Targeting the multifaceted HuR protein, benefits and caveats

Chiara Zucal[1*], Vito D'Agostino[1*], Rosa Loffredo[1], Barbara Mantelli[1], NatthakanThongon[1], Preet Lal[1], Elisa Latorre[1,2], Alessandro Provenzani[1+]

1 Laboratory of Genomic Screening, Centre for Integrative Biology, University of Trento, (CIBIO), Trento, Italy
2 Pharmacology Laboratory, Health Sciences Department, University of Milan, Italy

*These authors contributed equally

[+]*Corresponding author:*
Alessandro Provenzani
Laboratory of Genomic Screening, Centre for Integrative Biology (CIBIO),
University of Trento,
Via delle regole 101, 38123
Mattarello, Trento, ITALY
Phone: +39 0461 283094
Fax: +39 0461 283937
Email: alessandro.provenzani@unitn.it

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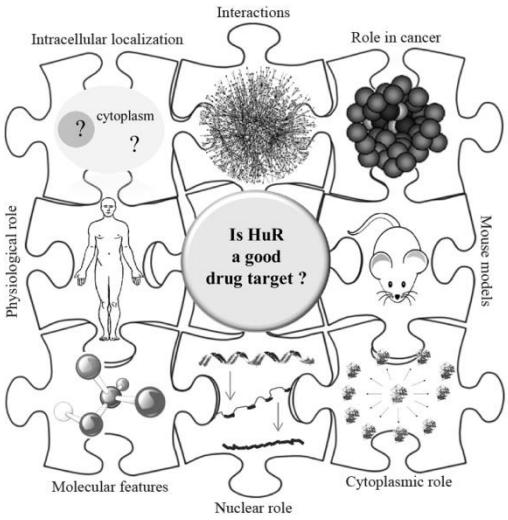
Abstract

The RNA-binding protein (RBP) HuR is one of the most widely studied regulator of the eukaryotic post-transcriptional gene expression and it plays a physiological role in mediating the cellular response to apoptotic, proliferating and survival stimuli. Following physiological or stress stimuli, HuR protein binds to Adenylate-Urydinilate rich elements (AREs) generally contained in the 3'UTR of transcripts, then it shuttles from the nucleus to the cytoplasm and regulates the half-life and/or translation of cargo mRNAs. Derangements in sub-cellular localization and expression of HuR have been associated with the pathophysiology of many diseases and this protein has been proposed as a potential drug target. Recent findings also re-evaluated HuR as a splicing and polyadenylation factor, expanding its *spectrum* of functional activity up to the maturation of pre-mRNAs. In this review we generate a comprehensive picture of HuR functionality to discuss the implications of considering HuR as pharmacological target and the detrimental or positive impact that can be expected upon its modulation. Firstly, we focus on the recent findings about the mechanistic role of HuR in the nucleus and in the regulation of long non coding RNAs; then we describe the animal models and the clinical association and significance in cancer; finally, we have reviewed the pharmacological tools that influence HuR's post-transcriptional control and the efforts made to identify specific HuR inhibitors.

Graphical Abstract



Introduction

Eukaryotes finely tune protein levels by regulating gene expression at many different steps involving transcription, post-transcription, translation and proteolysis. Specifically, post-transcriptional mechanisms are emerging to be much more complex than previously thought, mainly due to the identification of the regulatory role of non-coding RNAs and their interplay with a variety of RNA binding proteins, RBPs (1). HuR is among the most widely studied RBPs that regulates the splicing, stability and translation of thousands of coding and non-coding RNAs (2–4). HuR is a member of the embryonic lethal abnormal vision (ELAV)-like/Hu-protein family of RBPs. In contrast to three other family members, namely HuB/HelN1, HuC, and HuD which are expressed primarily in the neuronal tissue, HuR is ubiquitously expressed. In *Drosophila*, the *Elav* gene was discovered as a key regulator for the development and the maintenance of the visual system (5). In 1996 Ma and colleagues cloned and characterized the human homologue of HuR (hHuR) (6) that was mapped on the chromosome 19p13.2 and encoded a 326 amino acid long protein with a molecular weight of 36 kDa (7). Early studies demonstrated that HuR has the ability to tightly bind to AU Rich Elements (AREs) present in *c-FOS, N-MYC, c-MYC* and *IL3* mRNAs (8–11). The HuR coding region comprises three RNA recognition motifs (RRMs) and a hinge region where the HuR nucleo-cytoplasmic shuttling sequence (HNS) is located. RRM1 and RRM2 mediate HuR recognition of mRNA targets (i.e. U and AU rich sequences mainly, but not exclusively, present within the 3'UTR of mRNAs) (11). The third RRM, RRM3, is thought to bind to the poly (A)-tail and to maintain the stability of the RNA-protein complex. Indeed, HuR mutants in the RRM3 regions are unable to bind to target mRNAs, hence showing a major role of all RRM domains in mediating HuR functionality (12–14). Together with structural features, post-translational modifications can also affect HuR binding to the cargo mRNAs and influence its function. For example, HuR-mRNAs interactions are generally affected by modifications of residues, mainly phosphorylation, within the RRMs, while the sub-cellular localization is altered by modification of residues within or near the HNS. According to the stress condition or to the physiological stimulus, HuR can be phosphorylated by a number of kinases such as Chk2, Cdk1, PKCs (α, β and δ), and by p38 MAPK (15–22). HuR can be also methylated by CARM1 and degraded by the proteasome after ubiquitination (17,23–25).

Recognized role of HuR

Since HuR is involved in the nuclear export and post-transcriptional regulation of thousands of mRNAs, its correct function is critical for cell survival and proliferation. Among the transcripts, *p21, c-FOS,* the vascular endothelial growth factor *(VEGF), SIRT1, TNFα, Bcl-2, Mcl-1, COX-2, p53, cyclins A2, B1, E and D1* are just some examples (8–11,15,22,26–33). In un-stressed cells HuR is primarily located in the nucleus, its translocation to the cytoplasm occurs in response to various stimuli (e.g. UV irradiation, heat shock, cytokines, viral infection and hormone treatment) and its cytoplasmic localization is required for its mRNA-stabilizing function. Mechanistically, HuR can either favor or repress the translation of target mRNAs: it has been proposed that combinatorial binding of a variety of *trans-acting* factors to *cis* elements present in the target mRNA determines the fate of the mRNA itself. In addition, either the cooperation or the competition with microRNAs can significantly contribute to the gene expression program (25). In this context, it has been shown that HuR is able to displace the mRNA from the miRISC complex by interfering with the base pairing between miRNA and the messenger RNA, leading to attenuation of mi-RNA mediated repression (34).

HuR and Disease

Being a major regulator of gene expression, it is not surprising that HuR de-regulation can favor or sustain several cancer traits such as cell proliferation (35), cell survival (27), local angiogenesis (26), tumor cell invasion and

metastasis (36). Moreover, HuR has also been implicated in different pathologies, mainly cardiovascular diseases (37–39), nephropathy (40), inflammatory-related (41–43), retinal diseases (44,45), muscular disorders (46,47) and neurological diseases (48), due to its mRNA stabilizing function of many key mRNAs, such as cytokines (25,31,49,50). Therefore HuR has been proposed as a valuable drug target. The aim of this review is to discuss if this assumption can be generalized or if it is specific. We have browsed the literature to report the latest findings on HuR physiology, focusing on its function in the nucleus and its interplay with long non coding RNAs (lncRNA) and, exploiting prognostic studies and animal models, we have highlighted biological conditions in which the inhibition of HuR could be beneficial or, on the other hand, an activation of the protein would be desired. In addition we report and discuss the role of HuR in response to chemotherapeutic agents and chemicals used in research studies, illustrating that HuR enhances the efficacy of specific drugs. Finally, novel compounds identified as modulators of HuR will be also considered.

HuR nuclear functionality: splicing and polyadenylation

In recent years, several studies have investigated the role of HuR within the nucleus, demonstrating its direct involvement in the stabilization, alternative splicing and polyadenylation processes of pre-mature mRNAs. These investigations have provided a functional explanation for the strong nuclear presence of the protein.

