HuR status is a powerful clinical marker for resected pancreatic ductal adenocarcinoma patients and can bind to VEGF and HIF-1alpha mRNA

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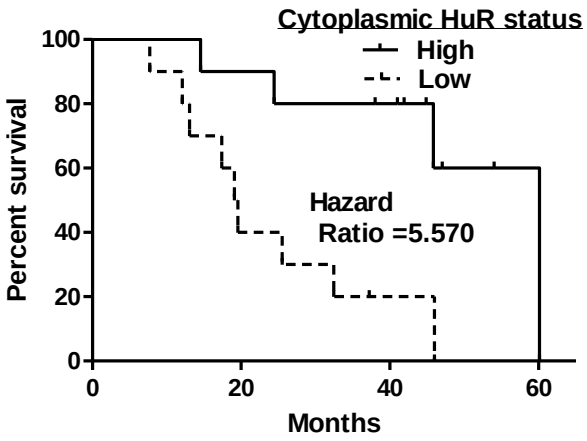
Objective: Treatment of pancreatic ductal adenocarcinoma (PDA) typically includes chemotherapy with gemcitabine. No reliable biomarker exists for overall prognosis or response to chemotherapy. Two previously proposed prognostic markers, COX-2 and VEGF, are regulated by HuR, an mRNA binding protein that we have demonstrated to be a promising predictive marker of gemcitabine response (*Cancer Research* 2009, 69:4567-72). This study was designed to evaluate a clinically useful biomarker for PDA and to explore the association of HuR to oncogenic mRNA target genes, HIF-1α and VEGF.

Methods: A tissue microarray of 53 PDA specimens, who underwent potentially curative resection, was analyzed. HuR, COX-2, and VEGF status were correlated with clinical data and compared for relative utility. Human pancreatic cancer cells were treated with gemcitabine for 6 hours, and cytoplasmic lysates were collected. Real-time (RT) quantitative PCR was performed with cDNA generated from bound RNA immunoprecipitated (RNP-IP) from an HuR or a non-specific IgG antibody. Relative quantification of VEGF and HIF-1α mRNA was assessed.

Results: Roughly 50% (27/53) of patients had elevated cytoplasmic HuR expression (HuR+). These patients had worse pathologic features (i.e. positive lymph nodes (75%) and AJCC pathologic stage 2 or greater (94%)) compared to HuR- patients. Cytoplasmic HuR status correlated with staging better than VEGF or COX-2 expression alone. When used in combination, HuR cellular positivity with VEGF+ status yielded 100% lymph node positivity. Additionally, HuR status was a robust positive predictive marker for overall survival in patients treated with gemcitabine pushing median survival over 40 months in the HuR+ patient population (p-value 0.0049). The described RT and RNP-IP assay demonstrated mechanistically that VEGF and HIF-1α mRNA transcripts are selectively bound after gemcitabine treatment to HuR as compared to a control by over 20 and 14 fold, respectively.



Conclusions: In pancreatic cancer cells, HuR associates with and likely regulates the expression of VEGF and HIF-1α, key proteins involved in pancreatic tumorigenesis. HuR status is a robust predictor of outcome for patients with resected PDA and should be used by clinicians for the individualized treatment of PDA. This study also validates our previous work in a separate clinical data set that HuR is a valuable predictive marker for gemcitabine response in patients with PDA.