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HuR function in disease

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1. ABSTRACT

The cytoplasmic events that control mammalian gene expression, primarily mRNA stability and translation, potently influence the cellular response to internal and external signals. The ubiquitous RNA-binding protein (RBP) HuR is one of the best-studied regulators of cytoplasmic mRNA fate. Through its post-transcriptional influence on specific target mRNAs, HuR can alter the cellular response to proliferative, stress, apoptotic, differentiation, senescence, inflammatory and immune stimuli. In light of its central role in important cellular functions, HuR's role in diseases in which these responses are aberrant is increasingly appreciated. Here, we review the mechanisms that control HuR function, its influence on target mRNAs, and how impairment in HuR-governed gene expression programs impact upon different disease processes. We focus on HuR's well-recognized implication in cancer and chronic inflammation, and discuss emerging studies linking HuR to cardiovascular, neurological, and muscular pathologies. We also discuss the progress, potential, and challenges of targeting HuR therapeutically.

2. INTRODUCTION

Mammalian gene expression is regulated at many levels, both transcriptional and post-transcriptional. Post-transcriptional gene regulation can occur at the levels of pre-mRNA splicing and maturation, as well as mRNA transport, editing, storage, stability, and translation (1, 2). Among these steps, the cytoplasmic control of mRNA turnover and translation is particularly effective in eliciting rapid adaptive changes in expressed proteins in response to environmental alterations and internal cues. The *t*urnover and *t*ranslation *r*egulatory *R*NA-*b*inding *p*roteins (TTR-RBPs) and noncoding RNAs (particularly microRNAs) are two main classes of *trans* factors that associate with specific *cis* elements present in mRNAs whose stability and translation are subject to regulation (3-5).

Some TTR-RBPs control one specific post-transcriptional process; for example tristetraprolin (TTP), butyrate response factor-1 (BRF1), and KH domain-containing RBP (KSRP) selectively accelerate mRNA degradation (6-9). However, most TTR-RBPs, including AU-binding factor 1 (AUF1), T-cell intracellular antigen-1 (TIA-1) and TIA-1-related (TIAR) proteins, polypyrimidine tract-binding protein (PTB), nuclear factor 90 (NF90), and other TTR-RBPs (10-14; reviewed in reference 15), can influence both mRNA turnover and translation. In addition, most TTR-RBPs often function jointly, cooperating, competing, or acting sequentially on shared target mRNAs. As many disease-associated proteins (e.g., tumor suppressors, oncoproteins, cell cycle factors, and cytokines) are encoded by mRNAs which bear TTR *cis* elements, their aberrant post-transcriptional expression in disease processes has been the focus of intense research over the past decade (see accompanying articles in this issue).

First described in Drosophila as *elav* (embryonic lethal abnormal vision), the mammalian Hu/elav family of TTR-RBPs comprises the ubiquitous HuR (HuA) and the primarily neuronal proteins HuB, HuC and HuD (16). The neuronal Hu proteins have been implicated in neuronal development, neuronal plasticity, and memory (reviewed in 17, 18). Since its identification in 1996 (19), HuR has been found to interact with dozens of mRNAs, many of them encoding proteins linked to specific pathologies. Although HuR was originally described as a stabilizing TTR-RBP (20), it was later shown to modulate the translation of target mRNAs, generally promoting translation, but sometimes inhibiting it (reviewed in 15, 16). The regulation of HuR function, the fate of [HuR-mRNA] ribonucleoprotein (RNP) complexes, and the impact of HuR-mediated gene regulation in disease processes are the focus of this review.

3. HuR TARGET mRNAs

Through its three RNA recognition motifs (RRMs), HuR interacts with target mRNAs. Many HuR target mRNAs bear U- and AU-rich elements in their 3'-untranslated region (UTR), where they are termed AREs, but HuR has also been found to interact with U- and AU-rich sequences in the 5'UTR of some target mRNAs (15, 16, 19-22). Although HuR is predominantly nuclear, its influence upon the expression of target mRNAs is linked to its localization in the cytoplasm, a process controlled by numerous transport mechanisms (see section 4.2).

3.1. Stabilized HuR target mRNAs

HuR stabilizes a large subset of target mRNAs, including many which encode proteins implicated in different pathologies, particularly cancer and inflammation. These mRNAs are the templates for proteins such as c-Fos, the cyclin-dependent kinase (cdk) inhibitor p21, cyclins (A2, B1, E1, D1), inducible nitric oxide synthase (iNOS), granulocyte macrophage-colony stimulating factor (GM-CSF), eukaryotic initiation factor (eIF)-4E, murine double minute (mdm)2, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, sirtuin 1 (SIRT1), tumor necrosis factor (TNF)-α, B-cell leukemia (Bcl)-2, myeloid leukemia cell differentiation protein (Mcl)-1, oncostatin M (OSM), cyclooxygenase (COX)-2, γ-glutamylcysteine synthetase heavy subunit (γ-GCSh), survival of motor neuron (SMN), SH2D1A, the regulator of G-protein signaling 4 (RGS4), parathyroid hormone-related protein (PTHrP), Fas ligand (FasL), Myogenin, MyoD, acetylcholinesterase (AChE), p53, ARHI [aplasia Ras homolog member I (DIRAS3)], nitric oxide/soluble guanylyl cyclase (sGC), urokinase plasminogen activator (uPA) and its receptor (uPAR), neurofibromatosis type 1 (NF1), von Hippel-Lindau protein (pVHL), toll-like receptor 4 (TLR4), Snail, matrix metalloprotease (MMP)-9, c-Fms, the mitogen-activated protein kinase (MAPK) phosphatase (MKP)-1, interferon (IFN)-γ, HuR itself, and interleukin (IL)-3, IL-4, IL-6, and IL-8 (Table 1 and discussed below). The exact mechanisms whereby HuR protects mRNAs from decay are unknown; however, binding of HuR to a target mRNA is widely believed to block the association of other TTR-RBPs or microRNAs (associated with the RNA-induced silencing complex or RISC) capable of recruiting the mRNA to sites of mRNA decay like the exosome or processing bodies (PBs) (e.g., 5, 23).