Importantly, two pivotal studies have proposed that HuR post-transcriptional function is a *continuum* of activities, from the binding of HuR to messenger RNAs during, or immediately after, transcription as the initial step of the HuR-dependent metabolism of target mRNAs, to cytoplasmic export and translation (4,51,52). Using different high-throughput targeting technologies, Lebedeva et al (2011) and Mukherjee et al (2011) suggested that one of the main nuclear function of HuR is to couple pre-mRNA processing and mRNA stability. Both studies showed that HuR functional ablation impacts on the expression of HuR target mRNAs, containing HuR binding sites in intronic regions as well as in the 3'UTRs. Previously, Hu proteins have been found to compete with TIA/TIAR in the exon definition of several mRNAs (53,54), while HuR was found to be associated with the spliceosome complex (55) and to regulate the splicing of a reporter gene (56). A direct role of HuR in the regulation of alternative exons regarded the ability to exclude exon 6 of *Fas* pre-mRNA by the association with the U2 snRNP auxiliary factor 65 kDa on the 3'upstream splice site. This effect was antagonized by the RNA binding protein TIA1/TIAR that was, on the contrary, favoring recognition of the splice site (57,58). The same players have been also involved in the splicing regulation of *SIRT1* pre-mRNA. Under UV-induced cellular stress, HuR over-expression increased the skipping of exon 8 of SIRT1 pre-mRNA, while TIA1/TIAR antagonized SIRT1-ΔExon8 mRNA production (59). Nevertheless, the binding of HuR to intronic regions, responsible of the splicing events, has been firstly reported in the above mentioned genome wide investigations by Lebedeva et al (2011) and Mukherjee et al (2011) (4,51). Both studies have also shown that intronic HuR binding preferentially occurs close to exons with a strong bias to bind just upstream of the 3'UTR. However alternative splicing events correlated with HuR depletion and could depend on the enrichment of pre-mRNA processing factors, as FOX2, NOVA, PTB and hnRNPC, in the set of functionally responsive HuR target and not solely to the alteration of the inclusion of some exons containing adjacent HuR-binding sites (51). On these footsteps, Akaike and colleagues have shown that arsenite induced phosphorylation of HuR (at S88 and T118) is able to initiate the association of HuR at the 39-nt proximal region of *TRA2*β exon 2. This favored the production of *TRA2*β4 mRNA, that included multiple premature stop codons and led to Tra2β protein loss. Importantly, this mechanism was exclusively ascribed to a nuclear HuR functionality in the regulation of alternative splicing programs (60). Another breakthrough in the elucidation of the nuclear function of HuR came from Dutertre and colleagues (61). By the utilization of pan-genomic exon-arrays they

have shown that doxorubicin modulates the utilization of alternative 3' terminal exons by the cell, also called alternative last exons (ALEs), and leads to the alternative use of intronic poly(A)-sites in a splicing-dependent manner. This mechanism was mediated, at least in part, by HuR binding to the conserved, doxorubicin-repressed, internal ALEs. In their model, they proposed that, for a subset of human pre-mRNAs, internal ALEs require HuR association for inclusion in mature mRNAs. However, following cell response to DNA damage, as in the case of doxorubicin treatment, HuR association was reduced and the production of specific isoforms decreased. This has been shown for *CENPN, KIF1B* and *MBD1* mRNAs, involved to various extent in G2/M transition and DNA damage response. Meanwhile, HuR relocated to the cytoplasm, where it regulated the stability and translation of various mRNAs through binding to 3'UTRs, therefore showing the HuR *continuum* of post-transcriptional activity between the nucleus and the cytoplasm (61). Accordingly, it has been shown that HuR plays an important role also in alternative polyadenylation regulation because it leads to a different expression abundance of polyadenylated variants. HuR bound only to polyadenylated transcripts containing U-rich elements and stabilized them by inhibiting the recruitment of cleavage stimulating factors such as CstF64 (62–64). As an example, it has been observed that HuR regulates integrin β1 mRNA isoforms using different polyadenylation sites during mammary gland development (65). Moreover, different studies have demonstrated that HuR mRNA isoforms are regulated through alternative polyadenylation. In neuronal cells, other ELAV family members bound to the U-rich sequences flanking the 2.4-kb polyadenylation site and prevented the access of the polyadenylation machinery (66). HuR itself (67) was reported to autoregulate its own expression level by competing with CstF64 for binding to one of the alternative poly(A)-sites (reviewed in (68)). Similarly, HuR sequestration in the cytoplasm by RNA Sindbis virus led to an alteration in the stabilization and in the splicing/polyadenylation events of cellular pre-mRNAs in infected cells (69,70).

As it has been outlined, the nuclear function of HuR is in tight connection with its widely studied cytoplasmic functions and HuR can be considered a relevant processing factor encompassing the entire post-transcriptional maturation process. However a detailed, genome wide, investigation of the reciprocal influence between HuR and specific transcriptional factors could be beneficial to elucidate the level of coupling between transcriptional and post-transcriptional networks, eventual feed-back or feed-forward loops, and to clarify the HuR nuclear role.

HuR and lincRNA

Recent investigation of the RNA species bound by HuR has led to the identification of the association of HuR to lincRNAs. Indeed HuR ability to bind lincRNA transcripts, such as lincRNA-p21, MALAT1, NEAT1 and lincRNAs involved in X chromosome inactivation, has been anticipated by genome wide studies that exploited photo activatable ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) analyses (4,51,71). Therefore, mechanistic clues and biological meaning of specific lincRNA-HuR interactions have been described. Yoon and colleagues (2012) showed that HuR binds to lincRNA-p21 and influence its decay, in turns regulating the translation of pro-survival protein-encoding transcripts such as *JUNB* and *CTNNB1* (β-catenin) in HeLa cells (72). The increment in lincRNA-p21 level after the knock-down of HuR expression level surprisingly indicated that HuR, differently to the canonical mRNA stabilization function, enhances lincRNA-p21 destabilization. Accordingly, in mouse embryonic fibroblasts (MEFs) derived from $HuR^{-/-}$ mice, the linc-RNA-p21 expression level was higher if compared with the wild type. In this article the mechanism by which HuR cooperates with let7/AGO2 in the elicitation of lincRNA-p21 decay has been precisely described. In this model HuR rendered lincRNA-p21 unstable through the recruitment of let-7/Ago2 and then promoted the association of the targets *CTNNB1* and *JUNB* mRNAs with polysomes for their translation. In

the absence of HuR, lincRNA-p21 accumulated, and associated with the RNA helicase RCK, to form a RNP complex, that repressed the translation of *CTNNB1* and *JUNB* mRNAs through a mechanism that included a reduction in polysome size, indicating a destabilizing function of HuR-lincRNA-p21 interaction correlated with the cytoplasmic localization of HuR.

In another case, the role of HuR appeared to rely on its nuclear localization, since it normally blocked processing of miR-675 in the early phase of placenta development. During the second half of gestation, close to parturition, HuR expression levels were reduced and miR-675, embedded in the first exon of lincRNA H19, started to be processed leading to a suppression of cell proliferation. In this work, Keniry and colleagues (2012) demonstrated that the binding of HuR to specific AU-rich regions blocks miR-675 excision from the pre-processed lincRNA H19 (pre-miRNA), affecting miR-675 production and placenta cell proliferation control (73).

With a similar mechanism, interaction between HuR and lincRNA-MD1 has been pointed out in the early phases of muscle differentiation, during which linc-MD1 acts as a sponge for miR-133, that represses HuR, and miR-135 (74). Legnini and colleagues (2014) showed that HuR specifically regulates the expression of miR-133b, which is contained in the linc-MD1 transcript. In the frame of Competing Endogenous RNA (ceRNA) theory (75), HuR has been proposed to control linc-MD1 cytoplasmic accumulation at the expense of miR-133b synthesis by competition with the cleavage processing by Drosha. Consistently, HuR was also required in the cytoplasm to ensure efficient linc-MD1 sponge activity by facilitating specific miRNA recruitment to maintain a positive regulatory loop allowing progression to later stages of muscle differentiation.

Therefore, these two reports have shown the existence of a specific circuitry in which the time-and-spatial control of linc-H19/ or linc-MD1 and HuR are reciprocally regulated in order to establish a correct progression of muscle differentiation and placenta development, respectively. These important observations indicate that the stabilizing or destabilizing properties of HuR have to be functionally contextualized to a specific tissue, therefore the intricate regulation of non coding RNA species needs further elucidation to understand the framework of post-transcriptional mechanisms and should be taken in consideration in the future animal model studies. In an additional layer of complexity, HuR post-transcriptional functionalities are not limited to the associations with coding or non-coding RNAs, but it is also influenced by the interactions with other regulatory proteins.

HuR and protein interaction

In physiological conditions, HuR associates with many others protein forming ribonucleoprotein (RNP) complexes via direct protein-protein interactions and/or RNA-mediated interactions. Recent studies have shown that HuR cooperates with other RBPs determining dynamic interplays that regulate the fate of target mRNAs (76–78). In particular, recently it has been shown that HuR interacts with AUF-1 in a cooperative manner regulating the turnover of *p16(INK4)* mRNA (79), of phosphoenolpyruvate carboxykinase (*PEPCK*) mRNA in response to pH (80), of *VEGFA* mRNA under normoxic conditions or hypoxia (81) and also it interacts with β-catenin in response to hypoxia for the regulation of *SNAI2* and *CA9* mRNAs (82).

