis that whether losartan can also attenuate the atrophic response caused by spinal cord injury through the same pathways. Daily administration of losartan induced increase of wet weight of gastrocnemius and muscle fiber size compared with SCI group without treatment. Besides, SCI increased variation of smaller muscle size and formed approximate circle shape of muscle fiber, and losatan saved the shrink and partly reversed that change. However, losartan cannot result in recovery of motor function as no significant difference existed on Basso, Beattie, and Bresnahan (BBB) score between groups during 2 weeks following SCI. On the other hand, expectedly, the expression of IGF1 increased by administration of losartan, as well as phosphor-Akt and phosphor-mTOR. Meanwhile, as important E3 ubiquitin ligases in ubiquitin-proteasome system, the overexpression of Muscle atrophy F-box (MAFbx) and muscle ring finger (MuRF)-1 following SCI was suppressed and further down-regulated to close to the level of control group by losartan after SCI. These results indicate that losartan can attenuate atrophy caused by SCI and this protective mechanism may be mediated through activation of IGF1/Akt/mTOR signaling pathways and inhibition of expression of MAFbx and MuRF-1.

Protective Mechanism of Liproxstatin-1 on Glutamate Induced Neuronal Injury

Jian hao
Tianjin Medical University General Hospital

Objective: To investigate the protective effects of Liproxstatin-1 on glutamate induced neuronal injury and the mechanism. Methods: We use 14-15 day pregnant rats, executed by cervical dislocation and then using 75% alcohol sterilized for 5 minutes, then we take out the fetal and separate of fetal brain, after digestion, digestion terminated, centrifugation, resuspended, after count to 5 x 104 cells were seeded in 24 well plate and cultured for 7 days, the cell state is good. Cultured nerve cells randomly divided into 1, the control group without any treatment. 2, glutamate damage group to adjust the concentration of glutamate 5mM/L, culture 24 hours. 3, glutamate and Liproxstatin-1 co culture group to regulate the concentration of glutamate 5mM/L, and adding Liproxstatin-1 to regulate the final concentration of 200nM/L, coincubation for 24 hours. Each grope was set for 5 repeat. After 24 hours, the living cells and dead cells were counted by the use of the trypan blue staining. The intracellular reactive oxygen species (ROS) were detected by immunofluorescence. Results: After trypan blue staining then observed with 40 power microscope., random five vision was photographed: group 1: The survival rate is 34.8% of average; group 2 The survival rate with an average of 17.6%; group 3 The survival rate of the average 30.2%. Group 1, group 2 and group 2, group 3 with statistical significance ($p<0.01$), group 1, group 3 was not statistically significant ($p>0.01$). Reactive oxygen species (ROS) showed that the green fluorescence was significantly increased in group 2, while no significant increase in fluorescence was increased in group 1 and group 3. Conclusion: Liproxstatin-1 can protect the nerve cell damage induced by glutamate, and its protective mechanism is to reduce the production of reactive oxygen species (ROS). Liproxstatin-1 is an inhibitor of ferroptosis, then the protection mechanism of Liproxstatin-1 could inhibit ferroptosis.

Gene Expression Analysis at Multiple Time-Points Identifies Key Genes for Nerve Regeneration

Yi Liu
Tianjin Medical University General Hospital

Objective: The purpose of this study was to provide a comprehensive understanding of gene expression during Wallerian degeneration and axon regeneration after peripheral nerve injury. Methods: A microarray was used to detect gene expression in the distal nerve 0, 3, 7, and 14 days after sciatic nerve crush. Bioinformatic analysis was used to predict function of the differentially expressed mRNAs. Microarray results and the key pathways were validated by quantitative real-time polymerase chain reaction (qRT-PCR). Results: Differentially expressed mRNAs at different time-points (3, 7, and 14 days) after injury were identified and compared with a control group (0 day). Nine general trends of changes in gene expression were identified. Key signal pathways and 9 biological processes closely associated with nerve regeneration were identified and verified. Conclusions: Differentially expressed genes and biological processes and pathways associated with axonal regeneration may elucidate the molecular-biological mechanisms underlying peripheral nerve regeneration.

Cervical Disc Arthroplasty for Symptomatic Cervical Disc Disease: Traditional and Bayesian Meta-Analysis with Trial Sequential Analysis

Shun-Li Kan
Tianjin Medical University General Hospital

Objective: Cervical disc arthroplasty (CDA) was designed as a substitute for anterior cervical discectomy and fusion (ACDF) for the treatment of symptomatic cervical disc disease (CDD). Several researchers have compared CDA with ACDF for the treatment of symptomatic CDD; however, the findings of these studies are inconclusive. Based on the lately appearing evidence, this meta-analysis was conducted to further verify the benefits and harms of CDA for symptomatic CDD. Methods: Relevant trials were searched from PubMed, EMBASE, and the Cochrane Library databases. Outcomes were reported as odds ratio or standardized mean difference. Both traditional frequentist and Bayesian approaches were used to synthesize evidence with a random effect model. The trial sequential analysis (TSA) was applied to test the robustness of our findings and get more conservative estimation. Results: Nineteen trials were included. The findings of this meta-analysis demonstrated better overall success, lower neck disability index (NDI) scores, better NDI success, lower neck and arm pain scores, greater 36-Item Short Form Health Survey (SF-36) Physical Component Summary (PCS) and Mental Component Summary (MCS) scores, better neurological success, more patient satisfaction, greater ROM at the operative level, fewer secondary surgical procedures ($P < 0.05$) in the CDA group compared with the ACDF group. CDA was not statistically different from ACDF in adverse events ($P > 0.05$). The TSA for overall success exhibited that the cumulative z-curve crossed both the conventional boundary and the trial sequential monitoring boundary for benefit, indicating sufficient and conclusive evidence had been ascertained. Conclusions: For treating symptomatic CDD, CDA showed superiorities over ACDF in terms of overall success, NDI scores, NDI success, neck and arm pain scores, SF-36 PCS and MCS scores, neurological success, patient satisfaction, ROM at the operative level and secondary surgical procedures. Additionally, there were no significant differences between CDA and ACDF in adverse events.