3.2. Translationally upregulated target mRNAs

HuR also promotes the translation of several target mRNAs encoding proteins which are involved in disease processes, including Cyclin A2, prothymosin α (ProTα), hypoxia-inducible factor (HIF)-1α, Bcl-2, VEGF, thrombospondin (TSP)-1, MKP-1, p53, the cationic amino acid transporter (CAT)-1, the intrinsic cellular caspase inhibitor XIAP, and cytochrome *c* (24, 25, Table 1 and discussed below). It is also unclear how HuR promotes the translation of each of these target mRNAs, but HuR was recently shown to associate with the internal ribosome entry site (IRES) of the *XIAP* 5'UTR and directly enhanced XIAP translation (24). Models of HuR-elicited exclusion of translational repressors (other TTR-RBPs or microRNA/RISC) from target mRNAs have been reported in some instances of translational upregulation by HuR [e.g., *CAT-1* and *cytochrome* c mRNAs (25-27)].

3.3. Translationally repressed target mRNAs

HuR inhibits the translation of a small subset of target mRNAs that encode disease-associated proteins.

Table 1. Influence of HuR upon target mRNAs involved in disease processes

HuR target mRNA	Influence of HuR on mRNA	Processes Regulated	Cell/Disease Model	References
c-Fos	↑ Stability	Proliferation	Oral squamous carcinoma	(109)
c-Myc	↓ Translation	Proliferation, survival	Cervical carcinoma	(31)
p21	↑ Stability	Proliferation, survival	Carcinoma (breast, colon)	(68, 69)
p27	↓ Translation	Proliferation, survival	Cervical carcinoma	(28)
cyclin A2	↑ Stability ↑ Translation	Proliferation	Carcinoma (colon, gastric, oral)	(66, 107, 109)
cyclin B1	↑ Stability	Proliferation	Oral carcinoma	(109)
cyclin E1	↑ Stability	Proliferation	Breast carcinoma	(86)
cyclin D1	↑ Stability	Proliferation	Carcinoma (oral, colon)	(109, 24)
OSM	↑ Stability	Proliferation	Lymphoma	(117)
eIF4E	↑ Stability	Proliferation, survival	Pharyngeal carcinoma	(67)
EGF	↑ Stability	Proliferation	Prostate carcinoma	(98)
VEGF	↑ Stability ↑ Translation	Angiogenesis, proliferation	Carcinoma (colon, non-small cell lung, kidney, pancreatic, prostate), glioma, meningioma, astrocytoma, ischemia, amyotrophic lateral sclerosis)	(59, 63, 102, 104, 105, 150, 154,155, 168)
HIF-1α	↑ Stability ↑ Translation	Angiogenesis, survival	Cervical carcinoma, ischemia	(70, 150)
COX-2	↑ Stability	Angiogenesis, survival, inflammation	Carcinoma (colon, ovarian, gastric, oral, prostate), central nervous system malignancies, rheumatoid cartilage, osteoarthritic cartilage, inflammatory, bowel disease	(63, 64, 93-95, 97, 100, 106, 109)
iNOS	↑ Stability	Angiogenesis, survival, inflammation	Colon carcinoma, muscle wasting	(121, 123)
TSP1*	↑ Translation	Angiogenesis	Breast carcinoma	(73)
TGF-β	↑ (n.d.)	Immunity, inflammation	Tumors of the central nervous system	(63)
MKP-1	↑ Stability ↑ Translation	Signaling, immunity	Cervical carcinoma	(74)
Mdm2	↑ Stability	Survival	Intestinal epithelium function	(170)
SIRT1	↑ Stability	Survival, stem cell development	Carcinoma (cervical, prostate)	(56, 97)
Bcl-2	↑ Stability ↑ Translation	Survival	Carcinoma (cervical, prostate, epidermoid), leukemia, ischemia-reperfusion injury	(71, 72, 151)
Mcl-1	↑ (n.d.)	Survival	Cervical carcinoma	(71)
XIAP	↑ Translation	Survival	Untransformed cells	(24)
Cyto c	↑ Translation	Survival	Cervical carcinoma	(27)
uPA	↑ Stability	Invasion, migration	Breast carcinoma	(80)
uPAR	↑ Stability	Invasion, migration	Breast carcinoma	(80)
MMP-9	↑ Stability	Invasion	Fibrosarcoma, myeloid leukemia, fibrosis, left ventricular function and remodeling	(81, 82, 128)
Snail	↑ Stability	Invasion	Breast carcinoma	(83)
dCK	↑ (n.d.)	Chemotherapy	Pancreatic carcinoma	(92)
ARHI/DRAS3*	↑ Stability	Tumor suppression	Ovarian carcinoma	(94)
p53	↑ Stability ↑ Translation	Tumor suppression	Carcinoma (cervical, gastric, liver, colon), intestinal epithelium function, myocardial infarction	(25, 171)
pVHL	↑ Stability	Tumor suppression	VHL syndrome, kidney carcinoma	(40, 173)
BRCA1	(↓) n.d.	Tumor suppression	Breast carcinoma	(174)
ER	↑ Stability	Tumorigenesis	Breast carcinoma	(175)
Wnt5a	↓ Translation	Tumorigenesis	Breast carcinoma	(90)
c-Fms	↑ Stability	Tumorigenesis	Breast carcinoma	(87)
GATA3	↑ Stability	Tumorigenesis	Breast carcinoma	(176)
GM-CSF	↑ Stability	Inflammation, immunity	Asthma, T cell activation, atherogenesis	(115, 138)
TNF-α	↑ Stability	Inflammation, immunity	Muscle wasting, malignant glioma, rheumatoid arthritis, atherosclerosis	(63, 115, 135)
TM	↓ Translation	Inflammation	Sepsis	(30)
RGS4	↑ Stability	Inflammation	Smooth muscle contraction, cardiac development	(119)
TLR4	↑ Stability	Inflammation, immunity	Vascular smooth muscle hyperplasia	(139)
IL-6	↑ Stability	Inflammation, immunity	Tumors of the central nervous system, viral infection, atherosclerosis	(63, 111, 113)
IL-8	↑ Stability	Inflammation, immunity	Carcinoma (breast, colon, gastric), glioma	(63, 64, 89)
IL-13	↑ Stability	Inflammation	Allergy	(132)
SMN	↑ Stability	Neuropathology	Spinal muscle atrophy	(156)
SH2D1A	↑ Stability	Proliferation	X-linked lymphoproliferative disease	(163)
NF1	↑ Stability	Signaling	Neurofibromatosis	(153)
PROX1	↑ Stability	Endothelial differentiation	Kaposi's sarcoma	(143)
Eotaxin	↑ Stability	Inflammation	Asthma	(118)
ProTα	↑ Translation	Tumorigenesis	Cervical carcinoma	(177)
IGF-1R	↓ Translation	Proliferation	Breast carcinoma	(29)
HuR	↑ Stability ↑ Translation	(above processes)	(above cell/disease models)	(3, 34, 35)