The interplay of HuR together with KSRP (KH-type splicing regulatory protein) and TTP has been reported for the competitive regulation of the *iNOS* mRNA expression after cytokine treatment due to the ability of these three RBPs to bind to the same 3'UTR region (83). Interestingly, Cammas et al (2013) showed that HuR can directly interact with KSRP during the early steps of myogenesis to regulate the decay of the cell cycle promoter nucleophosmin (*NPM*) mRNA (84). Notably, in this context the interaction of HuR with KSRP conferred de-stabilizing properties to HuR binding, opposite to its canonical stabilizing activity. In response to different stress stimuli (UV-irradiation, viral

infection, heat shock), HuR translocates from the nucleus to the cytoplasm forming transient cytoplasmic aggregates with bulk mRNA-RBPs, namely stress granules (SGs), regulating mRNA stability and/or translation (85). HuR is a disposable marker of SGs formation (86) and it has been shown to direct interact with hnRNP complexes following heat-induced stress (77). The general function of HuR to facilitate the stabilization of SG-associated ARE-containing mRNAs (87) has been also evidenced by its un-mobilization to processing bodies (P-bodies), cytoplasmic formations that contain RNA decay elements (88), following arsenite treatment (89). Recently, it has been shown that HuR preserves *TNF* mRNA from miR-181-induced degradation but recruiting it into SGs with important implication for reversing sepsis-induced immunoparalysis (90). Intriguingly, a specific phosphorylation of HuR at tyrosine 200 by JAK3 upon arsenite treatment has been associated with cytoplasmic relocalization of the protein and accelerated decay of *SIRT1* and *VHL* mRNAs (72). HuR is also involved in the trafficking of cargo ARE-bearing mRNAs to polysomes through interaction with cytoskeleton proteins. In particular, Doller et al (2014) demonstrated that, in renal mesangial cells, phosphorylated cytoplasmic HuR, induced by angiotensin II, interacts with myosin IIA in an RNA-dependent manner and is transported to polysomes *via* a myosin-driven transport; this trafficking was inhibited by the utilization of cytoskeletal inhibitors as latrunculin and blebbistatin (91,92). In summary, these few data indicate the existence of a complex network of protein interactions where HuR is a central hub for differential RNA binding activity after undergoing post-translational modifications and for transcript selection upon binding with other proteins. For this reason, dissection of post-translational modification's profile may contribute to predict spatio-temporal HuR functionality and define protein cooperators. Speculatively, targeting specific HuR-protein interaction might be considered to selectively inhibit functions of HuR within the nucleus or the cytoplasm, alternative to the modulation of its sub-cellular localization. However, to fully elucidate the complexity of HuR-mediated post-transcriptional regulation, up to now addressed with cell-based systems, animal models have been required and important information about HuR relevance in the body are emerging.

Lessons from Animal models

In vivo studies are needed to support *in vitro* data and therefore transgenic and knock-out mice have been recently produced to study the role of HuR in diseases, in particular its involvement in the inflammatory response. A major problem to study HuR *in vivo* is that its complete knock-out results in embryonic lethality (93,94), thus preventing the assessment of its involvement in immune processes and diseases. Therefore conditional knock-out animals are necessary to evaluate the role of HuR in each tissue (Figure 1).

HuR in the development and tissue homeostasis

Animal models have been really helpful in unravelling some important roles of HuR in development. In 2011, it was shown that HuR epiblast-induced deletion in mice blocks lung branching morphogenesis and that HuR is an essential regulator of mesenchymal FGF networks (95). The function of HuR during gametogenesis was investigated by Chi et al (2011) using conditional loss- and gain-of-function approaches in mice (96). Mutant males have been shown to be azoospermic because of the extensive death of spermatocytes at meiotic divisions and failure of spermatid elongation. HuR was demonstrated to specifically bind to the heat shock protein *Hspa2* mRNA, a key regulator of spermatogenesis, controlling its expression at the translational level in germ cells. Hematopoietic function for HuR was investigated in zebrafish by Li and coworkers (2014), using specific morpholino to knock-down *elavl1a*, the predominant isoform during embryogenesis (97). In this work, they demonstrated a striking loss of primitive embryonic erythropoiesis due to a missing post-transcriptional control by which HuR maintains appropriate levels of gata1 expression. An evolutionarily

conserved post-transcriptional mechanism controlling angiogenesis was also revealed by Chang et al (2013) (98). This mechanism involved a competitive interactions between HuR and miR-200b in the regulation of Vascular Endothelial Growth Factor-A (VEGF-A) expression. A myeloid-specific HuR knock-out mouse model was used to show that HuR expression in bone marrow-derived macrophages is needed to promote VEGF-A expression. In this model, HuR knock-out attenuated angiogenesis, vascular sprouting, branching, permeability and tumor growth therefore suggesting that HuR blockade could be beneficial to inhibit macrophage-dependent tumor vascularization. Zebrafish embryos injected with an *elavl1* morpholino also showed defects in angiogenesis in the sub-intestinal vein plexus. It has been also recently reported the fundamental role of HuR in promoting the growth of small intestinal mucosa (99). HuR function was investigated by tissue-specific deletion in the intestinal epithelium (IE), using IE-HuR-/- mice generated by crossing $HuR^{fl/fl}$ mice with mice carrying *Villin-Cre*. The deletion resulted in a significant small intestinal mucosal atrophy and the epithelial crypt progenitors also displayed decreased regenerative potential after irradiation. The phenotype was linked to the alteration of Wnt signaling activity through post-transcriptional regulation of LRP6 expression by HuR interaction with the 3'UTR of its mRNA. Conditional HuR knock-out mice obtained by crossing *Emx1-Cre X* $HuR^{fl/fl}$, allowed the selective deletion of HuR at approximately E11 in Radial Glia, resulting in selective HuR deletion in all primary projection neurons in the neocortex. By comparison of polysome profiling of neuronal cells, Kraushar and colleagues (2014) revealed that HuR association with eIF2ak4 (GCN2) was responsible of the temporally specific association of functionally related mRNAs in the polysomes of the developing neocortex. Loss of HuR altered the spatio-temporal loading of mRNAs, the phosphorylation status of initiation factors as eIF2α and eEF2, phenotypically disrupting the neocortical lamination and corpus callosum formation (100).

From the animal model studies so far conducted, we can conclude that HuR plays a pivotal role in the post-transcriptional control of gene expression *in vivo* that is necessary during the organism development and in the maintenance of the tissue homeostasis. Therefore an hypothetical pharmacological HuR inhibition would be detrimental for those stem cell compartments, as the hematopoietic or the intestinal crypt, that require HuR for their maintenance. In fact, when HuR is removed from the cell or its function modulated, the equilibrium with other *trans*-acting factors or non-coding RNAs is broken and so is the cell commitment, with strong impact on the development of the embryo, its tissues and annexes. In a pathological setting, such as inflammation and cancer, HuR over-expression allows its targeting to achieve control of the expression of specific mRNAs encoding key pathogenic proteins.

HuR and Inflammation

The post-transcriptional regulation exerted by HuR plays important roles in inflammation, by quickly altering the expression levels of key mediators such as TNFα, COX-2, and interferon-γ. Interestingly, 90% of chemokines and cytokines mRNAs contain AREs, illustrating the importance of post-transcriptional gene regulation during the immune response (101). Papadaki et al (2009), using the Cre-loxP system, showed that early HuR deletion in thymocyte development during the double-negative stage, leads to a loss of peripheral T cells. This implicated the involvement of HuR in the physiological thymocyte maturation, selection and normal traffic from the thymus to the periphery (102). For this reason, in order to study the post-transcriptional mechanisms by which HuR regulates $CD4^+$ T-cell differentiation and cytokine production *in vivo*, a conditional HuR knock-out (KO) mouse was used, in which HuR was deleted in activated T cells after thymic selection and egress from the thymus (103). In this work, by the conditional knock-out of HuR in T-cells, the authors have revealed a complex regulation of cytokines dependent on HuR dosage. In fact, Th2-polarized cells from heterozygous HuR conditional KO mice, decreased steady-state levels of *Gata3, Il4* and *Il13* mRNAs with little changes at the protein level. On the contrary, Th2-polarized cells from homozygous HuR

