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Complex HuR function in pancreatic cancer cells

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers with dismal patient outcomes. The underlying core genetic drivers of disease have been identified in human tumor specimens and described in genetically engineered mouse models. These genetic drivers of PDAC include KRAS signaling, *TP53* mutations, and genetic loss of the SMAD4 tumor suppressor protein. Beyond the known mutational landscape of PDAC genomes, alternative disrupted targets that extend beyond conventional genetic mutations have been elusive and understudied in the context of PDAC cell therapeutic resistance and survival. This last point is important because PDAC tumors have a unique and complex tumor microenvironment that includes hypoxic and nutrient-deprived niches that could select for cell populations that garner therapeutic resistance, explaining tumor heterogeneity in regards to response to different therapies. We and others have embarked in a line of investigation focused on the key molecular mechanism of posttranscriptional gene regulation that is altered in PDAC cells and supports this pro-survival phenotype intrinsic to PDAC cells. Specifically, the key regulator of this mechanism is a RNA-binding protein, HuR (*ELAVL1*), first described in cancer nearly two decades ago. Herein, we will provide a brief overview of the work demonstrating the importance of this RNA-binding protein in PDAC biology and then provide insight into ongoing work developing therapeutic strategies aimed at targeting this molecule in PDAC cells.

Keywords

ELAVL1; HuR; pancreatic cancer; pancreatic ductal adenocarcinoma; RNA binding proteins

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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1 | INTRODUCTION

Polymerase chain reaction (PCR)-amplified based sequencing of isolated pancreatic ductal adenocarcinoma (PDAC) genomes followed by high throughput next generation sequencing (NGS) platforms have provided a genetic landscape of the progression model of PDAC (Knudsen, O'Reilly, Brody, & Witkiewicz, 2016). These later studies have subtyped PDAC tumors, which may have implications for prognostic clinical value and for therapeutic targeting (Bailey et al., 2016; Collisson et al., 2011). To highlight how far this work has come, NGS sequencing of PDAC tumors have now become routine in some institutions and numerous commercial platforms exist. In fact, a pioneer in this field and one of the leading advocacy groups in the country, the Pancreatic Cancer Action Network (PanCAN), has launched into a program attempting to democratize sequencing of PDAC tumors for the ultimate purpose of matching patients with the appropriate clinical trial (Pishvaian et al., 2016). Additionally, PanCAN is pushing the limits on personalized medicine for pancreatic cancer patients, by initiating the launch of the *Precision Promise Program*—a match your tumor-to-therapy initiative that brings together a multidisciplinary group of investigators, institutions, and platforms to accelerate the pace of molecular tailored trials and research. Certainly, these *tour de force* clinically driven initiatives are timely, logical, and cutting-edge, and the amount of data the research community will obtain will be invaluable. However, these personalized medicine initiatives will take time and will most likely not directly translate into clinical practice in the near future.

The long term future of precision medicine for pancreatic cancer seems more promising than the short term, in part, because the targeted therapies available for treating pancreatic cancer cells and their complex tumor microenvironment lack specificity and activity. For instance, a *one size fits all* approach of targeting *KRAS* mutations have proven to be challenging most likely due to the PDAC cell's ability to compensate through different signaling pathways. Besides compensation, many genetic lesions that have been identified as critical for tumor progression may not be the most valuable targets at the time the tumor presents clinically. Therefore, a target that meets the following criteria should be considered in order to target PDAC cells efficiently: (a) an available target: one that is abundant in cancer cells compared to normal cells, (b) having a functional purpose that can be targeted in cancer cells, (c) activated by the unique tumor microenvironment in PDAC tumors (e.g., hypoxia and low glucose), and (d) provide an important survival advantage for PDAC cells. Herein, we will provide a brief account of the evidence describing the role of a target that meets these criteria, the RNA-binding protein HuR that is a master regulator of pancreatic tumorigenesis and cell survival.

