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Review

HuR, a key post-transcriptional regulator, and its implication in progression of breast cancer

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Summary. HuR, an ubiquitously expressed member of the Hu family, selectively binds and stabilizes ARE-containing mRNAs encoding proto-oncogenes, cell cycle regulators, cytokines and growth factors. The mechanism of HuR stabilization on target mRNAs is believed to be mediated through competition with destabilizing ARE-BPs. HuR is mainly localized within the cell nucleus and the nucleo-cytoplasmic shuttling of HuR is generally assumed as the initial and critical step of its stabilizing effects. A number of signaling pathways are believed to be involved in HuR shuttling. Due to the pivotal role played by HuR in stabilizing the mRNA of key factors or cytokines involved in carcinogenesis and subsequent progression, its implication and therapeutic potential in cancer have been investigated intensively since its discovery in 1996. This review discusses the role of HuR in the stabilization of key mRNAs and it's the nucleo-cytoplasmic shuttling. The review also covers the current knowledge of HuR's role in carcinogenesis, particularly its involvement in breast cancer, and the feasibility of using HuR as a therapeutic target for the treatment of breast cancer.

Key words: HuR, AU- and U-rich elements (AREs), Nucleo-cytoplasmic shuttling, Carcinogenesis, Breast cancer

Introduction

The human (Hu) antigen R (HuR) was first cloned and identified as a member of the Hu family of RNA-binding proteins in 1996 (Ma et al., 1996). Analyzing the fluorescence signals on banded chromosomes, the human HuR gene was localized to human chromosome 19p13.2 (Ma and Furneaux, 1997). The Hu protein family comprises four vertebrate members, the primarily neuronal proteins HuB (Hel-N1), HuC (PLE21) and HuD, and the ubiquitously expressed 34 kDa protein HuR (Ma and Furneaux, 1997). Hu proteins were first identified as target antigens of paraneoplastic neurological syndrome and found to play a role in neuron-specific RNA processing (Szabo et al., 1991). The Hu proteins were found to share homology with the *Drosophila* embryonic lethal abnormal vision (ELAV) proteins and are also regarded as the Hu/elav family (Yao et al., 1993). In humans, HuR is widely expressed in all proliferating cells, whereas HuB, HuC and HuD are expressed in terminally differentiated neurons and are therefore called the neuronal Hu proteins (Peng et al., 1998). The Hu protein family have been identified as playing an important role in the development, plasticity and memory of neurons (Lazarova et al., 1999; Deschenes-Furry et al., 2006). Two recent studies by Ghosh et al and Katsanou et al have demonstrated the importance of HuR *in vivo*. These studies describe the generation of conventional and conditional HuR knockout mice and demonstrate the importance of HuR in embryonic development and in the survival and homeostasis of adult mice (Ghosh et al., 2009; Katsanou et al., 2009).

The HuR protein (Fig. 1) contains three classic RNA recognition motifs (RRMs) that share greater than 90% amino acid sequence identity among family members (Okano and Darnell, 1997). RRM1 and RRM2 are found to bind and mediate the AU- and U-rich element (collectively termed AREs) recognition (Ma et al., 1997; Inoue et al., 2000). RRM3 is believed to bind the poly (A) tail and help maintain the stability of the RNA-protein complex (Ma et al., 1997; Beckel-Mitchener et al., 2002). In addition to the common characteristic structure of the three highly conserved RRMs, HuR

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contains a variable basic hinge region between its RRM2 and RRM3 (Brennan and Steitz, 2001). The less conserved hinge region contains a sequence called the HuR nucleo-cytoplasmic shuttling sequence (HNS) that allows HuR to shuttle back and forth between the nucleus and cytoplasm.

