

RNA-BINDING PROTEIN HUR ENHANCES SURVIVIN MRNA STABILITY AND CONTRIBUTES TO SURVIVIN OVEREXPRESSION IN ESOPHAGEAL CANCER

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Background: The inhibitor of apoptosis protein survivin is overexpressed in esophageal cancer. We have previously shown that the increased expression of survivin is mediated, in part, at the level of transcription by the loss of p53, which negatively regulates survivin expression. We observed that overexpression of p53 in esophageal cancer cells was associated with decreased survivin transcription, but levels of survivin protein were not significantly changed. Based on this finding, we hypothesized that stability of survivin mRNA was an additional control point for survivin expression. HuR is an RNA-binding protein that binds to the 3' untranslated region (UTR) of many labile mRNAs bearing AU-rich elements (AREs). Analysis of the 3' UTR of survivin mRNA documented multiple AREs. The purpose of this study is to determine whether HuR binds to survivin mRNA and if this interaction stabilizes survivin mRNA.

Methods: Studies were conducted in normal human esophageal mucosal cells and in TE-7 human esophageal adenocarcinoma cells. Levels of protein expression were measured by Western blot. Survivin mRNA binding to HuR was examined by biotinylated RNA pull-down and RNP-IP assays. Survivin mRNA stability was examined by measuring its half-life. HuR function was tested by its silencing using siRNA.

Results: HuR is overexpressed in TE-7 esophageal cancer cells in comparison to normal esophageal cells. HuR directly bound to the survivin 3' UTR as measured by RNA-pull down and RNP-IP assays. mRNA stability assays demonstrate that the half-life of survivin mRNA in normal esophageal cells is 2.6 hours and 17.5 hours in TE7 cells. HuR silencing in TE-7 resulted in both decreased levels of survivin protein and in a reduction of survivin mRNA half life to 2.1 hours.

Conclusions: These findings demonstrate that HuR is overexpressed in esophageal cancer cells and directly binds survivin mRNA. This interaction stabilizes survivin mRNA and may play an important role in survivin overexpression in esophageal cancer.