2 | AN ALTERNATIVE TO DRIVER GENETIC EVENTS: A CRITICAL POSTTRANSCRIPTIONAL GENE REGULATORY MECHANISM

Many laboratories, including our own work, have established a line of investigation that dates back over 10 years, demonstrating that gastrointestinal (GI) cancer cells and tumors (i.e., PDAC and colorectal cancer) survive under cancer-associated stress conditions (e.g., chemotherapy) through posttranscriptional gene regulation (Blanco et al., 2016; Costantino

et al., 2009; Dixon et al., 2001; Lal et al., 2014; Lal et al., 2017; McAllister et al., 2014; Pineda et al., 2012; Richards et al., 2010; Romeo et al., 2016; Subbaramaiah, Marmo, Dixon, & Dannenberg, 2003; Williams et al., 2010; Young et al., 2009; Young, Moore, Sokol, Meisner-Kober, & Dixon, 2012). Primarily through the actions of RNA-binding proteins (RBPs) and micro-RNAs (miRNAs), posttranscriptional gene regulation can rapidly alter the proteome in response to cellular signals by directly impacting gene expression at the level of mRNA stability (Cheadle et al., 2005; Fan et al., 2002; Garneau, Wilusz, & Wilusz, 2007). While the majority of RBPs and miRNAs are noted for their effects on promoting mRNA decay and/or suppressing translation, the RBP Human antigen R (HuR, *ELAVL1*) is the most intriguing and prominent antagonist of cancer-associated mRNA degradation.

HuR is a member of the ELAV family of RBPs (for an extensive review of this family of proteins please see Hinman & Lou (2008)) and consists of two-tandem RNA recognition motif (RRM) domains, followed by a hinge region and a third RRM domain (Brennan & Steitz, 2001; Meisner & Filipowicz, 2010). HuR binds to adenylate uridylylate (AU)-rich elements (AREs) typically located in the 3'-untranslated regions (3'UTRs) of specific target genes involved in cell survival and tumorigenesis (Abdelmohsen & Gorospe, 2010; Blanco, Jimbo, et al., 2016; Brody & Gonye, 2011; Costantino et al., 2009; Khabar, 2017; Lal et al., 2014). HuR is predominantly localized to the nucleus (>90%) and can shuttle between the nucleus and the cytoplasm. Nucleocytoplasmic trafficking is mediated through a basic 32-amino acid HuR Nucleocytoplasmic Shuttling (HNS) sequence contained in the hinge region and involves several transport machinery components including exportin-1 (XPO1, CRM1), transportins, and importins (Fan & Steitz, 1998a; Fan & Steitz, 1998b; Gallouzi & Steitz, 2001; Rebane, Aab, & Steitz, 2004; Wang et al., 2004). It is hypothesized that the ability of HuR to promote mRNA stabilization requires its translocation to the cytoplasm (Brennan & Steitz, 2001; Keene, 1999). In the context of cancer, HuR becomes functionally active as an mRNA stability factor in response to various cancer-associated stressors (e.g., DNA damage, low glucose), where it binds to selective mRNAs and translocates to the cytoplasm.

3 | HUR IN PANCREATIC CANCER

The Gorospe and Dixon laboratories have initiated the discovery that HuR is both abundant in GI cancer cells and tumors, and is functionally active as a pro-survival network important for tumorigenesis (Dixon et al., 2001; Lopez de Silanes et al., 2003; Mazan-Mamczarz et al., 2003; Wang et al., 2000). HuR has been identified as a prognostic marker or relevant to at least a dozen tumor types (Abdelmohsen & Gorospe, 2010; Kotta-Loizou, Giaginis, & Theocharis, 2014; Wang et al., 2013; Zucal et al., 2015). Mechanistically, it was shown that HuR is upregulated and dysregulated in cancer cells, in part, through posttranscriptional gene regulation (Abdelmohsen et al., 2010; Abdelmohsen, Srikantan, Kuwano, & Gorospe, 2008; Pullmann Jr. et al., 2007).