conditional KO mice, increased *Il2, Il4* and *Il13* mRNA and protein levels *via* different mechanisms. In addition, HuR conditional KO mice mounted a robust inflammatory response similar to mice with wild-type HuR levels when an allergic airway inflammation model was used, therefore highlighting the complex and contradictory, to some extent, effects of HuR deletion during inflammation onset. A more straightforward role of HuR in the modulation of IL-17 has been reported: HuR deletion in distal lung epithelium attenuated IL-17–induced neutrophilia and pulmonary inflammation *in vivo* (104). HuR depletion substantially impaired IL-17–induced chemokine expression (CXCL1 and CXCL5) due to increased mRNA decay. Upon IL-17 stimulation, Act-1 mediated HuR ubiquitination and co-shifted towards the polysomes. The post-transcriptional gene regulation of the proinflammatory cytokine IL-17 by HuR has been also reported in another manuscript, by using conditional KO mice in which HuR was deleted in activated $CD4^+$ T cells (105). HuR knock-out mice provided evidences of the regulation of IL-17, produced by activated Th17 cells. HuR was reported to directly bind to and stabilize IL-17 3'UTR mRNA, which in turn resulted in increased *Il-17* mRNA and protein levels. Additionally, HuR ablation in $CD4^+$ Th17 cells interfered with Th17 differentiation and decreased IL-17 expression in an adoptive transfer model of Experimental Autoimmune Encephalomyelitis (EAE), reducing the disease initiation and early severity. Therefore, HuR was proposed as a novel therapeutic target for the treatment of Th17-mediated autoimmune neuro-inflammation. Apparently in contrast with this finding, HuR transgenic expression in astrocytes has been reported to attenuate EAE (106). Astrocytes express many ARE-containing cytokine and chemokine mRNAs involved in the inflammatory response. In these transgenic mice, HuR was expressed in spinal cord astrocytes using the human glial fibrillary acidic protein (GFAP) promoter. Female transgenic mice, and to a lesser extent male mice, had significantly less clinical disability and histopathological changes in the spinal cord. Reversal of protection after ovariectomy strongly suggested that the effect was modulated by E2, progesterone or both and that transgenic HuR expression in astrocytes could be linked to hormone-dependent attenuation of EAE. The paradoxical protection of HuR over-expression in astrocytes is another case in which HuR functional significance is strictly dependent on cell context, as is mediating, in this case, the protective effects of steroids hormones. In the brain, HuR was found to play a neuroprotective role by Skliris et al (2014). By using KO mice lacking HuR in the hippocampal neurons of the central nervous system ($CamKII\text{-}Cre^+Elavl1^{fl/fl}$) they reported an higher neuronal sensitivity to glutamatergic excitation and more hippocampal lesions. The lack of HuR in hippocampal neurons resulted in mitochondrial dysfunction, increase in oxidative ROS such as SOD1/2and COX2 and programmed necrosis. Thus, HuR protected neurons from degeneration by controlling oxidative metabolism and regulating fundamental mRNAs involved in this process such as Bcl-2, NQO1, Ppargc1a (107). Examples have been reported also linking HuR, inflammation and cancer. Yiakouvaki et al (2012) used mice lacking HuR in myeloid-lineage cells. Macrophages deficient in HuR had increased expression of pro-inflammatory cytokines, enhanced CCR2-mediated macrophage chemotaxis and enhancements in the use of inflammatory mRNAs (including *Tnf, Tgfb, Il10, Ccr2,* and *Ccl2*) due to a lack of inhibitory effects on their inducible translation and/or stability. In addition, these mice developed chemical-induced colitis and were susceptible to colitis-associated cancer (108) suggesting that HuR plays different roles in macrophages and in T cells. Giammanco et al (2014) generated conditional intestine-specific HuR knock-out mice (HuR^{IKO}) and demonstrated that epithelial HuR plays an important role in small intestinal epithelial homeostasis and proliferation (109). In $Apc^{Min/+}HuR^{IKO}$ mice, a transgenic model of intestinal tumorigenesis, they showed that intestinal HuR deletion attenuates small intestinal polyposis and caused a three-fold decrease in tumor burden. In addition, HuR^{IKO} mice subjected to an inflammatory colon carcinogenesis protocol exhibited a two-fold reduced colon tumor burden. These mice models revealed that intestinal HuR deletion attenuates spontaneous intestinal polyposis and colitis-associated cancer, protecting from tumorigenesis by promoting apoptosis (109). These studies indicate that HuR mediates the pro-inflammatory process in

$CD4^+$ lymphocytes but an anti-inflammatory and protective program in macrophages, astrocytes and neurons, addressing the possibility of HuR inhibition in T-cell lineages. On the same line, its inhibition would be beneficial in colon cancer, although its loss of function may have important drawbacks in the maintenance of the intestinal mucosa. Notably, HuR inhibition would be beneficial to attenuate macrophage-dependent tumor vascularization. These observations might be linked with clinical data addressing the role of HuR to predict outcome in cancer patients.

Indication from the clinic: prognostic role of HuR

HuR has been implicated in many different diseases (25,110) and in the upcoming session we focus our attention on cancer pathology and discussing those cases in which HuR expression level or cellular localization is endowed with a prognostic significance. In the past decades, cumulative data have shown that over-expression of HuR or aberrant nucleus/cytoplasm ratio is related to carcinogenesis and tumor progression in various cancer types including colon cancer (111,112), ovarian carcinoma (113) and cervical carcinoma (114). Interestingly, neither HuR has been found mutated in tumors nor its polymorphisms associated with overall survival in breast cancer patients (115). Consequently several retrospective studies have been conducted in the recent years to investigate the sub-cellular localization of HuR and its clinical predictive and prognostic value in human malignancies, as extensively described in a recent review (116). Here we report only the newest studies.

Cytoplasmic accumulation of HuR is a predictor of negative clinical outcome

From the majority of the association studies, convincing data have shown that cytoplasmic HuR accumulation is correlated with unfavorable clinical outcome. Cytoplasmic HuR accumulation, in patients with primary renal cell carcinoma (RCC), upper urinary tract urothelial carcinoma, esophageal squamous cell carcinoma (ESCC), non-small cell lung cancer (NSCLC) and bladder carcinoma was associated with reduced RCC-specific survival and with other negative clinical features as higher histological grade, presence of lymph node metastasis and pattern of cell's invasion (117–121). In other cases cytoplasmic HuR has been associated with the over-expression of oncogenes or pro-tumoral factors. In oral squamous cell carcinoma (OSCC), nuclear and cytoplasmic HuR was over-expressed in tumor samples and high cytoplasmic HuR levels were correlated with high expression of cellular inhibitors of apoptosis proteins-2 (cIAP2) and COX-2 expression with a reduced progression-free and overall survival rate (122,123). In samples from non-muscle invasive bladder cancer (NMIBC), only cytoplasmic HuR accumulation has been positively associated with primary tumor stage, grade and with malignant behavior such as angiogenesis and lymphangiogenesis (expression of VEGF-A, –C and COX-2, microvessel density and lymph vessel density). In a large cohort of primary resected gastrointestinal stromal tumors (GISTs), the cytoplasmic accumulation of HuR has been significantly correlated with some adverse clinico-pathological features and with the increase of cyclin A expression. Both HuR cytoplasmic localization and cyclin A contributed to confer an aggressive phenotype as proved by the fact that they were independent predictor of low rate of disease-free survival (124). In a breast cancer study, Zhu and colleagues (2013) demonstrated that the simultaneous cytoplasmatic localization of HuR and P-glycoprotein (P-gp) over-expression correlates with a significant lower disease-free survival rate and overall survival rate compared to patients with cytoplasmic expression of either one of the two protein, showing a higher risk to relapse (125). In limited cases cytoplasmic HuR localization has not been correlated with poor survival, as in primary pulmonary adenocarcinoma (ADC) and squamous cell carcinoma (SCC) samples and OSCC (126,127). Beside the role of HuR in the cytoplasm, the nuclear localization of HuR is also important for clinical outcome, as in gallbladder carcinoma. Both nuclear and cytoplasmic HuR were significantly associated with increment of primary tumor, tumor stage, histological grade and

vascular invasion. Moreover patients with cytoplasmic HuR localization had poor disease-specific survival (DSS) and disease-free survival both in univariate and multivariate analysis, whereas nuclear HuR has been correlated only with low disease-free survival in univariate analysis (128). These data are consistent with another study in ovarian cancer in which high nuclear HuR localization was correlated with high histological grade, invasive cancer and a reduced disease-free survival (113). A new insight came from perspective study on early stage of lung adenocarcinoma. This clinical study showed that the nuclear/cytoplasmic ratio of HuR seems to be a more sensitive negative prognostic factor of poor overall and metastasis-free survival than individual staining of nuclear and/or cytoplasmic HuR localization. Moreover the evaluation of HuR nuclear/cytoplasmic ratio seemed to allow the identification of a subset of patients with higher risk of metastasis and death (129).

