Delivery of hsa-let-7b-5p by PAMAM (G5)-TPP nano-conjugates to mitochondria in NSCLC cells

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Introduction: Making disturbance in mitochondrial respiratory chain function in NSCLC cells could result in higher production of ROS levels leading to suppressing the tumor cells proliferation, as NSCLC cells use heavily mitochondrial respiration to survive [1]. Tumor suppressor microRNAs (miRNAs) such as Let-7b are surprisingly present in mitochondria (mitomiRs) playing a significant role in regulation of mitochondrial functions by targeting mitochondrial-encoded genes [2]. Mitochondriotropics such as Triphenylphosphonium cation (TPP) possess the ability of specifically targeting into the mitochondria. PAMAM dendrimers are the most available agents in gene delivery and they can conjugate to the molecules of interest such as TPP due to their large density of surface functional groups [3]. In this research, TPP-modified PAMAM (G5) dendrimers were designed to deliver Let-7b miRNA to mitochondria, as a novel treatment of NSCLC cells by targeting mitochondrial oxidative phosphorylation function.

Methods: PAMAM (G5)-TPP conjugates was synthesized and hsa-let-7b-5p was loaded forming nano-particles. Gel retention assay of nano-particles, cell viability and ability of apoptosis induction were analyzed in A549 cells via gel electrophoresis, MTT assay test and Annexin apoptosis kit, respectively. Mitochondrial co-localization of conjugates were observed using a confocal laser scanning microscopy. Transfection efficiency of hsa-let-7b-5p loaded in nano-particles and MT-CO1 gene expression were studied by qRT-PCR method.

Results: hsa-let-7b-loaded-PAMAM (G5)-TPP nano-particles were developed, successfully delivered to A549 cells and localized in mitochondria. They showed a significant reduction of tumor cells by inducing apoptosis process. Interacellular abundance of Let7-b in treated-cells exhibited a significant increasement of Let-7b level in comparison with un-treated A549 cells. It is resulted in effectiveness of this delivery system for delivery of Let7-b to mitochondria. MT-CO1 gene expression analysis indicated reduction of this gene in the respiratory chain complexes of mitochondria. This analysis could be related to regulation effect of hsa-let-7b-5p on its mitochondrial target (MT-CO1 gene) and by reducing expression of this subunit of Complex IV in mitochondrial respiratory chain, oxidative phosphorylation could be disturbed leading to increasing of ROS, mtDNA damage and inhibition of NSCLC cells growth.

Conclusion: NSCLC cells proliferation and MT-CO1 expression were reduced by hsa-let-7b-5p-loaded-PAMAM (G5)-TPP nano-particles. PAMAM (G5)-TPP nano-conjugates could be considered as an

efficient gene delivery system to deliver microRNAs to mitochondria. mitomiRs could have an effect on mitochondrial oxidative function by regulation of mitochondrial genes expression related to mitochondrial respiratory chain leading to inhibition of tumor growth and could be considered as a novel way of NSCLC cells treatment.

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Learning Objectives:

Explain the strategies for targeting mitochondria in cancer.

Evaluate the role of mitochondrial respiratory chain in cancer progress.

Discuss the presence of miRNAs in mitochondria and influence of mitomiRs on cancer bioenergetics.