Listed are HuR target mRNAs encoding proteins linked to disease (column 1), the influence of HuR on mRNA stability and/or translation [enhanced (↑) or reduced, (↓), column 2], the cellular processes affected by the HuR-mRNA interactions (column 3), and the cell model or disease model in which [HuR-mRNA] regulation has been studied (column 4). In column 1, * indicates mRNAs whose regulation by HuR primarily involves dissociation of HuR from the mRNA. See text for further details.

HuR binds to the 5'UTRs of *p27*, *IGF-1R*, and thrombomodulin (*TM*) mRNAs and represses their translation; this inhibitory action was proposed to result from disruption of IRESs in these 5'UTRs (28-30). HuR was also found to bind to the 3'UTRs of *Wnt5a* and *c-Myc* mRNAs and repress their translation; the mechanism of Wnt5a repression is not yet known, but the reduced translation of c-Myc was linked to HuR's recruitment of the let-7/RISC complex to the *c-Myc* 3'UTR (31).

4. REGULATION OF HuR FUNCTION

The function of HuR is controlled at multiple levels. The initial studies focused on HuR cytoplasmic export as a critical way to control expression of HuR target mRNAs, but recent work has revealed that the abundance and integrity of HuR protein, as well as post-translational modifications affecting HuR binding to mRNAs all potently influence HuR function (Figure 1).

4.1. Regulation of HuR abundance

The steady-state levels of HuR protein are regulated in a number of ways. The transcriptional control of HuR expression is poorly understood, but HuR transcription is positively regulated by the nuclear factor (NF)-κB (32) and by Smads (33). By contrast, the abundance of HuR mRNA and HuR protein are subject to multiple regulatory mechanisms (Figure 1).

4.1.1. HuR auto-regulation

HuR binds the *HuR* mRNA, in keeping with the ability of many TTR-RBPs to associate with the very mRNAs that encode them (3). Among different polyadenylation variants of the *HuR* mRNA, HuR was found to bind to and stabilize a long *HuR* mRNA bearing a distal AU-rich element; this effect that was opposed by the mRNA decay-promotion actions of TTP (34). HuR binding to the *HuR* 3'UTR also enhanced the cytoplasmic export of the *HuR* mRNA (35).

4.1.2. Downregulation of HuR by microRNAs

The *HuR* mRNA is the target of two microRNAs. miR-519 was computationally predicted to associate with the *HuR* mRNA coding region (CR) and a distal segment of the *HuR* 3'UTR, but only the CR interaction was functional (36). Acting upon the *HuR* CR, miR-519 selectively repressed HuR translation; in turn, cells which overexpressed miR-519 showed reduced cell proliferation in culture, displayed features of cellular senescence, and developed into significantly smaller tumors in a xenograft model (37, 38). miR-125a associated with the *HuR* 3'UTR and similarly repressed HuR production by inhibiting HuR translation. In breast cancer cells, miR-125a overexpression enhanced apoptosis and suppressed cell proliferation and cell migration (39).

4.1.3. HuR ubiquitination

In response to moderate heat shock, HuR protein levels declined transiently. This reduction was linked to HuR ubiquitination at residue Lys-182 followed by proteasome-mediated proteolysis. The transient degradation of HuR, which enhanced cell survival after heat shock, was antagonized by phosphorylation of HuR by Chk2 (40).

4.1.4. Caspase-mediated HuR cleavage

Recently, cleavage of HuR at Asp-226 was identified as a key component of the apoptotic cell death program (41). In response to lethal damage by staurosporine, HuR cleavage involved the apoptotic proteins FADD, caspase-8, and caspase-3 (42). In muscle cells, the larger HuR cleavage product (a 24-kDa fragment named CP1) was shown to bind to transportin 2 and to block the nuclear import of HuR, thereby promoting myogenesis (43).

4.2. Regulation of HuR localization

Although HuR is predominantly nuclear, its nuclear function is largely unknown except for a poorly defined role in pre-mRNA splicing, as shown for the pre-RNAs encoding Fas and HuD (44, 45). The transport of HuR across the nuclear envelope requires a specific HuR domain (the HuR nucleocytoplasmic shuttling domain or HNS) and several transport machinery components, including transportins 1 and 2, the chromosome region maintenance 1 (CRM1), and importin-1α (46-49). HuR nucleocytoplasmic transport is also influenced by kinases [Cdk1, AMP-activated protein kinase (AMPK), PKC, and p38] that phosphorylate HuR and HuR transport proteins (50-55), as explained in 4.3. and shown in Figure 1.