HuR has a variety of biological functions which are all based on its ability to stabilize mRNAs bearing AU- and U-rich elements and hence, affect their expression (Myer et al., 1997). Many AREs-containing mRNAs may contribute to carcinogenesis since they encode functionally diverse proteins promoting proliferation, angiogenesis, tissue invasion, and other characteristic features of cancer cells. In this review, we discuss HuR's ability to stabilize mRNA and its nucleo-cytoplasmic shuttling. The review also focuses on the current knowledge of HuR's role in oncogenesis, particularly its involvement in breast cancer and the feasibility of using HuR as a therapeutic target for breast cancer.

HuR stabilizes AREs-containing mRNA

HuR recognize and bind to AREs of mRNAs

The growth and development of eukaryotic organisms require that gene expression be regulated (Standart and Jackson, 1994; Guhaniyogi and Brewer, 2001). An important mechanism of post-transcriptional gene regulation in mammalian cells is the control of cytoplasmic mRNA stability mediated by ARE in its 3' untranslated region (3' UTR) (Peng et al., 1998). In an effort to understand the mechanism and regulation of ARE-signalled mRNA degradation a number of laboratories have looked for proteins that selectively bind AREs. Only two of these proteins, AUF1 (hnRNP D) and HuR, have been demonstrated to alter the stability of ARE-containing mRNAs *in vivo* (Zhang et al., 1993; Ma et al., 1996; Myer et al., 1997). Later studies suggest that AUF1 can bind AREs of target mRNAs and have a destabilizing effect (Lal et al., 2004). HuR however, can recognize and bind to AREs of target mRNAs and prevent their degradation, thus, indirectly enhancing protein production (Ma et al., 1996; Myer et al., 1997). AREs are divided into three classes based on sequence similarities: Class I AREs contain multiple copies of an AUUUA motif within U-rich regions; class II AREs contain two or more overlapping nonamers containing the AUUUA motif; and class III AREs are U-rich regions without AUUUA pentamers (Chen and Shyu, 1995). Although HuR has been shown to bind to representative mRNAs from all three ARE classes (Dean et al., 2001), most of the specific mRNAs bound by HuR *in vitro* contain class I or class II AREs (Brennan and Steitz, 2001). Interestingly, whilst considerable studies have indicated HuRs role in stabilizing target mRNAs there is emerging evidence to suggest that it may also be capable of regulating translation and studies have shown HuRs ability to alter translational levels of type I insulin-like growth factor receptor (IGF-IR) (Meng et al., 2008), hypoxia-inducible factor-1 alpha (HIF-1α) (Galban et al., 2008) and p53 (Mazan-Mamczarz et al., 2003).

Target mRNAs of HuR

ARE mRNAs represent as much as 5-8% of human genes (Bakheet et al., 2001). The development of *in vivo* and *in vitro* systems has facilitated the study of ARE mRNAs stabilized by HuR. Numerous ARE-containing mRNAs have been reported either to associate with HuR or, to be post-transcriptionally regulated by HuR (Table 1). Many of them encode functionally diverse proteins that are important in many biological processes including cell growth and differentiation, signal transduction, transcriptional and translational control, haematopoiesis, apoptosis, nutrient transport and metabolism (Bakheet et al., 2003).

Competing with destabilizing ARE- binding proteins

Although numerous target mRNAs of HuR have been identified, the mechanisms by which HuR stabilizes mRNAs is still only partially understood. One possible mechanism for HuR stabilization of target mRNAs is believed to be mediated through competition with destabilizing ARE- binding proteins (ARE-BPs). Destabilizing ARE-BPs, such as the protein tristetra-prolin (TTP) and its homolog BRF-1, can enhance the rate of degradation of mRNAs through multiple mechanisms (Barreau et al., 2005). TTP is shown to stimulate the deadenylase PARN (poly (A)