More sporadic studies have reported a favorable prognostic value of HuR. In breast cancer specimens high *HuR* mRNA levels, measured by Q-PCR, have been associated with a favorable outcome. Indeed patients with metastasis, bone metastasis and poor prognosis had significantly reduced transcript levels of *HuR* (130).

In conclusion, these retrospective studies, using different quantitative techniques and score analysis, show that high HuR cytoplasmic accumulation predicts a malignant behavior when correlated with increased stabilization of oncogenes and/or cell cycle factors and can be an independent marker of poor outcome and tumor aggressiveness in colon and ovarian cancer, RCC, ESCC, NSCLC, NMIBC, GIST, urothelial and bladder cancer. On the other side, the clinical significance of nuclear HuR remains unclear. In view of the latest findings suggesting a significant role of HuR in the maturation of pre-mRNAs of coding and non-coding mRNAs, HuR nuclear accumulation could be investigated in correlation with mRNA processing factors or with the presence of splicing and polyadenylation variants of cancer-associated mRNAs. Notably, HuR sub-cellular distribution could be also influenced by direct interaction with factors determining its post-translational modifications, i.e. kinases that are aberrantly regulated in cancer pathologies, causing the prevalence of either nuclear or cytoplamisc HuR location according to the genetic background of the tumor type. However, no systematic analyses have been reported so far, and clarification of these molecular aspects can concur to better address and explain the extent of general HuR over-expression in these tumors.

HuR and resistance to treatment

In line with its pro-tumoral role and with the fact that it mediates the expression of anti-apoptotic and pro-survival genes, other reports have shown how HuR is opposing to the efficacy of some pharmacological agents. In patients with breast cancer stage II and III that underwent paclitaxel and anthracycline-based neoadjuvant therapy (NACT), high expression of cytoplasmic HuR has been associated with lower overall survival and malignant phenotype. Cytoplasmic HuR was not indicated as a predictive marker of response to NACT, but a better predictor in combination with pathologic complete response (pCR) of clinical outcome (131). Consistently HuR silencing produced a significant decrease in tumor volume in an intracranial tumor model with primary glioblastoma cells whereas over-expression of HuR generated chemoresistance to topotecan, cisplatin and etoposide due to the abundance of the anti-apoptotic factor Bcl-2 (132). Similarly, mitomycin C, oxaliplatin, cisplatin, carboplatin and PARP inhibition efficacy was reduced in PDAC given the post-transcriptional stabilization of WEE1 by HuR (133). In breast cancer MCF-7 cells, tamoxifen resistance was due to the cytoplasmic accumulation of HuR bringing to the stabilization of transcripts encoding drug-resistant proteins and to the following activation of MAPK and JNK signaling (134). Therefore in these cases an eventual pharmacological inhibition of HuR could increase the efficacy of the chemotherapy.

HuR is a positive predictor of chemotherapy efficacy in specific cases

In apparent contrast with the previous observation suggesting a negative prognostic role of HuR, high cytoplasmic HuR localization in high-risk patients with upper urinary tract urothelial carcinoma (stage T3 and T4) has been correlated with better DSS after adjuvant therapy (118). This finding suggested that high cytoplasmic HuR level could be a tool to identify subset of patients that can benefit from adjuvant therapy, as shown in other research study on pancreatic ductal adenocarcinoma (PDAC) in which HuR cytoplasmic location has been identified as a positive indicator of gemcitabine treatment efficacy (135,136). Therefore it appears that cytoplasmic HuR accumulation could become a robust tool for the identification of patients that can benefit from adjuvant therapy (Table 1), specifically in the case of gemcitabine, doxorubicin or 5-FU. The efficacy of these chemotherapeutic molecules requires the presence of HuR for their maximum efficacy. In particular gemcitabine, the reference chemotherapeutic drug used against pancreatic ductal adenocarcinoma (PDAC), increased the binding of HuR to *dCK* (deoxycytidine kinase) mRNA, which encodes the enzyme that metabolizes and activates gemcitabine, and consequent up-regulation of the dCK protein levels in cancer cells. Accordingly, HuR over-expressing cancer cells have been shown to be more sensitive to treatment with gemcitabine and Ara-C (136). The expression levels of dCK and HuR for prediciting sensitivity to gemcitabine and 5-flurouracil (5-FU) have been evaluated by immunohistochemistry analysis using tissue microarray taken from resected PDACs belonging to a large study of radiation therapy oncology group (RTOG) 9704 trail, a large phase III adjuvant trial of pancreatic ductal adenocarcinoma (PDAC) (137). They showed that dCK expression renders cells more sensitive to 5-FU and that 5-FU increases HuR activity by enhancing HuR translocation from the nucleus to the cytoplasm, similar to the effect of gemcitabine in PDAC cells. In a breast cancer cell line, knock-down of HuR strongly abrogated 5-FU induced apoptosis, detected by caspase-3 activity, suggesting that 5-FU therapeutic function was dependent on HuR presence. In this case, the proposed mechanism required the HuR mediated stabilization of the tumor suppressor FOXO1 (the Forkhead box O) transcription factor as over-expression of FOXO1 partially rescued 5-FU-induced apoptosis (138). A third chemotherapeutic agent, doxorubicin, widely used in breast cancer treatments showed similar behavior. This genotoxic agent is a DNA intercalator and a Topoisomerase 2 inhibitor that stabilizes the complex of TOP2A bound to the double-strand DNA breaks. *TOP2A* post-transcriptional regulation is dependent on the binding competition of two *trans*-factors, HuR and miR-548c-3p, on its 3'UTR with opposite functionality. Lowering *TOP2A* mRNA by overexpressing miR-548c-3p or silencing HuR or *TOP2A* itself reduced the levels of doxorubicin-induced DNA damage, therefore decreasing the drug efficacy (139). Importantly this effect was specific to doxorubicin, as cisplatin-induced DNA damage did not change according to HuR presence. Interestingly, Dutertre and colleagues (2014) showed that doxorubicin-induced alternative polyadenylation is due to the loss of function of HuR, therefore suggesting that doxorubicin, in this perspective, could also be considered an inhibitor of nuclear HuR functionality (61). Summing up, HuR over-expression and/or cytoplasmic location in cancer tissues, could be a negative condition limiting the efficacy of agents as platinum drugs, but a favorable event when using other agents such as gemcitabine or doxorubicin.

HuR and acquired resistance

A well-known phenomenon associated with chemotherapy is the insurgence of pharmaco-resistance and during the isolation of tumor cells resistant to anti-cancer drugs, HuR was found to be differentially regulated. Using *in vitro* selected doxorubicin-resistant breast cancer cells, over-expressing the multidrug resistance (MDR) related ABCG2 transporter, a significant HuR down-regulation was observed. This was paralleled by the corresponding down-regulation of the HuR target, Topoisomerase 2A (TOP2A). Interestingly, PKCδ, one of the kinases that phosphorylates and regulates HuR localization, has been also involved in the HuR mediated doxorubicin-induced apoptotic response.

Restoration of HuR or PKCδ expression resensitized resistant cells to doxorubicin (140,141). By contrast triple negative breast cancer cells, selected for resistance to lapatinib, developed increased expression of Epidermal Growth Factor Receptor (EGFR) and aggressiveness. The mechanism underlying augmented metastasis potential of resistant cells involved the overexpression of COX2 protein, through the stabilization of its mRNA by HuR. This effect was greatly facilitated by the interaction of the EGFR protein with HuR, and was not due to the enzymatic activity of the EGFR (142). Therefore, further randomized controlled clinical trials aimed at evaluating the predictive and prognostic significance of HuR in different stages of human malignancies and its relevance to specific chemotherapy response are needed to confirm these data. The choice of the chemotherapeutic regimen should, then, take in consideration the level of HuR expression, its sub-cellular location and, possibly, the insurgence of pharmacoresistance as new indicators predicting chemotherapy efficacy.