4.3. Post-translational modification of HuR

Phosphorylation of HuR at different residues by a number of kinases as well as methylation by the methyltransferase CARM1 affect the subcellular localization of HuR and/or its interaction with target mRNAs. In general, modification of residues within the RRMs affect HuR binding to target mRNAs, while modification of residues within or near the HNS alter HuR subcellular localization (Figure 1).

4.3.1. Chk2

Phosphorylation by the checkpoint kinase Chk2 at HuR residues Ser-88, Ser-100, and Thr-118 (located between and within RRM1 and RRM2) modulates HuR binding to several target mRNAs. Oxidative damage activated Chk2, which in turn phosphorylates Ser-100, triggering the dissociation of HuR from *SIRT1* mRNA and other mRNAs (56).

4.3.2. Cdk1

Also known as Cdc2, this kinase phosphorylates HuR at Ser-202, triggering the nuclear retention of HuR mediated by nuclear 14-3-3θ, which interacts with HuR. Under conditions of stress, Cdk1 is inactive, the Ser-202 residue of HuR is unphosphorylated, and the protein can be mobilized to the cytoplasm (53).

4.3.3. PKC

HuR is a substrate for protein kinase C. Phosphorylation by PKCα at HuR Ser-158 and Ser-221 in response to ATP treatment, and phosphorylation by PKCδ of Ser-221 and Ser-318 in response to angiotensin II (AngII) have been shown to promote the cytoplasmic

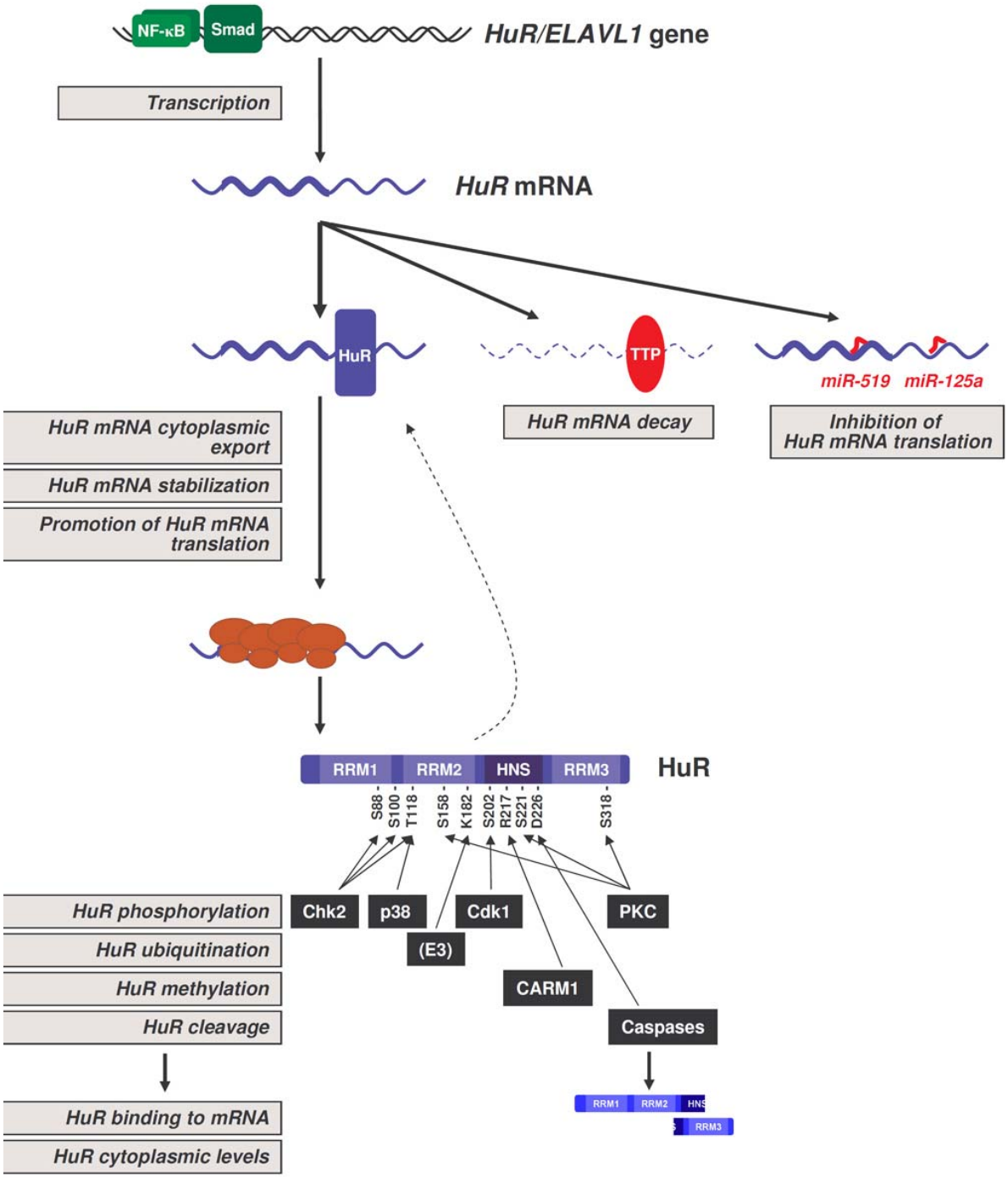

Figure 1. Regulation of HuR expression and function. The schematic depicts the current understanding of HuR regulation. Transcription of the *HuR/ELAVL1* gene is controlled by the transcription factor NF-κB. The *HuR* mRNA is positively regulated by enhanced export to the cytoplasm, stabilization, and enhanced translation but HuR itself; the *HuR* mRNA is negatively regulated by TTR-RBP tristetraprolin (TTP), which promotes *HuR* mRNA decay, and by microRNAs miR-125a and miR-519, which repress HuR translation. HuR protein is subject to phosphorylation by Chk2, which affects [HuR-mRNA] interactions, by Cdk1, which affects HuR levels in the cytoplasm, and by p38 and PKC, which affect both [HuR-mRNA] interactions and cytoplasmic HuR levels. Methylation by CARM1 can also affect HuR subcellular distribution and binding to mRNAs. Ubiquitination of HuR by an as-yet unknown E3 ligase controls HuR protein stability, and caspases can cleave HuR into two fragments with different cellular properties. Gray squares indicate steps in which HuR expression or function are regulated. See text for further details.