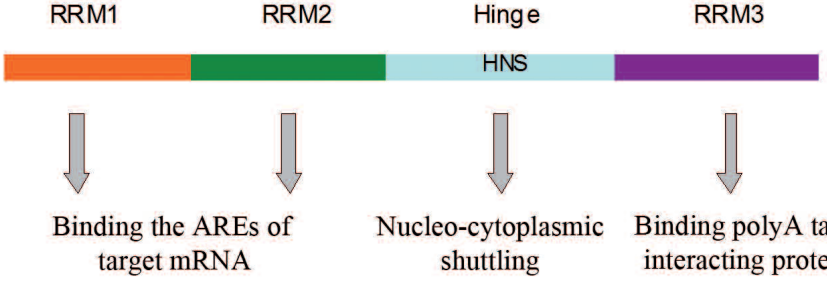

Fig. 1. HuR protein contains three RNA recognition motifs (RRMs), a hinge region and HuR nucleo-cytoplasmic shuttling (HNS) sequence. The function of certain domains are listed below.

ribonuclease), subsequently increasing the rate of removal of the 3' poly (A) tail and thus, contributing to the rate-limiting step of mRNA degradation (Lai and Blackshear, 2001). It has also been shown that TTP and BRF-1 associate with decapping enzymes, suggesting that they may enhance decapping-dependent 5'-3' degradation of their target mRNAs (Lykke-Andersen and Wagner, 2005). The mechanism by which HuR potentially blocks the association of destabilizing proteins may be through the formation of oligomers on their target mRNAs. Deletion analysis indicates that both the hinge region and RRM3 are important for assembly of HuR oligomers (Hinman and Lou, 2008). In addition, a truncated form of HuR without RRM3 fails to stabilize reporter mRNAs containing AREs (Fan and Steitz, 1998; Anderson et al., 2000). It is conceivable that without RRM3, HuR cannot oligomerize on their target mRNAs and its ability to prevent mRNA decay may also be weakened.

HuR and AUF1

Recent studies suggest that HuR may be able to antagonize the effects of destabilizing proteins through some complicated mechanism other than simply preventing their binding to the same sites on target mRNAs. HuR and AUF1 bind to many common AU-rich target mRNAs and exert an opposing influence on mRNA stability, with HuR having a stabilizing effect and AUF1 having a destabilizing effect. Using common target RNAs encoding p21 and cyclin D1, Lal et al provided evidence that HuR and AUF1 can simultaneously bind target transcripts on both distinct, nonoverlapping sites, and on common sites in a competitive fashion (Lal et al., 2004). In the nucleus, both proteins were found together within stable ribonucleoprotein complexes; in the cytoplasm, HuR and AUF1 were found to bind to target mRNAs individually, HuR co-localizing with the translational apparatus and AUF1 with the exosome (Lal et al., 2005). The simultaneous binding of HuR and AUF1 was observed mainly in the nucleus and the authors propose a model in which either HuR or AUF1 dissociates from the mRNA once it reaches the cytoplasm, allowing the mRNA to enter either the stabilization or destabilization pathway (Lal et al., 2004). However, David et al obtained slightly different results, suggesting that HuR and AUF1 could functionally interact both within the nucleus and in the cytoplasm (David et al., 2007). These studies suggest that the precise mechanism whereby HuR antagonizes the effects of destabilizing proteins is only partially understood.

Table 1. A list of previous known target mRNAs of HuR.

mRNA (type or function)	Reference
c-myc (proto-oncogene)	Lafon et al., 1998
c-fos (proto-oncogene)	Mili and Steitz, 2004
cyclin A (cell cycle)	Wang et al., 2000a
cyclin B1 (cell cycle)	Wang et al., 2000a
cyclin D1 (cell cycle)	Wang et al., 2000a
cyclin E1 (cell cycle)	Guo and Hartley, 2006
Cyclooxygenase (inflammation)	Lasa et al., 2000
EGF (proliferative factors)	Sheflin et al., 2004
Estrogen receptor α (hormone mediator)	Pryzbylkowski et al., 2008
Galectin-1 (cytokines)	Nabors et al., 2001
GM-CSF (proliferative factors)	Esnault and Malter, 2003
HIF-1α (angiogenesis promoter)	Sheflin et al., 2004
MMP-9 (matrix metalloproteases)	Lopez de Silanes et al., 2004b
MTA1 (matrix metalloproteases)	Lopez de Silanes et al., 2004b
p21 (cell cycle regulator)	Wang et al., 2000b
ProTα (anti-apoptotic)	Lal et al., 2004
TGF-ß (cytokines)	Lopez de Silanes et al., 2004b
uPA (matrix metalloproteases)	Tran et al., 2003
VEGF (angiogenesis promoter)	Levy et al., 1998

