The RNA binding protein HuR differentially regulates unique subsets of mRNAs in estrogen receptor negative and estrogen receptor positive breast cancer

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Abstract

Background: The discordance between steady-state levels of mRNAs and protein has been attributed to posttranscriptional control mechanisms affecting mRNA stability and translation. Traditional methods of genome wide microarray analysis, profiling steady-state levels of mRNA, may miss important mRNA targets owing to significant posttranscriptional gene regulation by RNA binding proteins (RBPs).

Methods: The ribonomic approach, utilizing RNA immunoprecipitation hybridized to microarray (RIP-Chip), provides global identification of putative endogenous mRNA targets of different RBPs. HuR is an RBP that binds to the AU-rich elements (ARE) of labile mRNAs, such as proto-oncogenes, facilitating their translation into protein. HuR has been shown to play a role in cancer progression and elevated levels of cytoplasmic HuR directly correlate with increased invasiveness and poor prognosis for many cancers, including those of the breast. HuR has been described to control genes in several of the acquired capabilities of cancer and has been hypothesized to be a tumor-maintenance gene, allowing for cancers to proliferate once they are established.

Results: We used HuR RIP-Chip as a comprehensive and systematic method to survey breast cancer target genes in both MCF-7 (estrogen receptor positive, ER+) and MDA-MB-231 (estrogen receptor negative, ER-) breast cancer cell lines. We identified unique subsets of HuR-associated mRNAs found individually or in both cell types. Two novel HuR targets, CD9 and CALM2 mRNAs, were identified and validated by quantitative RT-PCR and biotin pulldown analysis. Interestingly, calmodulin is encoded by three different genes with identical ORFs, located at different chromosomal locations. All these transcripts must be simultaneously targeted by siRNA to affect a knock-down. Though all three genes are expressed at high levels in both ER+ and ER- breast cancer, HuR only binds to and regulates CALM2, suggesting a fine tuning of cellular calcium metabolism.

Conclusion: This is the first report of a side-by-side genome-wide comparison of HuR-associated targets in wild type ER+ and ER- breast cancer. We found distinct, differentially expressed subsets of cancer related genes in ER+ and ER- breast cancer cell lines, and noted that the differential regulation of two cancer-related genes by HuR was contingent upon the cellular environment. Furthermore, many HuR targets genes do not have any known cancer roles and thus well represent novel targets.

Introduction

There is a poor correlation between steady state mRNA levels and gene products. The AU-rich (AREs) elements found in the 3' UTR of mRNAs have been implicated in control of nuclear transport, stability and translation. Eight percent of the human genome consists of ARE-containing genes, indicating a central role for this motif in gene expression. Not surprisingly, these genes are found in areas of transient biologic processes, including cell growth and differentiation, immune responses, signal transduction, transcription and translational control, hematopoiesis, apoptosis, nutrient transport and metabolism. The RNA binding protein, HuR, is a paraneoplastic antigen, often over expressed in many malignancies. HuR binds to target mRNA and acts to stabilize and translationally up regulate their expression. RNA immunoprecipitation applied to microarray analysis (**RIP**-ChiP) methods can be used to identify genes whose steady state mRNA levels do not significantly change. These genes may be over looked by traditional microarray profiling methods. *The posttranscriptional operon hypothesis* states that RBPs, like HuR, are coordinately regulating expression of biologically related genes. Therefore, the hypothesis has been suggested that HuR may act as a tumor maintenance gene, which enables the cancer cells to survive, expand and perhaps metastasize. We asked whether HuR is regulating different mRNA targets in ER- and ER+ breast cancer. We were surprised to find that HuR differentially regulates the same targets differently depending upon cellular milieu. Furthermore, HuR RIP-Chip analysis may be used to discover novel cancer genes which are candidate members in the acquired capabilities model of cancer.

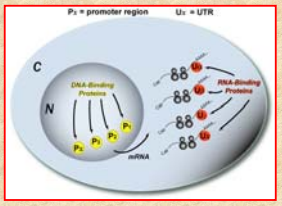

Figure 1

Reducing the Complexity of Gene Expression by Partitioning at the level of Regulation

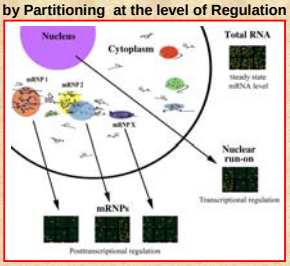

Figure 2

Properties of ELAV/Hu Proteins

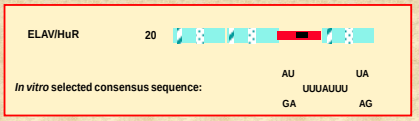

Figure 3

- Bind to AU-rich sequence elements (AREs) found in 3'UTRs of growth regulatory mRNAs (eg. *c-myc*, *c-fos*, GM-CSF, IL-2, NF-M, tau, others)
- Paraneoplastic antigens over-expressed in various malignancies
- Affect stability and translation of target mRNAs, thereby influencing regulation of cell growth and differentiation
- Increased cytoplasmic HuR expression correlates with worse outcomes in breast cancers