The first study to demonstrate that HuR was abundant in PDAC cells was published in 2009 (Costantino et al., 2009). This study showed that overexpression of HuR in PDAC cell lines did not dramatically change the in vitro phenotype, but did specifically make PDAC cells highly sensitive to the commonly utilized chemotherapeutic agent, gemcitabine. These data

were also supported by a retrospective study of patient data that showed if patient's tumor specimens had a high cytoplasmic HuR score they were more likely to respond to gemcitabine adjuvant therapy. These findings were expanded to show that PDAC cells exposed to gemcitabine induced HuR's translocation to the cytoplasm. This allowed for binding of the HuR target mRNA encoding deoxycytidine kinase (dCK) and its subsequent upregulation, leading to enhanced metabolism and efficacy of the prodrug (gemcitabine) in PDAC cells. These results provided a mechanistic explanation when PDAC cells are exposed to the stressor gemcitabine. This study laid the framework for future studies linking a pancreatic cancer-associated stress with a novel HuR target that conferred a pro-survival PDAC phenotype (see Table 1 and Figure 1).

3.1 | Pre-clinical evidence that HuR is a good target in PDAC cells

More recently, we generated and evaluated HuR-null cells in two different PDAC cell lines by CRISPR/Cas9 deletion of the *ELAVL1* (HuR) gene (Lal, Cheung, et al., 2017). While HuR(−/−) cells displayed a mild growth phenotype in vitro, deletion of HuR generated a dramatic xenograft lethal phenotype in vivo, supporting the notion that HuR is required for tumor initiation and progression in vivo. Specifically, deletion of HuR in two PDAC cell lines were unable to engraft in mice, compared to isogenic proficient HuR PDAC lines. To demonstrate that this phenotype was directly due to the loss of HuR, addition of a HuR cDNA rescued the ability of the PDAC cells to establish tumors in nude mice (Lal, Cheung, et al., 2017). These data support our previous published work that inducible knockdown of HuR using short hairpin RNA-based silencing in PDAC cells in vivo can reduce the tumor size by roughly fourfold (Jimbo et al., 2015). Similarly, we recently demonstrated the efficacy of targeting HuR in GI cancer in vivo by using small molecule inhibitors of HuR function (Blanco et al., 2016; Lal et al., 2017).

4 | HUR IS A POTENT MODULATOR OF PDAC DRUG RESISTANCE WITHIN THE ELEMENTS OF THE PDAC ENVIRONMENT

The selective pressure imposed by the harsh tumor microenvironment favors growth of the most aggressive and fit PDAC cells, which tend to be the most resistant to cytotoxic chemotherapeutic agents (Jones et al., 2008; Vineis, 2000; Von Hoff et al., 2013). Previously, it has been shown that PDAC tumors are embedded in a highly *hypoxic and nutrient-deprived tumor microenvironment* wherein clonal populations of PDACs with aggressive traits thrive and expand (Anderson, Mack, & Silverman, 2006; Koong et al., 2000; Vineis, 2000). In order to overcome the harsh stress imposed by chronic hypoxia (e.g., low oxygen pressure [pO₂] and intratumoral perfusion) PDAC cells orchestrate a multifaceted response by activating hypoxia-inducible factors (HIFs) such as HuR and PIM1 (Bertout, Patel, & Simon, 2008; Burkhart et al., 2013). Additionally, we have recently demonstrated that HuR translocation from the nucleus to the cytoplasm occurs under nutrient deprivation (e.g., low glucose) (Zarei et al., 2017). Specifically, under these conditions HuR regulates the key metabolic enzyme, wild-type IDH1 (Table 1). These potent acute cellular reprogramming events regulated by HuR activates pathways responsible for regulating cell motility, intracellular pH, mitochondrial function, angiogenesis, cellular metabolism, DNA repair, and cell survival (Buchler et al., 2004; Chang, Jurisica, Do, & Hedley, 2011; Humar, Kiefer,

Berns, Resink, & Battegay, 2002; Zarei et al., 2017). Indeed, we have shown that HuR plays critical roles in both hypoxia and low glucose-induced chemoresistance that represents a major barrier for the clinical efficacy of chemotherapeutic regimens in PDAC (Blanco, Jimbo, et al., 2016; Zarei et al., 2017; Figure 1 and Table 1). In future, more sophisticated studies will address the additive effect of hypoxia and nutrient deprivation on HuR's subcellular localization and function.