Nucleo-cytoplasm shuttling of HuR

Cytoplasmic abundance of HuR

Previous studies have found that HuR is predominantly (>90%) localized in the nucleus of most unstimulated cells and, upon stimulation, it can translocate to the cytoplasm where it binds target mRNAs and prevents their decay (Wang et al., 2000; Chen et al., 2002). In a global survey of HuR abundance and subcellular localization in human tissues containing approximately 300 paired cancer and normal specimens, it is shown that cytoplasmic HuR expression is elevated in a wide variety of human carcinomas compared with normal tissue samples and this is found to be especially evident in colon cancer (Lopez de Silanes et al., 2003). An increased cytoplasmic HuR abundance is characteristic of various types of cancer and, therefore, the level of cytoplasmic HuR seems to be an eligible indicator of patient survival prognosis in some specific cancers (Dixon et al., 2001; Erkinheimo et al., 2003; Lopez de Silanes et al., 2003; Denkert et al., 2004a,b). However, some studies suggest a contrasting view with low HuR expression being predictive of higher recurrence risk in early stage breast cancer patients (Ortega et al., 2008). The stimulus-dependent transport between the nucleus and the cytoplasm and vice-versa, denoted as "HuR shuttling" is generally assumed as the initial and critical step of HuR-controlled mRNA stabilization (Ma et al., 1996; Antic and Keene, 1997). Further work identifying pathways and mechanisms involved in HuR shuttling will be of substantial interest.

HNS and HuR ligands

Nucleo-cytoplasmic shuttling of HuR structurally relies on a sequence in the hinge region located between its second and third RRM, which is denominated as HNS (HuR nucleocytoplasmic shuttling sequence) and contains both nuclear localization and export signals (Fan and Steitz, 1998). The association of HuR with four ligand proteins is believed to influence HuR export from

the nucleus and possibly to also modulate HuR's affinity for its target mRNAs (Brennan et al., 2000). The four ligands are SETα/ß (von Lindern et al., 1992; Matsumoto et al., 1993), pp32 (Malek et al., 1990) and acidic protein rich in leucine (APRIL) (Brennan et al., 2000). Three of these HuR ligands (SET·, SET , and pp32) have previously been identified as inhibitors of protein phosphatase 2A (PP2A) (Li et al., 1996; Saito et al., 1999).

Signaling pathways involved in HuR shuttling

Although, numerous signaling cascades have been demonstrated to play an important role in nucleo-cytoplasmic shuttling of HuR, the precise mechanism underlying HuR trafficking is not well understood. In recent studies, a discrete number of signaling pathways have been demonstrated and are believed to be involved in the nucleo-cytoplasmic shuttling of HuR. These include the AMP-activated kinase (AMPK) (Wang et al., 2002), the protein kinase C (PKC) family (Doller et al., 2007, 2008), the mitogen-activated protein kinases (MAPKs) (Winzen et al., 1999; Ming et al., 2001) and its upstream kinase and the MAPK activated protein kinase-2 (MK2) (Subbaramaiah et al., 2003; Tran et al., 2003). AMPK can influence mammalian protein expression in numerous ways including transcriptional activation (Zheng et al., 2001), modulation of both protein synthesis (Bolster et al., 2002) and mRNA turnover. The latter is mainly attributed to an AMPK-dependent inhibition of nucleo-cytoplasmic HuR shuttling (Wang et al., 2002, 2003). Increased AMPK activity, by elevations in the AMP/ATP ratio, seems particularly relevant to cellular senescence through mechanisms which include an impaired HuR function (Wang et al., 2003). Conversely, AMPK inhibition by UVA and ATP substantially increases the cytoplasmic HuR levels concomitant with an elevation in the half-lives of HuR-regulated mRNAs encoding prominent cell growth regulating genes including p21, cyclin A and cyclin B1 (Wang et al., 2002, 2003).