export and the binding activity of HuR (50, 51, 57, 58). PKCβ was also recently shown to phosphorylate HuR in a model of diabetic retinopathy, although the specific residues were not identified (59). These regulatory processes were associated with an increase in the expression of HuR target mRNAs encoding angiogenic, proliferative and proinflammatory proteins like cyclins, VEGF, and COX-2.

4.3.4. p38^{MAPK}

In cells exposed to DNA damage (γ irradiation), this MAPK phosphorylates HuR at Thr-118, triggering its translocation to the cytoplasm and increasing its association with *p21* mRNA (60).

4.3.5. CARM1

HuR methylation at Asp-271 by CARM1 (coactivator-associated arginine methyltransferase 1) in response to lipopolysaccharide stimulation of macrophages results in stabilization of *TNF-α* mRNA (61).

5. HuR IN CANCER

With the discovery that HuD was an antigen in paraneoplastic encephalomyelitis associated in patients with small-cell lung cancer (62), Hu/ELAV proteins were among the first TTR-RBPs found to be implicated in carcinogenesis. The earliest reports of HuR being elevated cancer were from the King and Prescott laboratories; their findings of upregulated HuR in brain and colon cancers were linked to the enhanced expression of COX-2, VEGF, TGF-β, IL-8, and other cancer-associated proteins (63, 64). Subsequent studies revealed that HuR was broadly elevated in virtually all malignancies tested, including cancers of the breast, colon, stomach, kidney, pancreas, esophagus, prostate, skin, lung, and thyroid (65). HuR was proposed to play a causal role in tumor development, since cultured carcinoma cells with ectopically elevated HuR developed into larger tumors in a mouse xenograft model, while forced reduction of HuR reduced the tumor size (38, 65). The work of numerous laboratories has led to the identification of dozens of additional HuR target mRNAs encoding cancer-related proteins (as reviewed in 22).

5.1. HuR modulates cancer traits

HuR binds to many mRNAs that encode proteins responsible for implementing five major cancer-associated phenotypes: enhanced cell proliferation, increased cell survival, elevated local angiogenesis, evasion of immune recognition, and facilitated cancer cell invasion and metastasis (22).

5.1.1. Enhanced proliferation

For a tumor to grow, cells must divide actively. Many HuR target mRNAs encode proteins implicated in cell cycle progression and cell division. In this manner, HuR promotes the expression of several cyclins (D1, E1, A2, B1) and other factors that enhance cell proliferation [c-Fos, epithelial growth factor (EGF), ProTα, eIF4E]. Additionally, HuR represses expression of proteins with growth inhibitory roles, such as p27 and Wnt5a. HuR was linked to elevation in these proteins in numerous malignancies, including cervical, colorectal, breast, and ovarian cancers (66, 67; reviewed in 22).

5.1.2. Anti-apoptotic phenotype

Tumor cells must also acquire resistance to death signals. HuR associates with and promotes the expression of numerous mRNAs that encode pro-survival proteins, as shown for ProTα, Bcl-2, Mcl-1, SIRT1, p21, XIAP, and Mdm2 in various tumor types (22, 24, 68-72). Moreover, HuR binds to the *c-Myc* mRNA, and represses the synthesis of the encoded protein c-Myc, which can have a pro-apoptotic function (31).

5.1.3. Increased angiogenesis

The development of local vasculature delivers oxygen and nutrients needed for the tumor to thrive. HuR interacts with the mRNAs that encode the pro-angiogenic factors HIF-1α, VEGF, and COX-2 and promotes their expression (22, 70). HuR can also associate with the mRNA that encodes TSP1, an inhibitor of angiogenesis, but this interaction declines in a model of breast carcinogenesis (73).

5.1.4. Reduced immunosurveillance

As the immune system can eliminate tumor cells, escaping immune recognition is advantageous for tumor cells. By binding to the *MKP-1* mRNA and potently enhancing MKP-1 expression (70), HuR could suppress the function of immune cells, a key action of MKP-1 (75, 76). Another important HuR target, *TGF-β* mRNA, encodes a cytokine that enables advanced-stage tumor cells to escape immune recognition (77-79).

5.1.5. Invasion and metastasis

Tumor cells will often invade adjacent tissues and colonize distant organs. HuR promotes the expression of extracellular proteases and proteins which alter the interaction of the cancer cell with its local environment and can promote epithelial-to-mesenchymal transition. Examples include HuR target mRNAs encoding Snail, MMP-9, uPA and the uPA receptor (22, 80-83).

5.2. Implication of HuR in specific cancer types

HuR interacts with and regulates many mRNAs encoding cancer-related proteins. Since HuR is upregulated in virtually all cancer types (65), it has been proposed to coordinate the expression of cancer genes and thereby impact upon phenotypic traits central to tumorigenesis (section 5.1). Over the past decade, numerous studies have examined the levels of HuR in individual cancers and cancer cell models (Table 1).