IP/Westerns from MDA-MB-231 and MCF-7 cells identify HuR protein

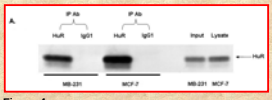

Figure 4

HuR RIP-Chip identifies a known target, b-actin

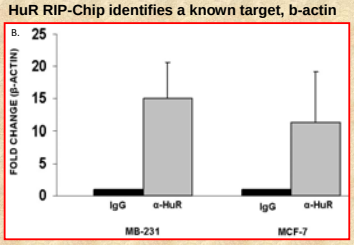

Figure 5

HuR RIP-Chip analysis reveals different subsets of targets in ER- and ER+ breast cancer

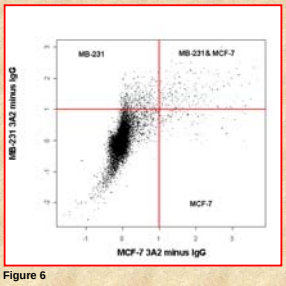

Figure 6

GO Analysis of HuR targets over-expressed in ER- vs. ER+ breast cancer

Figure 7

Biotin pull downs reveal that HuR binds to CD9 and calm2 mRNA 3' UTRs

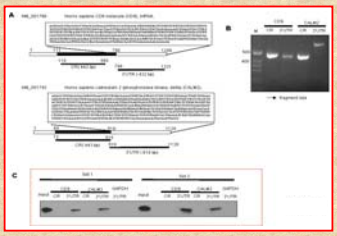

Figure 8

HuR over and under expression in breast cancer cell lines

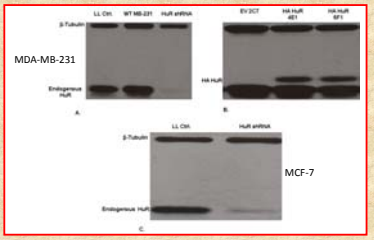

Figure 9

HuR over expression reduces CD9 mRNA and protein but not calm2 in MDA-MB-231 cells

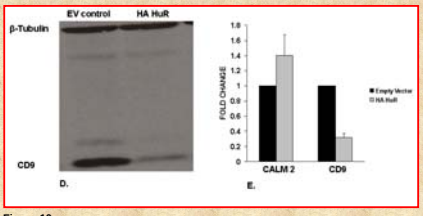

Figure 10

HuR shRNA knock down in MDA-MB-231 increases CD9 and calm2 mRNA levels

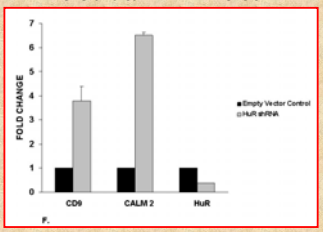

Figure 11

HuR shRNA knock down in MCF-7 decreases CD9 and calm2 mRNA levels

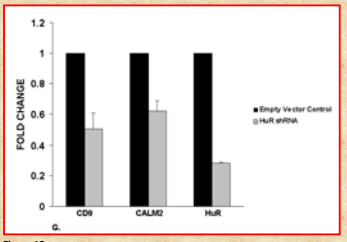

Figure 12

HuR over expression in MDA-MB-231 cells alters cell cycle kinetics

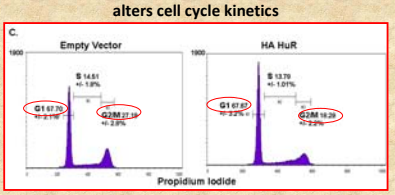

Figure 13

Proposed Candidate Genes to the Acquired Capabilities Model

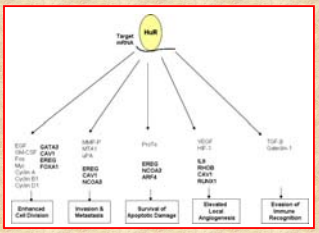

Figure 14

Conclusions

- HuR RIP-Chip analysis can be used to identify novel cancer target genes
- HuR regulates both CD9 and calm2
- Regulation of CD9 and calm2 is cell specific
- HuR coordinately regulates distinct subsets of genes in ER- and ER+ breast cancer

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