PKC is a family of serine/threonine protein kinases consisting of at least 10 different isoforms. Following treatment of rat mesangial cells (MC) with the stable ATP analog adenosine 5'Othiotriphosphate (ATPγS), Huwiler et al observed nucleo-cytoplasmic shuttling of HuR under confocal microscopy, which functionally lead to an increased mRNA stability of MMP-9 (Huwiler et al., 2003). The regulation of HuR by ATP has been shown to be a general principle occurring in human mesangial cells from different species (Doller et al., 2007). Using different pharmacological inhibitors, the ATP-dependent HuR shuttling in human MC is found to be strongly impaired by specific PKCα inhibitor species. In addition, silencing of PKCα by small interference (si) RNA is found to impair the ATP stimulated HuR translocation to the cytosol (Doller et al., 2007). These findings strongly indicate that PKCα is a crucial signalling pathway in HuR shuttling. However, the precise mechanism of PKCα's involvement in HuR shuttling needs to be addressed in future experiments.

Previous studies indicated that the MAP kinase (MAPK) family, namely the p38 MAP kinase (p38-MAPK), was involved in HuR-triggered stabilization of mRNAs encoding key inflammation mediators (Doller et al., 2008), such as tumour necrosis factor α (TNFα) (Dean et al., 2001), IL-8 (Winzen et al., 2004), cyclooxygenase-2 (COX-2) (Dixon et al., 2001; Nabors et al., 2001) and GM-CSF (Fan and Steitz, 1998). In addition, the p38-MAPK and its downstream target MAPKAPK-2 (MK2) have demonstrated a critical involvement in HuR-mediated stabilization of COX-2 in human mammary epithelial cells by the taxanes docetaxel and paclitaxel (Subbaramaiah et al., 2003; Dean et al., 2004). Stabilization of urokinase and urokinase receptor mRNAs by HuR is delineated to a MK2-induced cytoplasmic HuR accumulation (Tran et al., 2003). Furthermore, the angiogenesis inhibiting drug thalidomide was found to destabilize COX-2 mRNA by inhibiting p38 activity and this was accompanied by a reduction in HuR shuttling (Jin et al., 2007).

In summary, the AMPK, the PKC, the p38-MAPK and MK2 have demonstrated a critical involvement in the nucleo-cytoplasmic shuttling of HuR. However, the mechanism underlying HuR shuttling is complex and additional research is required to elucidate fully the underlying signaling pathways and interactions involved in this process.

HuR and carcinogenesis

Expression and intracellular distribution of HuR in cancer

The human HuR gene is localized on chromosome 19p13.2 (Ma and Furneaux, 1997), a locus associated with a number of translocations and oncogenic gains in human tumours (Ma and Furneaux, 1997). The first indication of elevated HuR expression in a human adenocarcinoma was seen in colorectal cancer (Dixon et al., 2001). Subsequent studies strongly indicate a correlation between the expression of HuR and cancer. Using immunohistochemical analysis, Lopez de Silanes et al. carried out a systematic comparison between HuR expression in a variety of cancers and their normal tissue counterparts, such as stomach adenocarcinoma, lung squamous cell carcinoma, colon adenocarcinoma and breast infiltrating duct carcinoma. Elevated HuR expression and cytoplasmic presence was detected in all malignancies examined, particularly colon cancer (Lopez de Silanes et al., 2003). In addition, the importance of HuR expression in human malignancies has been emphasized, and subsequent studies have shown cytoplasmic HuR expression to be a marker of poor prognosis in breast (Heinonen et al., 2005), ovarian (Erkinheimo et al., 2003), and gastric adenocarcinomas (Denkert et al., 2004; Mrena et al., 2005). However, low tumour expression of HuR has also been suggested as a predictive factor in disease recurrence in early stage

breast cancer patients (Ortega et al., 2008). Thus, HuR may have a complex role in cancer progression, potentially acting on a variety of target mRNAs with both cancer suppressive and promotive functions.

