The RNA-binding protein HuR protects PDCD4 mRNA from microRNA-21 induced silencing

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Introduction
Encoded by the PDCD4 gene in humans, the tumour suppressor Programmed cell death protein 4 plays a role in apoptosis and the development of cancer in humans. PDCD4 modulates the activity of the eukaryotic translation initiation factor (eIF)4A thus affecting global translation, and also plays a key role in repressing IRES-mediated translation of inhibitor of apoptosis proteins. The RNA-binding protein HuR has been shown to modulate the stability and translational efficiency of many messenger RNAs. We now describe the novel function of HuR regulating the expression of PDCD4 via miR-21 and ERK8.

Background
HuR
• RNA binding protein involved in regulating mRNA splicing, export and polyadenylation
• Binds to AU-rich elements in 3’UTRs to regulate stability

Figure 1: Left panel: HuR competes with micro-RNA/RISC complex binding to the mRNA. If miR/RISC binds, there is a loss in protein expression however, if HuR binds, there is an increase in protein expression. Right panel: HuR and microRNA/RISC complex cooperate to bind the mRNA and result in loss of protein expression.

Methodology
1. Tissue culture and transfection - HeLa cells were maintained in DMEM-high glucose completed with glutamine, heat-inactivated fetal calf serum, and penicillin-streptomycin antibiotics. Cells were seeded and transfected for knockdowns using siRNA (Ex: siCtrl, siERK8). Cells were treated with H2O2 then harvested for analysis.

2. Lysing cells for Western Blot analysis - Cells were washed with PBS, scraped, and transferred to an Eppendorf tube. Cells were pelleted by centrifugation and lysed in RIPA buffer on ice. Lysates were centrifuged at 13,000 x g to pellet cell debris and supernatant was transferred to a new Eppendorf tube.

3. Protein quantification - Bradford Assay was used to quantify protein concentration before loading the samples on SDS-PAGE gels.

4. Western Blotting analysis - Equal concentrations of proteins were loaded on SDS-PAGE gels. After running the gel for one hour at 150 V, proteins were transferred to a PVDF membrane and analyzed by antibody addition (Primary antibodies followed by secondary antibodies). Antibody complexes were detected using ECL and then exposed to X-ray film.

5. Immunofluorescence Cells were grown on coverslips and treated with H2O2. Cells were fixed with formaldehyde, permeabilized with Triton X-100 and blocked with 5% BSA for 30 min. Primary antibody to HuR was added overnight followed by secondary anti-mouse conjugated to alexa fluor-488. Nuclei were stained with Hoechst and coverslips were mounted on slides with Fluoromount. Images were obtained with the FV-1000 confocal microscope.

Results

Figure 2: (A) Knockdown of HuR results in a loss of PDCD4 protein. (B) Decrease in HuR levels results in a decrease of PDCD4 mRNA levels.

Figure 3: (A) Left panel: GST or GST-HuR was purified from E. coli cells and separated by SDS-PAGE gel and coomassie stained. Middle panel: Schematic of the RNA fragments of the PDCD4 3’untranslated region (UTR). Right panel: GST or GST-HuR binding assay with 32P-labelled PDCD4 3’UTR RNA fragments.

Figure 4: (A) Localization of HuR into the cytoplasm after treatment with H2O2 by immunofluorescence. (B) H2O2 treatment causes a loss in PDCD4 protein that is returned to normal levels if miR-21 is inhibited.

Conclusion

• HuR binds to the PDCD4 3’UTR to prevent miR-21 binding
• Activated ERK8 in response to H2O2 phosphorylates HuR causing it to lose its ability to bind to PDCD4 mRNA allowing miR-21 to bind
• miR-21 binding in response to H2O2 results in a loss in PDCD4 mRNA and protein expression

References

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