HuR and small molecules: activators

Besides genetic tools used to alter HuR expression, several pharmacological treatments have been functionally associated with modulation of HuR in terms of its sub-cellular localization, post-translational modifications or binding performances towards the RNA substrates. In this context, the functional versatility of HuR resides on the regulation of growth factors, cytokines and chemokines, inflammatory enzymes, apoptosis- and cell cycle-regulatory proteins and it has been demonstrated that modulation of HuR can bring to some benefits or caveats for the contextualized phenotype (13). Several enodogenous and exogenous molecules, for a comprehensive list (32), can trigger HuR cytoplasmic translocation and activate HuR. For example the activation of post-transcriptional HuR functionality by the p-38 MAPK pathway (22) was fundamental for the mechanism of action of many different substances. The positive wound healing effects of triterpene extract and betulin derived from birch bark transiently up-regulated pro-inflammatory cytokines and COX-2 at the gene and protein level through a process in which p38 MAPK activated HuR (143). Similarly a PPARβ agonist, L-165041, resulted in the induction of VEGF expression in HPV (Human Papillomavirus) positive Hela cells (144). The FDA approved, anti-inflammatory compound celecoxib showed a significant improvement of motor function and enhanced survival in a severe spinal motor atrophy (SMA) mouse model. This molecule increased the SMN protein levels in human and mouse neuronal cells by activating p38 that, at its turn, favored the HuR mediated stabilization of the *SMN* mRNA (48,145). Different types of signaling cascades were responsible, although not always elucidated, of HuR activation in other cases. The cell response to apigenin, a naturally occurring flavonoid with promising chemopreventive features against UV-induced DNA damage, required HuR. Indeed this molecule prompted HuR translocation to the cytoplasm enhancing p53 protein translation, the p53-dependent, p21 expression (146) as well as the Cyclooxygenase 2 (COX2) expression (147), therefore activating a protective reaction against UV. Another example came from the arachidonic acid analog 5, 8, 11, 14-eicosatetraynoicacid (ETYA), an activator of peroxisome proliferator-activated receptor PPARα. In IFNγ stimulated primary cultured rat astrocytes and microglia, ETYA suppressed *CCL2/MCP-1*transcription through up-regulation of MAPK phosphatase-1(*MKP-1*) mRNA levels, that, when translated, blocked IFNγ-induced c-JUN signaling cascade. In this case HuR cytoplasmic translocation was functional to the stabilization of the *MKP-1* mRNA (148). More controversial was the effect of 5-Aminoimidazole-4-carboxamideribonucleoside (AICAR), the most widely used AMPK activator with pleiotropic and beneficial effects on metabolic disorders. Although in RKO cells and human fibroblasts AICAR induced inhibition of the stabilizing function of HuR on target mRNAs as p21, cyclin B1 and cyclin A, with contemporary accumulation of HuR within the nucleus (149), in hepatocytes it gave opposite results (150). More recently, still in hepatocytes, it has been shown that

AICAR triggers HuR to stabilize the low density lipoprotein receptor (*LDLR*) mRNA through binding to its ARE1 region (151).

HuR and small molecules: inhibitors

A number of small molecules have been shown to interfere with the HuR activity; however their mechanism of action has not always been elucidated. For example MPT0B098 is a novel indoline-sulfonamide compound, 7-aryl-indoline-1-benzene-sulfonamide which was active against human cancer cells with IC50 values ranging from 70 to 150 nmol/L. It has been described as a potent microtubule inhibitor which suppressed hypoxia-inducible factor (HIF-1α) expression by accelerating the degradation of *HIF-1α* mRNA, a known target of HuR (152,153). MPT0B098 decreased translocation of HuR from the nucleus to the cytoplasm with parallel decrease of the stabilization of the *HIF-1α* mRNA (154). Pro-death HuR loss of function has been observed in hepatocellular carcinoma Hep3B cells during treatment with N-Benzylcantharidinamide, a novel cantharidin analog, that increased cytoplasmic HuR and *MMP-9* mRNA stability favoring the highly metastatic potential of these cells (11). N-Benzylcantharidinamide, abrogated cytosolic translocation of HuR and, consequently, the HuR dependent *MMP-9* mRNA stability (155). Similarly Triptolide, an oxygenated diterpene isolated from Tripterygium wilfordii herb, inhibited HuR shuttling from nucleus to cytoplasm and suppressed TNFα-induced *COX-2* expression by modulating *COX-2* mRNA stability in lung cancer cells (156). The beneficial effects of other natural compounds such as Green tea, Ginko Biloba and Kalopanax pictus extracts (157–159), have been shown to be dependent on HuR inhibition that led to the destabilization of key mRNAs. Finally, the anti-inflammatory activity of Methoxyphenolic compounds on human airway cells have been ascribed to the inhibition of binding of HuR to target mRNAs, indicating that they may act at the post-transcriptional level (160).

Summing up, it emerges that HuR is involved in the cell response to many different chemicals. According to the tissue and cell context, HuR can be therefore modulated mostly by changing its cellular location. However the major limitation of these studies is the elucidation of the specificity of the mechanism of action of these molecules, whilst, other efforts have been made to identify molecules that can directly inhibit the formation of the HuR-RNA complex.

Tailored strategies to identify small molecules affecting or mimicking HuR function

The *TNFα* mRNA post-transcriptional regulation represents one of the best example where the competition of different RBPs, HuR included, significantly impacts on the modulation of the cytokine protein levels (161); this competition strictly depends on the presence of *cis* AU-rich elements within the target mRNA. The interaction between recombinant HuR proteins and RNA probes corresponding to the ARE present in the 3'UTR of *TNFα* transcript has been evaluated and characterized with different biochemical assays, i.e. surface plasmon resonance (SPR) (162) and RNA electrophoresis mobility shift assay (REMSA) (163–165). The latter biochemical strategy has been applied to identify small molecules able to interfere with the protein-RNA complex formation and, consequently, in order to functionally mediate the destabilization of the HuR target *TNFα* mRNA. Among 179 chemicals screened by REMSA, two compounds, quercetin and b-40, have been identified as active chemicals *in vitro* with IC_{50} in the low micromolar range (163). Of interest, a previous work had shown that quercetin displays inhibitory activity against recombinant HuC protein (166), suggesting that, a high selective profile of low-molecular weight compounds is challenged by the structural redundancy of RNA recognition domains characterizing most of RBPs, including the ones of ELAV family. The post-transcriptional consequences of another flavonoid, the resveratrol, have emerged and have been functionally investigated in the work of Mukherjee et al (2009) (3). The authors quantified the HuR RNP dynamics during activation

of T-cells and inferred resveratrol for its induced gene expression signature in correlation with HuR dynamics by using a Connectivity MAP (CMAP), revealing the impact of this small molecule on HuR functionality. More recently, influences of resveratrol have been also associated with splicing of pre-mRNAs by modulating the expression level of different RNA processing factors, including HuR (167).The properties of the interaction between the mammalian recombinant HuR protein and the TNFα derived ARE-containing probe have been also characterized by AlphaScreen technology (164): in a high-throughput screening of a library of 2000 low molecular weight compounds cethylpiridinium choride and mitoxantrone have been identified as hits and confirmed as *in vitro* interferers of the protein-RNA complex formation also by REMSA. However, the biological effects of these molecules in virtue of their ability to inhibit RNA binding properties of HuR that they may also display *in vivo* have to be clarified. Another strategy that has been explored to specifically identify low-molecular-weight HuR inhibitors refers to the use of confocal fluctuation spectroscopic assay using a recombinant truncated HuR protein and fluorescence intensity distribution analysis (FIDA) (165). In this work 50,000 microbial, mycological and plant extracts have been screened and three compounds, dehydromutactin, MS-444 and okicenone, were identified for their capacity to interfere with HuR oligomerization and to inhibit nucleo-cytoplasmic redistribution of HuR during T-cell activation. Glioblastoma multiforme cell lines presented HuR up-regulation and the over-stabilization of HuR target mRNAs were necessary to sustain glioma growth (132). The small compound MS-444, was able to reduce targets half-lives and consequent reduced glioma cell growth properties (168), suggesting that HuR inhibition in glioblastoma cells could be a potential therapeutic strategy. MS-444 has been also used as a tool to demonstrate the mechanistic relation between HuR, miR-16 and the stabilization of *COX-2* mRNA. MS-444 inhibited HuR cytoplasmic accumulation of overexpressed HuR by preventing its oligomerization, in HeLa cells and consequent binding of HuR to mature miR-16 and *COX-2* 3'UTR (169). Derangement of mRNA stability has been reported in neurodegenerative disorders such as Alzheimer's disease (AD) and associated with down-regulation of ELAV proteins, in turn leading to destabilization of key mRNA responsible for brain development and memory processes. Taking advantage of *in silico* prediction based on the structure of the first two RNA Recognition Motifs (RRMs) which are highly conserved in the ELAV protein family, small peptides have been chemically synthesized and found able to stabilize *NOVA-1* and *VEGF* transcripts in SH-SY5Y cells when used in equimolar mixture (170). More recently, the same group has shown that the specific combination of two peptides exerts the *VEGF* mRNA stabilization (171), suggesting the possibility of designing small molecule potentially able to mimic the activity of the RBP and to be relevant in specific pathologic fields, although the selective profile, magnitude and spectrum of their biological effects has not completely covered yet. As we have highlighted a number of molecules, inhibitors and activators, have been identified with the ability to modulate HuR (Figure 2). Nevertheless, a detailed characterization of the inhibitory profile towards other RNA binding proteins and structural studies showing the binding sites of the molecules are still missing. In addition it should be important to evaluate the impact of these modulators in animal models representative of a HuR driven pathology.