The role of HuR in carcinogenesis

Subcutaneous injection of HuR-overexpressing RKO colorectal carcinoma cells into nude mice produced significantly larger tumours than those arising from control populations. Conversely, RKO cells expressing reduced levels of HuR, through small interference (si)RNA- or antisense HuR-based approaches, developed significantly slower (Lopez de Silanes et al., 2003). By comparing gene expression patterns, using cDNA arrays, in RKO wild type cells and those expressing differential levels of HuR, the influence of HuR on global steady-state mRNA in intact RKO cells was investigated. The effects of HuR on gene expression programs was also evaluated in mouse xenografts (Lopez de Silanes et al., 2004). In these two colon cancer studies, many HuR-regulated genes are identified which coded for proteins promoting proliferation, angiogenesis, tissue invasion, and other features of cancer cells, and may thus contribute to oncogenesis (Lopez de Silanes et al., 2004). Based on these observations, Lopez de Silanes et al proposed that HuR plays a pivotal role in cancer by binding to mRNAs encoding proteins involved in malignant transformation, and inducing their expression via mRNA stabilization and/or altered translation (Lopez de Silanes et al., 2005). These findings underscore HuR's function in regulating a number of key properties of malignant cells, a function that likely relies on its ability to modulate the target mRNAs involved in the malignant traits.

HuR and tumour progression

Normal human cells become neoplastic by progressively acquiring mutations in cancer genes (Vogelstein and Kinzler, 2004). These mutations provide the cell with a competitive growth advantage such as, enhanced cell division, resistance to apoptosis, maintenance of angiogenesis, tissue invasion and metastasis, and evasion of antitumour immune responses (Hanahan and Weinberg, 2000). Based on the previous findings, HuR was proposed to play a central role in tumour progression (Fig. 2). Through its association with target mRNAs, HuR was found to enhance the expression of many growth-promoting, proliferative, and proto-oncogenic factors like EGF (Sheflin et al., 2004), GM-CSF (Esnault and Malter, 2003), cyclin A, cyclin B1 and cyclin D1, c-myc and c-fos (Wang et al., 2000, 2001; Lal et al., 2004). HuR has also been shown to bind and stabilize pro-angiogenic factor mRNAs resulting in elevated expression of HIF-1α (Sheflin et al., 2004) and

