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Engineering a modified HsPUM1-HD in order to bind to internal ribosome entry site (IRES) of hepatitis C virus (HCV)

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Introduction: PUF (Pumilio/FBF) proteins are a family of RNA-binding proteins that bind to the 3¢-untranslated regions of specific mRNAs and induce translational repression. The presence of a common C-terminal domain consisting of eight tandem repeats, known as PUM-HD, for RNA binding is a typical characteristic of PUF proteins.
The eight repeats recognize eight nucleotides 1:1 while three conserved amino acid residues in each repeat bind specifically to each nucleotide. The specificity of PUM-HD can be changed by mutating these amino acid residues in each repeat. This versatile architecture provides opportunities to create PUF proteins with desired RNA specificities for a variety of purposes.
Objectives: In this study, we aimed to construct a modified HsPUM1-HD in order to bind sequence-specifically to HCV IRES to potentially inhibit its critical role in viral polyprotein translation.
Materials & Methods: To change the sequence specificity of PUM-HD from consensus UGUAUAUA, a series of mutations have been designed in the sequence of the repeats 2 and 6 of HsPUM1-HD to match the combination of amino acid side chains that bind to UGGAUAAA from loop 3 of HCV IRES.
The expression of modified HsPUM1-HD was induced in BL21(DE3) cells and cell pellets were lysed with sonication. The supernatant containing the modified HsPUM1-HD was used for pull-down assay with biotinylated RNAs containing the target sequence from HCV IRES (UGGAUAAA). The samples were analyzed by SDS-PAGE and western blot.
Results: In RNA-protein pulldown assay, the biotinylated RNA and its associated proteins were captured by the streptavidin beads (figure 1). Figure 1. Pull-down assay. The biotinylated RNAs pulled down the modified HsPUM1-HD (41kD) from cell lysates (figure 2), while the control RNAs did not (data not shown). Figure 2. Western blot analysis of pull-down assay results.
Conclusion: The results show that we are able to target HCV IRES by applying desired mutations in HsPUM1-HD sequence. The potential inhibitory effect of this binding on IRES-dependent translation is under investigation through dual luciferase reporter assay and HCV cell culture system. Targeting RNA transcripts involved in HCV replication may provide interesting tools as potential antiviral agents for treatment of HCV infection.

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