4.1 | Example 1 of a validated HuR target: The proto-oncogene PIM1

PIM1 (Proviral Integration site for Moloney murine leukemia virus 1), a serine-threonine kinase, has emerged as a prominent modulator of therapeutic resistance in head and neck squamous cell carcinoma, prostate carcinoma, and, most recently, PDAC (Blanco, Jimbo, et al., 2016). PIM1 drives chemoresistance by phosphorylating and inactivating key apoptotic and tumor suppressive proteins (Blanco, Jimbo, et al., 2016), thus rendering cells resistant to the stress imposed by DNA-damaging cytotoxic chemotherapy. Until recently (Blanco, Jimbo, et al., 2016), the mechanism behind PIM1 overexpression in PDAC was unknown, especially in the context of the hypoxic tumor microenvironment (1% oxygen). Histologic analysis of PIM1 expression in PDAC tumors revealed a strong correlation with tumor hypoxia markers (Blanco, Jimbo, et al., 2016). Since hypoxia-mediated PIM1 overexpression occurs in a HIF-1 α -independent manner (Blanco, Jimbo, et al., 2016), and no known *PIM1* mutations have been identified in PDAC, the contribution of posttranscriptional mechanisms was evaluated. We identified that a *cis*-acting AREs present in the 3'UTR of the PIM1 mRNA mediates interaction with HuR under conditions of hypoxic stress. This regulatory mechanism results in enhanced PIM1 mRNA stability and consequently PIM1 protein overexpression.

4.2 | Example 2 of validated HuR target: The metabolic enzyme IDH1

As discussed, HuR protects PDACs not only from hypoxia but also nutrient-related stress (Blanco, Jimbo, et al., 2016; Burkhart et al., 2013). Since nutrient deprivation and chemotherapy induce a surge in reactive oxygen species (ROS) (Chio & Tuveson, 2017; Zarei et al., 2017), the adaptive mechanisms required by PDAC cells to survive oxidative stress in the PDAC microenvironment likely also contribute to chemotherapy resistance. For example, HuR regulates a critical anti-ROS defense system through the posttranscriptional stabilization of the nicotinamide adenine dinucleotide phosphate (NAPDH)-generating enzyme, isocitrate dehydrogenase 1 (IDH1) (Zarei et al., 2017). It was observed that diminished nutrient availability (e.g., low glucose levels) promotes chemotherapy resistance in PDAC cells and mouse xenografts, as well as in a retrospective cohort of patients who underwent pancreatic resection for PDAC. HuR silencing by RNAi resulted in impaired PDAC cell viability under nutrient (glucose) withdrawal, and also abrogated chemotherapy resistance related to nutrient withdrawal. Ribonucleoprotein immunoprecipitation studies of HuR confirmed the binding interaction of HuR to IDH1 mRNA. Importantly, expression of IDH1 RNA and protein was almost completely absent in two different HuR-null PDAC cell lines, and transient IDH1 overexpression restored chemotherapy resistance under low nutrient conditions. Furthermore, stable expression of IDH1 in HuR-null PDAC cells restored the ability of these cells to successfully implant and grow in nude mice at the same rate as HuR-proficient control cells.