In breast carcinomas, elevated cytoplasmic HuR levels were associated with tumor grade and poor patient outcome (83, 85). In breast cancer cells, HuR increased expression of cyclin E1, IL-8, estrogen receptor, TSP1, and c-Fms (73, 86-89), while HuR repressed the translation of Wnt5a, a protein that inhibits tumor growth (90). In pancreatic cancer, high HuR levels correlated with high levels of VEGF (91) and with poor patient prognosis (92); paradoxically, however, high HuR was associated with improved survival of patients treated with the

chemotherapeutic drug gemcitabine, as discussed below (section 8.). This effect was linked to HuR's interaction with the deoxycytidine kinase (*dCK*) mRNA, leading to the increased expression of dCK, an enzyme that activates gemcitabine into an active drug (92).

The high abundance of HuR in colon cancer contributed to the increased expression of COX-2 and VEGF levels and was associated with advanced tumor stage (64, 93). Overexpression of HuR increased the growth of colon cancer cells in an athymic mouse xenograft model (65). COX-2 levels were also upregulated in ovarian carcinomas, where both nuclear and cytoplasmic HuR were found to be elevated. Interestingly, HuR association with the *ARHI/DRAS3* mRNA, which encodes a tumor suppressor, was reduced in ovarian cells (94). As in other tumors, increased HuR levels were associated with high ovarian tumor grade and poor prognosis (95, 96). In prostate cancers, HuR abundance was linked to increased levels of COX-2, prostate-specific antigen (PSA), SIRT1, and EGF (97-99). In keeping with the view that cytoplasmic HuR promotes prostate tumor development and relapse, patients who had elevated cytoplasmic HuR expressed higher COX-2 and adverse prognosis with shorter disease-free survival times (97, 100). HuR was also upregulated in oral, lung, gastric, and pharyngeal carcinomas, as well as in cancers of the central nervous system (e.g., meningioma, glioma, astrocytoma), where COX-2, c-Fos, VEGF, eIF4E, cyclin D1, cyclin A, and other HuR target mRNAs were found to be elevated (63, 67, 101-109).

6. HuR IN INFLAMMATION

HuR has been implicated in promoting inflammation and inflammatory diseases. The pro-inflammatory influence of HuR is linked to its interaction with mRNAs encoding pro-inflammatory proteins, leading to their increased production in a variety of cell types. In addition, HuR function was notably inhibited by anti-inflammatory factors (Table 1).

6.1. HuR promotes expression of pro-inflammatory factors

HuR associates with several mRNAs encoding pro-inflammatory cytokines, most prominently TNF-α and IL-6, stabilizes them and promotes the expression of the encoded proteins in different cell types, including fibroblasts, T-cells, and macrophages (63, 64, 110-113). In macrophages, endothelial cells, intestinal epithelial cells, and in colon, gastric and cervical carcinoma cells, HuR binds to mRNAs encoding the proinflammatory cytokines IL-8, TGF-β, and IFN-γ, and enhances their expression (63, 64, 92, 112, 114-116). HuR interacts with the oncostatin M (*OSM*) mRNA in lymphoma cells and induces expression of the encoded pro-inflammatory cytokine OSM (117) and with the *eotaxin* mRNA in lung epithelial cells, where it promotes expression of the encoded chemokine (118). Other proteins that modulate inflammatory responses, such as the G-protein signaling RGS4, are also regulated by HuR (119).

HuR binds to and stabilizes the mRNAs that encode two major pro-inflammatory mediators, the enzymes COX-2 and iNOS. An inducible enzyme, COX-2 catalyzes the conversion of arachidonic acid into prostanoids (including prostaglandins, prostacyclin and thromboxane) in many different cell types. HuR controls COX-2 expression in macrophages, renal mesangial cells, and many cancers, including carcinomas of the colon, breast, ovaries, prostate, larynx, and stomach (63, 64, 120, 121). Also an inducible enzyme, iNOS catalyzes the conversion of L-arginine into nitric oxide (NO), a major trigger of inflammation. Interestingly, NO in turn promotes the cytoplasmic localization of HuR and its association with target mRNAs (74). HuR enhances iNOS expression in muscle cells, in hepatocytes, and in carcinomas of the lung and colon (122-124). Besides sharing a common upstream regulatory (HuR), COX-2 and iNOS are functionally interconnected in different ways (125). In addition to inducing the expression of pro-inflammatory factors, HuR inhibits the production of anti-inflammatory proteins such as thrombomodulin (30); this effect was linked to the HuR-mediated disruption of the *TM* 5'UTR IRES (30).

6.2. HuR function inhibited by anti-inflammatory factors

Some anti-inflammatory cytokines can repress HuR function. IL-10, also known as human cytokine synthesis inhibitory factor (SCIF), is a pleiotropic cytokine that can potently repress inflammation (126, 127). As shown by the Kishore laboratory, IL-10 inhibits inflammation at least partly by repressing the HuR-mediated stabilization of mRNAs encoding proinflammatory cytokines in monocytes and inflammatory cells in the myocardium (128, 129). Similarly, the anti-inflammatory cytokine IL-19 can lower HuR abundance and repress HuR function, thereby reducing the inflammatory response of vascular smooth muscle cells (VSMCs) to injury (130). In a related regulatory paradigm, HuR binds to *IL-4* mRNA and promotes IL-4 expression in T-cells (131). Since IL-4 promotes the activation of repair macrophages which secrete IL-10, an inhibitor of inflammation, perhaps IL-4 can function as a negative feedback loop to shut off the production of pro-inflammatory factors by HuR. The biological effects of IL-4 are linked to the activity of transcription factor, signal transducer and activator of transcription 6 (STAT6); like IL-4, IL-13 is encoded by another HuR target mRNA and can also suppress pro-inflammatory responses and activate STAT6 (132, 133).