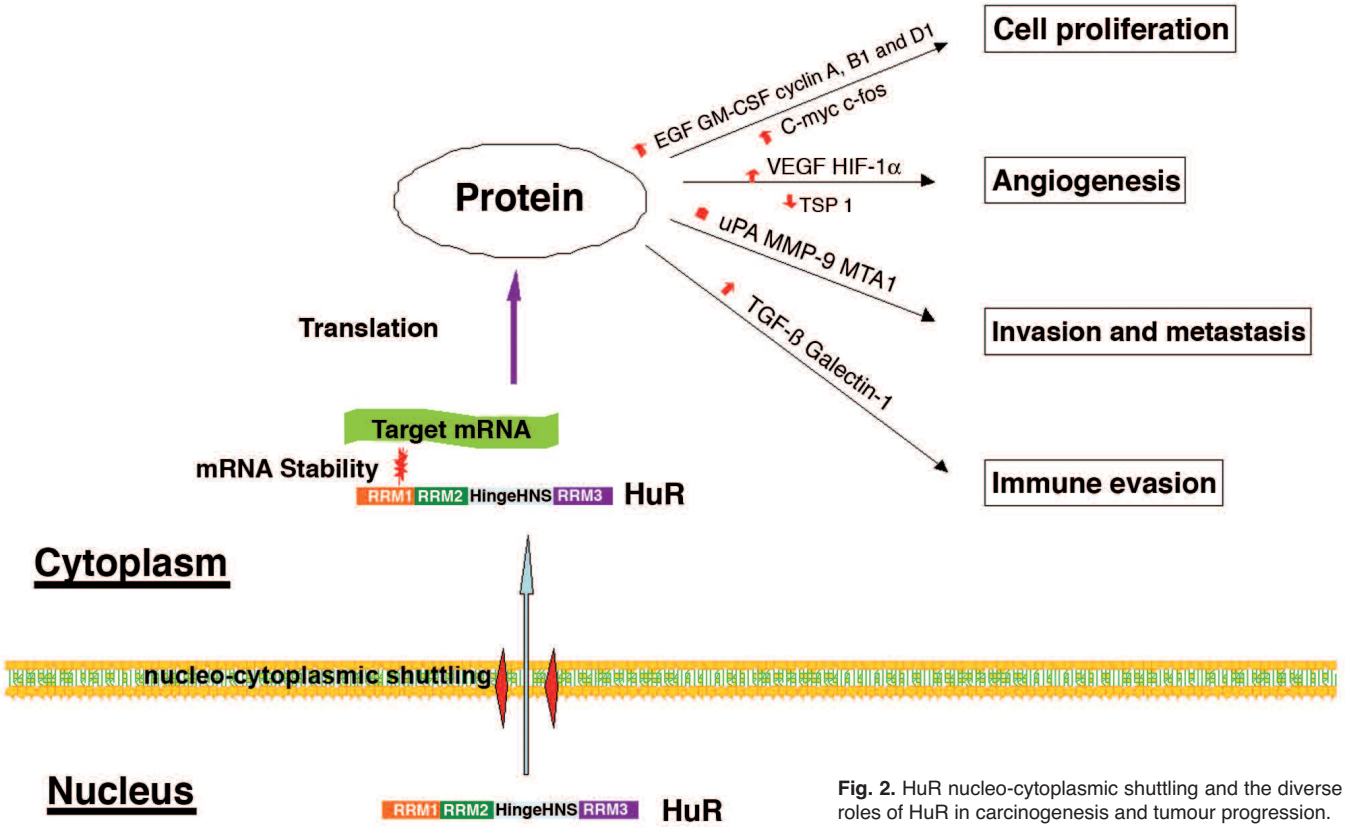

Fig. 2. HuR nucleo-cytoplasmic shuttling and the diverse roles of HuR in carcinogenesis and tumour progression.

VEGF (Levy et al., 1998). In contrast, HuR can also stablize mRNA of anti-angiogenic factors, such as thrombospondin 1 (TSP1) (Mazan-Mamczarz et al., 2008). This suggests that HuR is involved in the regulation of angiogenesis by both strengthened binding of HuR to pro-angiogenic factors and weakened association with anti-angiogenic factors in tumours and contributes to promotion of new vascularity. In addition, it has been postulated that HuR can expand the cell's invasiveness and metastatic ability by elevating the expression of mRNAs encoding uPA (Tran et al., 2003), MMP-9 and MTA1 (Lopez de Silanes et al., 2004). Furthermore, HuR was found to help the tumour to evade immune recognition by enhancing the expression of immunosuppressive cytokines such as TGF-ß (Lopez de Silanes et al., 2004) and the T-cell inhibitor galectin-1 (Nabors et al., 2001). Thus, HuR can influence the expression of numerous traits vital to the development and progression of cancer. It should also be noted however that HuR may, in some circumstances, have anti-tumourigenic roles. One such study has suggested a possible role for HuR in binding p53 mRNA and enhancing its translation in colorectal carcinoma cells following UV exposure (Mazan-Mamczarz et al., 2003)