4.3 | Example 3 of a validated HuR target: The mitotic checkpoint inhibitor WEE1

Previously it has been shown that conventional DNA damaging agents such as arsenite (Bhattacharyya, Habermacher, Martine, Closs, & Filipowicz, 2006), actinomycin D (Rattenbacher & Bohjanen, 2012), and hydrogen peroxide (Martin-Garrido et al., 2011) can activate (translocate) HuR (Wang et al., 2000). We have shown that other DNA damaging agents used clinically promotes activation of HuR, as assessed by the rapid translocation of HuR from the nucleus to the cytoplasm (Lal et al., 2014). Additionally, mitomycin C (MMC) treatment within in hours induced γ -H2AX foci in both control and HuR-silenced cells, indicating the presence of DNA damage breaks (Lal et al., 2014). Remarkably, after MMC treatment the number of foci per nuclei increased significantly in HuR-silenced cells compared to the control cells, demonstrating that DNA damage persisted and DNA repair was considerably delayed in the absence of HuR. Mechanistically to prove that this was a HuR-dependent event, HuR depletion resulted in significant downregulation of WEE1 (a mitotic inhibitor) mRNA and protein, both in treated and untreated cells. Ribonucleoprotein immunoprecipitation analysis validated that WEE1 mRNA was bound to HuR, demonstrating WEE1 mRNA as a novel HuR target in the DNA damage repair pathway. These observations were further supported by findings that showed silencing HuR followed by stress resulted in enhanced phosphorylation of CDK1 (a key cell cycle regulator) that phosphorylates HuR and restricts its localization to the nucleus (Kim et al., 2008). Together, these data indicate that HuR regulates the DNA damage repair in part via WEE1, which may lead to the inhibitory phosphorylation of CDK1 and G2/M cell cycle arrest.

In summary, it was demonstrated that the harsh tumor microenvironment (i.e., low glucose, hypoxia, and DNA damage) of PDAC renders these cells resistant to chemotherapy, and that posttranscriptional regulation of IDH1, PIM1, and WEE1 by HuR, in part, drives an underlying adaptive survival mechanism. These data may explain why conventional chemotherapy shows limited effectiveness against PDAC, and highlights HuR as a compelling therapeutic target in the context of the harsh and oxidative PDAC tumor microenvironment. Further effort to understand the significance of reported posttranslational modifications on HuR's subcellular localization status (i.e., specifically phosphorylation) (Abdelmohsen et al., 2007; Abdelmohsen & Gorospe, 2010; Chemnitz, Pieper, Gruttner, & Hauber, 2009; Doller et al., 2007; Doller et al., 2011; Doller, Schlepckow, Schwalbe, Pfeilschifter, & Eberhardt, 2017; Eberhardt, Doller, & Pfeilschifter, 2012; Grammatikakis, Abdelmohsen, & Gorospe, 2017; Kim et al., 2008; Liu et al., 2009; Scheiba, Aroca, & Diaz-Moreno, 2012; Wang et al., 2004; Yoon et al., 2014; Yu et al., 2011; Zou et al., 2008) is currently being investigated. Understanding the specific kinases and protein motifs affected under the above mentioned conditions may be important for targeting HuR in unique PDA tumor microenvironment niches. Based on this work, we hypothesize that this gene regulatory mechanism serves as a backbone of chemoresistance in PDAC that can be therapeutically disrupted using agents targeting HuR.

5 | HUR AS A BIOMARKER IN PDAC AND OTHER CANCERS: “TO BE OR NOT TO BE A BIOMARKER”