Conclusion: is HuR a good drug target?

According to the animal models so far produced, pharmacological HuR inhibition would be instrumental to relief the inflammation process induced by the IL-17 in pulmonary epithelium inflammation, in $CD4^{+}$Th17-mediated autoimmune neuro inflammation or in intestinal colitis. Similarly HuR inhibition could be beneficial to inhibit tumor vascularization or to decrease the tumor burden in colon cancer. Retrospective clinical data are indeed supporting the importance of HuR in the path to malignancy (Table I). By contrast, other animals models suggests that HuR inhibition would be detrimental in macrophages, spinal cord astocytes and hippocampal neurons in which HuR is promoting an

anti-inflammatory and neuroprotective function, respectively. In terms of prognostic significance, high levels of HuR or its cytoplasmic accumulation in cancer tissues is mostly related with unfavorable prognosis. In addition to these considerations, it is worth stressing the fact that in some cases HuR presence is necessary for the full efficacy of some drugs. Therefore, especially for cancer chemotherapy, special attention should be taken when evaluating the contemporary utilization of chemotherapy with a HuR inhibitor. Evaluation of its expression level or its cell localization can be used as a readout for considering HuR inhibition as a possible therapeutic strategy. A good drug target should be endowed with a number of features that allow its selective inhibition in the tissue of interest and whose loss of function is not detrimental to other organism tissues (172). HuR clearly owns some of these relevant features as summarized in Table 2, and can be considered a good drug target. Three dimensional X-Ray structure of HuR protein is available (173,174), biochemical tools have been set-up that allow the screening of thousands of molecules (the so called "assayability") and the identification of specific inhibitors is highly feasible. However some questions arise concerning the potential side effects of a general HuR inhibition. As outlined the pleiotropic role of HuR in post-transcriptional control of gene expression is very complex, so it is difficult to predict the consequences of HuR inhibition occurring all along the entire body. Lessons from animal models teach us that a complete HuR knock-out is not compatible with life and conditional knock-out in the T-cell and myeloid progenitors causes alteration in the cell maturation and functionality, so, a general and deep inhibition of HuR would probably be very stressful for body stem cell compartments, in particular for the hematopoietic stem cells. From a molecular point of view, being HuR at the crossroad of gene expression regulation in all of the body tissues, HuR inhibition would affect post-transcriptional control ubiquitously and differently, according to the cell and tissue context. Most importantly the binding of similar *trans*-acting factors, that modulate common mRNAs of HuR, would be altered resulting in an unpredictable disequilibrium of the transcripts fate. Indeed, it has been shown that HuR is a pivotal protein regulating the homeostasis of normal cells, its development and physiological processes. To conclude, although HuR is an attractive target and its pharmacological modulation feasible, the exact pathological setting in which this should be achieved is starting to emerge, possibly the generation of conditional animal models and a deeper *in vivo* investigation of HuR involvement would come in support.

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Captions to Figures:

Figure 1: The schematic figure indicates the mice tissues where conditional knock-out mice have been produced to study HuR role in cell physiology. Pictures of human lung and intestine are used to facilitate reader recognition of the organ itself.

Figure 2: Modulators of HuR functionality; the mechanism of action proposed for each molecule can be clustered in two categories: modulators of HuR sub-cellular localization, inhibitors of HuR binding to target mRNAs.

Figure 1

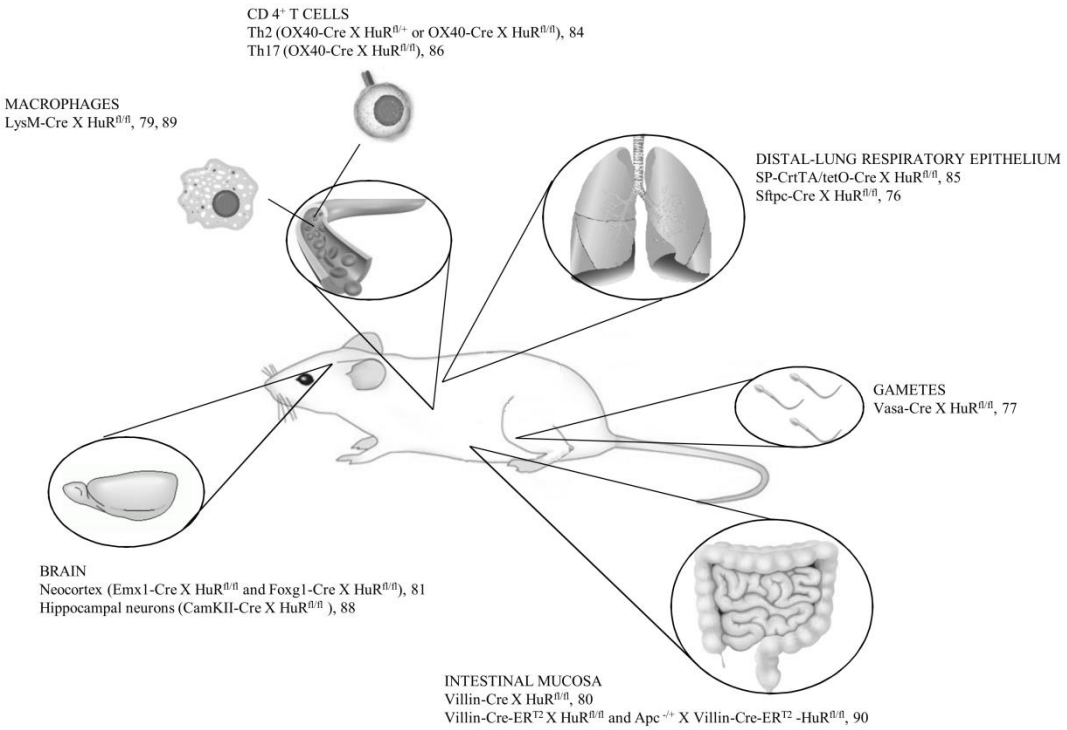
CD 4+ T CELLS
Th2 (OX40-Cre X HuR fl/+ or OX40-Cre X HuR fl/fl), 84
Th17 (OX40-Cre X HuR fl/fl), 86
MACROPHAGES
LysM-Cre X HuR fl/fl, 79, 89
DISTAL-LUNG RESPIRATORY EPITHELIUM
SP-CrtTA/tetO-Cre X HuR fl/fl, 85
Sftpc-Cre X HuR fl/fl, 76
GAMETES
Vasa-Cre X HuR fl/fl, 77
BRAIN
Neocortex (Emx1-Cre X HuR fl/fl and Foxg1-Cre X HuR fl/fl), 81
Hippocampal neurons (CamKII-Cre X HuR fl/fl), 88
INTESTINAL MUCOSA
Villin-Cre X HuR fl/fl, 80
Villin-Cre-ER T2 X HuR fl/fl and Apc -/+ X Villin-Cre-ER T2 -HuR fl/fl, 90