6.3. Implication of HuR in specific inflammatory diseases

Given its positive influence on the expression of inflammatory proteins, HuR has been implicated in different disease states. The involvement of HuR in rheumatoid arthritis was suggested based on its promotion of TNF-α expression (134, 135), while HuR-regulated COX-2 was associated with the development of rheumatoid and osteoarthritic cartilage (136). The upregulation of COX-2 by HuR in colonic epithelium was also linked to inflammatory bowel disease (137). In patients treated with

human immunodeficiency virus (HIV) protease inhibitors (PIs), the chronic inflammatory disease atherosclerosis was linked to the HuR-mediated upregulation of TNF-α and IL-6 in response to HIV PI therapy (113). Also a chronic inflammatory disease, asthma has been linked to HuR function through its upregulation of factors like TNF-α, GM-CSF (138). In response to lipopolisaccharide (LPS), HuR upregulation of toll-like receptor 4 (TLR4) mRNA was linked to hyperplasia of vascular smooth muscle cells in a model of vascular inflammation. (139). Although the vast majority of evidence supports a role for HuR in promoting inflammation, one study of HuR overexpression in mouse macrophages suggests that HuR may be anti-inflammatory (140).

7. HuR IN OTHER DISEASE PROCESSES

Many studies implicating HuR in additional diseases are rapidly emerging. In most of these, HuR elicits phenotypic traits previously described in sections 5 and 6, including the promotion of inflammation, proliferation, angiogenesis, and resistance to apoptosis.

7.1. Virus replication and infection

The alphavirus Sindbis virus expresses many U-rich RNAs and recruitment of HuR to these sequences helps to express viral proteins and is necessary to maintain a high viral yield and a productive viral infection (141). Yeast-two hybrid analysis identified HuR as a protein that interacted with the HIV enzyme reverse transcriptase (RT); this association was found to be necessary for successful HIV infection (142). The Kaposi's sarcoma herpes virus (KSHV) protein kaposin-B increased the cytoplasmic levels of HuR; in turn, HuR associated with the cellular mRNA encoding PROX1 (prospero homeobox 1), a key protein involved in the reprogramming of lymphatic endothelial cells (143). HuR also associated with the hepatitis C virus RNA, although the consequences of this interaction were not reported (144).

7.2. Cardiovascular disease

HuR was shown to associate with the β(2)-adrenergic receptor mRNA (145). Since HuR also associates with mRNAs that encode angiotensin receptors, iNOS, COX-2, and TNF-α, its putative involvement in cardiovascular diseases such as heart failure, myocardial infarction and hypertension has been long recognized (146). HuR binds to the mRNAs encoding the soluble guanylyl cyclase (sGC)-α1 and sGC-β1 and enhances their expression. In models of hypoxia-induced and spontaneous hypertension, the reduced expression of sGC was proposed to be due to the reduced ability of HuR to form complexes with the *sGCα* and *sGCβ* mRNAs (147-149). In an animal model of hypoxic adaptation involving enhanced angiogenesis, the levels of HuR, as well as those of its targets VEGF and HIF-1α were significantly upregulated; these observations prompted the authors to suggest a role for HuR in ischemia (150). The influence of HuR on Bcl-2 expression was also implicated in ischemia-reperfusion injury in the kidney (151). Elevated HuR levels were also detected in several vascular pathologies, including intimal hyperplasia, atherosclerosis, sclerosis of venous graft, and fibromuscular dysplasia (152).

7.3. Neurological pathologies

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease caused by deficiency of the *NF1* gene, which encodes the tumor suppressor protein neurofibromin. HuR was reported to interact with the 3'UTR of the *NF1* mRNA and was thereby proposed to regulate neurofibromin abundance post-transcriptionally (153).

Amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), a neurodegenerative disease caused by the degeneration of motor neurons, has been linked to mutations in copper-zinc superoxide dismutase 1 (SOD1). Mutant SOD1 competes with HuR for binding to the *VEGF* 3'UTR and leads to sequestration of HuR in RNA aggregates, effectively impeding HuR from enhancing the production of VEGF, a neuroprotective factor (154, 155).

Another neurodegenerative disease, spinal muscle atrophy, is characterized by the loss of alpha motor neurons leading to progressing muscle atrophy. The disease arises from mutation or deletion of the *SMN1* gene, which encodes the protein SMN (survival of motor neuron). Recent efforts to develop therapies that compensate for the loss of SMN1, aimed at expressing SMN from the *SMN2* gene, have revealed that p38 promotes HuR binding to the *SMN* mRNA, resulting in its stabilization (156).

Paraneoplastic encephalomyelitis is a disorder that causes inflammation of the central nervous system associated with a distant cancer, often small-cell lung carcinoma. The serologic hallmark of paraneoplastic encephalomyelitis is the presence of anti-Hu auto-antibodies which recognize the three neuronal Hu/ELAV proteins (HuB, HuC, and HuD), but also reacts against HuR (157, 158). As all Hu/ELAV proteins are highly abundant in the nervous system, the pathogenesis of this disorder is considered to be autoimmune.

7.4. Muscular disorders

A number of muscle pathologies have been linked to HuR function. Inclusion body myositis (IBM) is one of a group of muscle diseases known as inflammatory myopathies characterized by chronic, progressive muscle inflammation accompanied by muscle weakness. In muscle fibers from IBM patients, both the poly(A)-binding protein 1 and HuR were found to aggregate in RNA deposits; these observations were suggested to reflect an impairment in mRNA turnover and translation in IBM (159).