HuR is abundant in most cancers and reduced in normal cells, and the cytoplasmic HuR status in tumor specimens have been shown to correlate with poor prognostic value in many tumor types (Abdelmohsen & Gorospe, 2010; Kotta-Loizou et al., 2014; Wang et al., 2013; Zucal et al., 2015). Specifically, we have demonstrated that HuR is overexpressed and functionally active (i.e., cytoplasmic status) in patient-derived cell lines and in clinical samples (Costantino et al., 2009; Dixon et al., 2001; Young et al., 2009). Importantly, we have demonstrated that cytoplasmic HuR correlates with an identified HuR target in patient samples (Blanco, Jimbo, et al., 2016; McAllister et al., 2014; Pineda et al., 2012). In line with previous work in other tumor systems, HuR cytoplasmic expression was shown to be a poor prognostic marker, as it has been shown to correlate with tumor T staging (Richards et al., 2010). In regards to cytoplasmic HuR as a predictive biomarker for gemcitabine-based adjuvant therapy, we have followed up our evaluation of this with multiple publications (Costantino et al., 2009; McAllister et al., 2014; Richards et al., 2010; Tatarian et al., 2018). Many possibilities could account for why HuR status correlates as a predictive marker for gemcitabine and/or 5-fluorouracil-based therapies in these settings: (a) We performed these studies primarily in the adjuvant setting (postoperatively) in stage I and II patients. Thus, these studies analyzed the primary tumors, which were resected, and adjuvant therapy would most likely be targeting the micrometastatic and metastatic tumor cells, which may have a different cytoplasmic HuR status depending on the microenvironment. (b) Different therapeutic regimens may include radiation that we know from previous work may disrupt HuR's biology (Masuda et al., 2011). Ultimately, validating HuR as a predictive marker should be performed in a prospective fashion in a clinical trial focused in patients with advanced, metastatic disease.

6 | ONGOING THERAPEUTIC STRATEGIES TO TARGET HUR

Revisiting our proposed criteria for the therapeutic value of a target in PDAC, HuR checks off many of these boxes: (a) *it is an available target: HuR is overexpressed in PDAC cells compared to normal cells*, (b) *HuR has a functional purpose that can be targeted in PDAC cells as a pro-survival hub*, (c) *HuR is activated by the unique tumor microenvironment in PDAC tumors*, and (d) *HuR provides an important survival advantage for PDAC cells*. Therefore, based on nearly a decade's worth of published data, strategies to target HuR as a candidate molecular target are gaining significant attention (Zucal et al., 2015).

Our recent efforts have led the evaluation of lead small molecule HuR inhibitors (Blanco, Jimbo, et al., 2016; Blanco, Preet, et al., 2016; Lal, Cerofolini, et al., 2017; Romeo et al., 2016; Wu et al., 2015; Young et al., 2012). Currently, there are a number of HuR small molecule inhibitors that have been identified by high-throughput biochemical screens with the ability to inhibit HuR activity (Chae et al., 2009; D'Agostino, Adami, & Provenzani, 2013; Guo et al., 2016; Meisner et al., 2007; Wu et al., 2015; Zucal et al., 2015). MS-444 leads the small molecule inhibitors of HuR in citations and evaluation in different models and pathways. MS-444 is a small natural product produced by gram-positive bacteria

Micromonospora and chrysanthone-like in structure that was originally identified as an anti-tumorigenic, anti-inflammatory and anti-HIV agent (Aotani & Saitoh, 1995; Nakanishi, Chiba, Yano, Kawamoto, & Matsuda, 1995). MS-444 and a set of chemically related polyketides were identified as high-affinity small molecule HuR inhibitors by our collaborators (Novartis, Basel, Switzerland) (Meisner et al., 2007). Mechanistically, MS-444 has been shown to inhibit HuR homodimerization which prevents the binding of AREs (Meisner et al., 2007). This prevents translocation of HuR (and its associated mRNA cargos) to the cytoplasm. In a number of tumor cells, HuR inhibition by MS-444 leads to a dose-dependent reduction in cell proliferation by promoting apoptosis. Moreover, MS-444 inhibits HuR-dependent tumorigenic potential of colon cancer cells and GI tumorigenesis (Blanco, Preet, et al., 2016; Lang et al., 2017). While these studies provide proof-of-principal in small molecule targeting of HuR, limitations with MS-444 exist such as high IC50 values, large-scale drug solubility, and in vivo drug modifications (our personal observations). Other prominent small molecules have been described as HuR inhibitors include: CMLD-2, which maintains a coumarin-derived core and binds HuR to disrupt RNA binding (Wu et al., 2015); DHTS (15,16-dihydrotanshinone-I) derived from *Salvia miltiorrhiza*, is in the family of diterpenic tanshinones and inhibits the HuR-RNA complex formation (D'Agostino et al., 2015; Lal, Cerofolini, et al., 2017); and pyrvinium pamoate, an FDA-approved anthelmintic drug that blocks HuR nucleocytoplasmic translocation (Guo et al., 2016) and has been shown effectively reduce in vivo tumor growth for several different cancers, including pancreatic cancer (Esumi, Lu, Kurashima, & Hanaoka, 2004; Guo et al., 2016; Li et al., 2014; Lim et al., 2014; Sugimoto et al., 2015; Xu et al., 2013). Ongoing in vivo studies and chemical refinements of these and other compounds will aid in identifying a best-in-class molecule to test in the clinic.