Figure 2

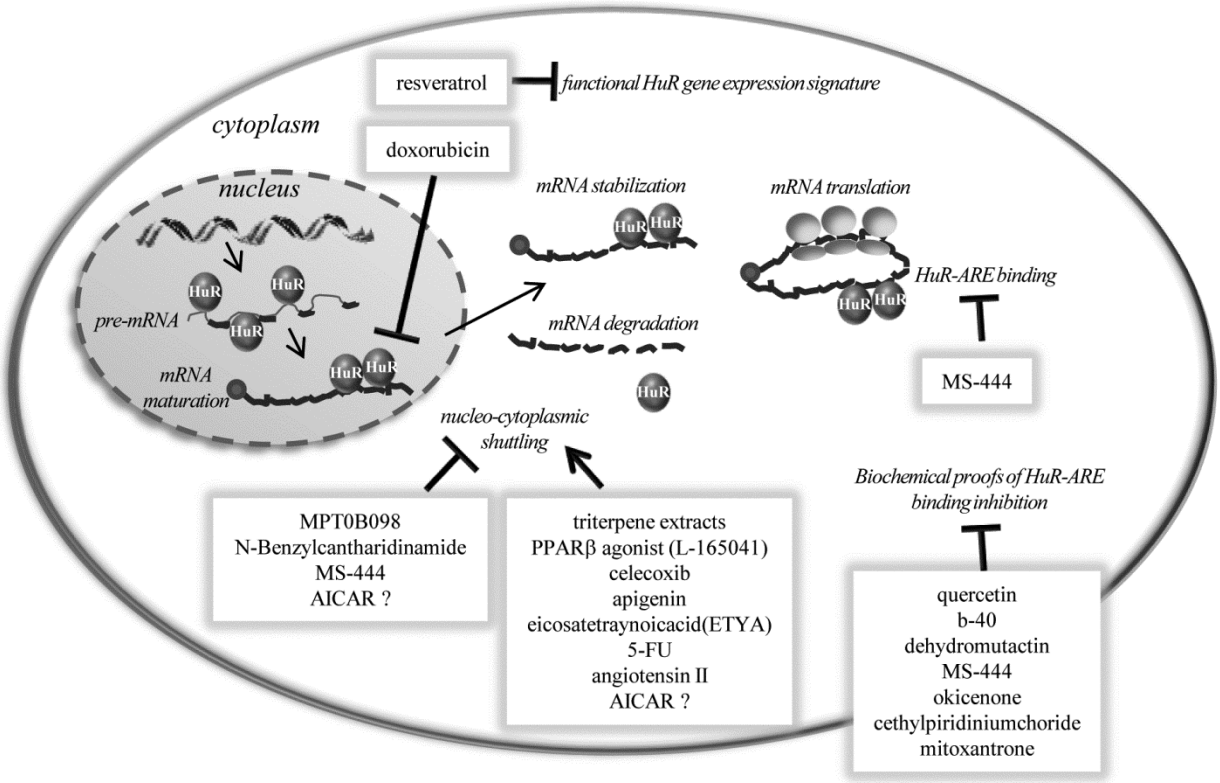
resveratrol
functional HuR gene expression signature
cytoplasm
doxorubicin
nucleus
mRNA stabilization
mRNA translation
HuR-ARE binding
pre-mRNA
mRNA degradation
mRNA maturation
MS-444
nucleo-cytoplasmic shuttling
Biochemical proofs of HuR-ARE binding inhibition
MPT0B098
N-Benzylcantharidinamide
MS-444
AICAR ?
triterpene extracts
PPARβ agonist (L-165041)
celecoxib
apigenin
eicosatetraynoicacid(ETYA)
5-FU
angiotensin II
AICAR ?
quercetin
b-40
dehydromutactin
MS-444
okicenone
cethylpiridiniumchoride
mitoxantrone

Table **1**. Association between HuR expression and localization with clinicopathological parameters and patient outcome in recent clinical studies

						Clinicopathological characteristic		**Survival analysis**		
	Type of cancer	**Year**	**Samples**	**Country**	**Method**	**High cytHuR**	**High nucHuR**	**High cytHuR**	**High nucHuR**	**Ref**
Negative prognosis	OSCC stage I-IV	2014	95	South Korea	IHC	↑Histological grade cIAP2[a]	ns ns	↓ Overall survival[b]	ns	103
		2011	103	South Korea	IHC	↑Histological grade LN metastasis Distant metastasis COX-2[c] expression	ns ns ns ns	↓ Overall survival[bd]	ns	104
	ESCC stage I-IV	2014	64	China	IHC	↑TNM stage Tumor invasion LN metastasis	ns ns ns	↓ Overall survival[bd]	ns	100
	NSCLC stage I-II	2012	54	Italy	IHC	acinar/papillary tumor histology LN involvment*	ns ns	↓ Metastasis-free survival[d] ↓ Overall survival[d] ↓ Metastasis-free survival[bd]* ↓ overall survival[bd]*	ns ns	110
	NSCLC stage I-III	2011	132	China	IHC	↑TNM stage LN metastasis ↑LVD ↑MVD	ns ns ns ns	↓ Relapse-free survival[bd] ↓ Overall survival[bd]	ns ns	101

	BC stage II-III	2013	139	China	IHC	invasive ductal tumor type ↑TNM stage ↑Histological grade negative estrogen receptor negative progesteron receptor	NR	↓ Local recurrence-free survival[bd] ↓ Distant recurrence-free survival[bd] ↓ Recurrence-free survival[bd] ↓ Overall survival[bd]	NR	112
	BC stage I-III	2013	82	China	IHC	↑Histological grade positive estrogen receptor positive progesteron receptor HER-2/neu[e] overexpression P-gp[f] expression	ns ns ns ns	↓ Disease-free survival[bd] ↓ Overall survival[bd]	ns ns	106
	GIST	2013	372	Taiwan	IHC	epithelioid histology ↑Tumor size Ki-67 labeling index Cyclin A expression ↑NIH risk level	ns ns ns ns ns	↓ Disease-free survival[bd]	ns	105
	GBC stage I-IV	2013	164	Taiwan	IHC	↑Histological grade ↑TNM stage pT status Ki-67 labeling index Vascular invasion	↑Histological grade ↑TNM stage pT status Ki-67 labeling index Vascular invasion Perineurial invasion Tumor necrosis	↓ Disease-free survival[bd] ↓ Disease-specific survival[bd]	↓ Disease-free survival[d] ns	109

	NMIBC stage I-III	2013	122	Japan	IHC	↑Histological grade ↑TNM stage COX-2[c] expression VEGF[g]-A and VEGF-C expression ↑LVD ↑MVD	ns ns ns ns ns ns	↓ Recurrence-free survival[d] ↓ Cause-specific survival[d] ↓ Metastasis-free survival[bd]	ns ns ns	102
	UTUC stage I-IV	2012	340	Taiwan	IHC	↑pT status ↑Histological grade LN metastasis Infiltrative or trabecular invasion Vascular and perineurial invasion Cyclin A expression	NR	↓ Disease-specific survival[bd] ↓ Metastasis-free survival[bd] ↓ Recurrence-free survival[d]	NR	99
PP	BC stage I-IV	2011	143	China	RT-PCR	↓ TNM stage**		↑ Overall survival** ↑ Disease-free survival**		111
NS	Lung ADC, lung SCC stage I-IV	2011	244	South Korea	IHC	Poor differentiation of tumor	ns	ns	ns	107
	OSCC stage I-IV	2011	96	South Korea	IHC	LN metastasis	ns	ns	ns	108

[a]cellular inhibitors of apopotosis protein-2, [b]Multivariate analysis, [c]Cyclooxygenase-2, [d]Univariate analysis, [e]Human epidermal growth factor receptor 2, [f]P-glycoprotein, [g]Vascular endothelial growth factor-, *Nuclear to Cytoplasm ratio, **Total HuR RNA

Abbreviations: ns non significative, LN = lymph node, LVD lymphatic microvessel density, MVD microvessel density, NR not reported, PP Positive prognosis, OSCC oral squamous cell carcinoma, ESCC esophageal squamous cell carcinoma, NSCLC non-small cell lung carcinoma, BC breast cancer, GIST gastrointestinal stromal tumor, GBC gallbladder carcinoma, NMIBC non-muscle invasive bladder cancer, UTUC upper urinary tract urothelial carcinoma, ADC adenocarcinoma, SCC squamous carcinoma.

Table 2. Features of HuR as drug target are reported, Table adapted from (135).

Properties of an ideal drug target	HuR fulfillment	HuR as drug target
Target is associated with the pathophysiology of a disease	YES	HuR is involved in the etiology of many different diseases (cancer, inflammation and neurological disease)
Modulation of target is less important under physiological conditions or in other diseases	YES	HuR inhibition seems to lead to clinical benefit in pathologic inflammation and cancer, more animal models are required
Presence of 3-D structure for the target protein for a druggability assestment	YES	HuR structure is well characterized
Target favorable 'assayability' enabling high throughput screening	YES	Quantitative and biochemical assays amenable for high throughput screening platforms have been developed for the identification of HuR molecular inhibitors
Target expression is not uniformly distributed throughout the body	NO	HuR is ubiquitously express
A target/disease-specific biomarker exists to monitor therapeutic efficacy	YES	Depends on the pathology, expression level of key mRNAs (COX2, cytokines) as biomarker. Alternatively HuR expression level or its subcellular localization could be used as markers for evaluate the possible therapeutic efficacy of HuR modulation
Favorable prediction of potential side effects according to phenotype data	NO	There is no knowledge about the possible impact of a systemic HuR-inhibition. Due to HuR central role in post-trascriptional regulation the consequences of its modulation are unpredictable. Moreover complete HuR KO is not compatible with life in mice models.