Muscle wasting (cachexia) is characterized by an excessive loss in skeletal muscle mass. It is often seen in patients with cancer, AIDS, congestive heart failure, and obstructive lung disease. The onset of cachexia is linked to the activation of transcription factors NF-κB and STAT1 by inflammatory cytokines TNF-α and IFN-γ, causing the transcriptional upregulation of *iNOS* mRNA. As reported by Gallouzi and colleagues, TNF-α and IFN-γ trigger the

association of HuR with *iNOS* mRNA, enhancing *iNOS* mRNA stability and promoting iNOS synthesis and NO production (122). NO accelerates the loss of MyoD, likely resulting from a reduction in the transcription of the *MyoD* gene or the stability of *MyoD* mRNA mediated by decay-promoting TTR-RBPs. While these observations indicate that HuR participates in cytokine-induced muscle wasting, other studies have shown that HuR associates with *MyoD* mRNA and promotes MyoD expression during myogenesis (160, 161). Therefore, in response to different extracellular stimuli, HuR can either promote muscle formation (160, 161) or trigger muscle decay (122); these distinct responses may be mediated by specific posttranslational modifications of HuR (section 4) and/or by the influence of other as-yet unknown post-transcriptional factors. It is interesting to note that treatment with NO triggers the association of HuR with numerous target mRNAs (162).

7.5. Lymphoproliferative disease

The X-linked lymphoproliferative disease (XLP) is a rare immunodeficiency condition characterized by frequent childhood mononucleosis triggered by Epstein-Barr virus (EBV), followed by hypogammaglobulinemia and a markedly higher risk of lymphoma and other lymphoproliferative diseases. The *SH2D1A* gene, which is altered or deleted in XLP patients, encodes the small protein SAP that is expressed in T and NK cells. HuR was shown to interact with the *SH2D1A* 3'UTR and was proposed to contribute to its stabilization (163).

8. HuR A THERAPEUTIC TARGET? CONCLUDING REMARKS

The heightened function of HuR in virtually all cancers examined to-date suggests that HuR could be a useful marker for cancer diagnosis. Indeed, the levels of HuR, its cytoplasmic abundance, and its binding to mRNAs are all regulated by several factors (Chk2, PKC, CARM1, Cdk1, p38, caspases, microRNAs) implicated in cancer. In addition, HuR appears to be a valuable prognostic indicator, since the vast majority of studies show significant correlations between HuR abundance in cancer and poor patient outcome.

Given HuR's capacity to promote protein expression programs advantageous to cancer cells (e.g., proliferative, pro-angiogenic, and pro-survival), HuR might also represent a useful therapeutic target. Interventions to modulate HuR kinases could be fruitful, although PKC, Cdc2, Cdk1, and p38 are broad-spectrum kinases which affect many cellular processes. Approaches to decrease HuR levels by small interfering (si)RNA or microRNAs are effective in cultured cells and could be attempted in tumors, provided that they are sufficiently specific. Mukherjee and colleagues recently reported that resveratrol triggered changes in the subcellular localization of HuR, and further implicated HuR in the changes in gene expression elicited by resveratrol (164). Small chemical inhibitors of HuR have also been reported, but their usefulness in organisms remains untested (165, 166).

Besides considering how targeting HuR might inhibit or reduce tumorigenesis, it is important to keep in mind that most studies on HuR and cancer have not examined how HuR affects anticancer therapy. In pancreatic cancer, the elevated presence of cytoplasmic HuR in pancreatic cancer cells paradoxically correlated with better prognosis for patients treated with the standard drug of choice, gemcitabine; the finding that HuR increased the expression of dCK, which metabolizes and thus activates gemcitabine, partly explained why elevated HuR correlated with positive response to therapy (92). Similarly, low levels of HuR were associated with high risk of breast cancer recurrence, although the specific mediators of this effect were not identified (167). Therefore, interventions to reduce HuR function should be designed carefully, since in some cases, the elevated presence of HuR may be advantageous for other therapies. It is possible to envision therapeutic regimens in which it is more appropriate to lower HuR *after* use of conventional chemotherapies whose effectiveness may rely, at least in part, on functional HuR.

Strategies to inhibit HuR function could also be beneficial for treating chronic inflammatory diseases. The ability of HuR to induce major proinflammatory factors, including TNF-α, IL-6, COX-2, suggests that lowering HuR levels or function might decrease inflammatory conditions. However, thus far, there has been little work assessing directly the usefulness of HuR as a therapeutic target in chronic inflammatory diseases. In one study, HuR was implicated in the response of rheumatoid arthritis patients to the drug infliximab, although only *HuR* mRNA levels were measured, it is unclear if HuR protein levels and HuR binding to mRNAs encoding inflammatory factors followed the same expression pattern (134). It is also important to remember that TNF-α and other pro-inflammatory factors are necessary for clearing infections, so like with cancer therapy, HuR-directed interventions to avoid tissue damage during chronic inflammation must be devised thoughtfully.

The studies examining the therapeutic potential of HuR in other diseases are also still very limited. Patients receiving immunosuppressive therapy show high cancer incidence. Some of these therapies can mobilize HuR to the cytoplasm causing increases in expression of VEGF and other pro-tumorigenic proteins, suggesting that targeting HuR could lower the development and aggressiveness of cancers in patients undergoing immunosuppressive therapy (168).

In closing, while the use of cultured cells has advanced greatly our understanding of HuR function and influence on proteins associated with cancer, chronic inflammation, and other diseases, more efforts must now focus on mammalian models of disease. HuR overexpression in mouse macrophages suggested a systemic anti-inflammatory role for HuR, but HuR-null thymocytes showed aberrant cell division cycle, activation, selection, and survival (169). A role for HuR in the establishment of a physiologic thymocyte pool was confirmed in another mouse model with inducible global HuR-null phenotype, which showed widespread death of progenitor cells in hematopoietic organs and in the

intestinal epithelium (170). While the mouse phenotypes generally agree with HuR's roles in proliferation and survival, more studies are needed to fully understand the influence of HuR on tumorigenesis, chronic inflammatory diseases, and other human pathologies.

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Abbreviations: ARE, AU-rich element; CR, coding region; TTR-RBP, turnover and translation regulatory RNA-binding protein; UTR, untranslated region

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