7 | FUTURE TARGETING OF HUR

Working in collaboration with Genisphere LLC (Hatfield, PA), we are using the novel 3DNA dendrimer for siHuR delivery; it is unique both in structure and functional capabilities as compared to other nanomaterials (Huang et al., 2016). Its dendrimeric structure is built entirely from interconnected monomeric subunits of CpG-free DNA through a directed assembly process that yields a single molecular species. Derivatization of individual DNA arms with therapeutic payloads (e.g., siRNA, miRNA, DNA, or small molecules), targeting moieties (e.g., antibodies or ligands), and imaging tags allows for versatility in the design of nanocarriers having potential for a wide range of applications. The ability to easily modify the targeting moiety (e.g., targeting epidermal growth factor receptor [EGFR], transferrin) opens the possibility to move towards a realistic personalized approach. Current efforts have tested PDAC-specific targeting moieties for delivery of HuR-specific siRNAs and showed promising results both in vitro and in vivo indicating the feasibility of this novel targeting approach (Brody, Dixon laboratories unpublished).

8 | CONCLUSION

As the clinical medicine field continues to advance the concept of targeting a nonmutated gene or pathway, HuR is emerging as an attractive target in PDAC and other cancers. For example, HuR has been demonstrated to either be a marker for malignancy or have an

oncogenic role in numerous tumor systems including breast, ovarian, and colon (Heinonen et al., 2005; Huang et al., 2016; Young et al., 2009). As a means to halt tumorigenesis or treat cancer, HuR functions analogous to other gene regulatory mechanisms and proteins that govern these mechanisms, allowing it to be considered as a candidate target. However, the concept of targeting HuR is a new approach and many questions are still unanswered. For instance, what are the most critical HuR-target mRNAs in PDAC? Will targeting HuR be effective as a monotherapy or serve best in the context of combination therapy? How long lasting is the HuR pro-survival effect in a given tumor (e.g., minutes, hours, and days), and will inhibition of HuR be effective enough to compensate for this effect? Are the other elements that will compensate for HuR inhibition and render a PDAC cell resistant to this type of therapy? These and other questions are intriguing, but leave plenty for the field to explore and prioritize. As we and others continue to understand HuR's role in tumorigenesis and cancer cell survival, we strongly believe enough evidence exists to initiate therapeutic strategies, some described herein, to target this molecule in pancreatic cancer.

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Step 1: Identify PDAC-associated stressors that translocate HuR.

Step 2: Identify HuR targets related to the above identified stress.

Step3 : Demonstrate HuR and its target have a related role to the above identified stress.

FIGURE 1.

Framework for linking a pancreatic cancer-associated stress with a novel HuR target that confers a pro-survival pancreatic ductal adenocarcinoma (PDAC) phenotype

TABLE 1

Pancreatic cancer-associated stressors associated with HuR target mRNAs

PDAC stress	HuR target identified
Gemcitabine	dCK (deoxycytidine kinase)
Hypoxia	PIM1 (Pim-1 proto-oncogene, serine/threonine kinase)
Apoptosis	DR4/5 (TNF receptor superfamily member 10a and 10b; death receptors 4 and 5)
DNA damage	WEE1 (WEE1 G2 checkpoint kinase)
Low glucose	IDH1 (isocitrate dehydrogenase (NADP(+)) 1, cytosolic)
PARP inhibitor	PARG (poly(ADP-ribose) glycohydrolase)