HuR and breast cancer

Based on previous findings HuR has, similar to other cancers, been proposed to be involved in breast carcinogenesis and tumour progression. Besides the functional roles of HuR in cell growth and proliferation of breast cancer, the post-transcriptional effects of HuR on the expression of various target genes are believed to contribute to breast carcinogenesis (Lopez de Silanes et al., 2005). Through its post-transcriptional regulation of target mRNA, HuR has been shown to mediate cellular response to DNA damage in various cancer cells (Wang et al., 2000; Lafarga et al., 2009). Recent studies indicate that HuR enhances the expression of various anti-apoptotic proteins, such as the apoptosome inhibitor prothymosin α (Lal et al., 2005). HuR has been proposed to play a pivotal role in breast cancer by binding to target mRNAs encoding proteins involved in malignant transformation and altering their expression via post-transcriptional mechanisms (Nabors et al., 2001; Lopez de Silanes et al., 2005). Additionally, it has recently been found that a microRNA (miR-125a) can translationally repress HuR, decreasing HuR protein levels and inhibiting the growth of breast cancer cells (Guo et al., 2009).

Association of cytoplasmic HuR expression with poor outcome, potential links to breast cancer susceptibility genes BRCA1 and BRCA2

Approximately 7% of newly diagnosed breast cancers are due to hereditary predisposition (Claus et al., 1996). Two tumour-suppressor genes, BRCA1 and BRCA2, have been identified and shown to predispose to breast cancer (Miki et al., 1994; Wooster et al., 1995). Examination of HuR abundance and subcellular localization in breast infiltrating ductal carcinoma and normal tissue counterparts revealed higher HuR expression and cytoplasmic presence in breast carcinoma samples (Heinonen et al., 2005). Additionally, high cytoplasmic HuR expression in breast invasive ductal carcinoma was found to be associated with a poor histologic differentiation, large tumour size and poor patient survival rates in ductal breast carcinoma (Heinonen et al., 2005). Subsequent studies undertaking immunohistochemical analysis of HuR expression in familial non-BRCA1/2 and BRCA1/2 mutation breast carcinoma specimens indicated that cytoplasmic HuR expression was found to be more frequent in patients with BRCA1 or BRCA2 mutations (Heinonen et al., 2007). BRCA1 is a breast cancer susceptibility gene which is down-regulated in a significant proportion of sporadic breast cancers and is post-transcriptionally regulated by RNA-binding proteins (Rice and Futscher, 2000). Using bioinformatic analysis of the BRCA1 3'UTR and RNA-protein assays of HuR protein and BRCA1 3'UTR, Saunus et al. describe the identification of two predicted HuR-binding sites in the BRCA1 3'UTR. One of these sites binds specifically to HuR, and this interaction is disrupted by singular nucleotide substitions in BRCA1 3'UTR in breast cancer cells (Saunus et al., 2008). HuR selectively stabilizes target mRNA by binding its 3'UTR; conversely, expression of ectopic HuR results in a significant decrease in BRCA1 protein expression and also BRCA1 3'UTR activity (Saunus et al., 2008). These results demonstrate that HuR may be an important post transcriptional regulator of BRCA1 in human breast cancer, however the precise underlying mechanism needs to be further understood. Collectively, these studies suggest that high cytoplasmic HuR expression is associated with poor patient outcome in breast cancer and this may in some part due to its potential role in the regulation of the pre-dispositional factors BRCA1 and BRCA2

Interaction between HuR and estrogen receptor and its implication in hormone therapy

Estrogen receptor (ER) is one of the most important molecular factors used in clinical practice to predict the prognosis and response to therapy of breast cancer patients (Shao and Brown, 2004). The control of ER mRNA stability is mediated by HuR through binding to its 3'UTR (Pryzbylkowski et al., 2008). Treatment of ER positive breast cancer cells with the DNA methyl-transferase inhibitor, 5-aza 2' deoxycytidine (AZA), and histone deacetylase inhibitor, trichostatin A (TSA), results in decreased cytoplasmic accumulation of HuR leading to a reduction in ER mRNA stability and subsequently reduced ER mRNA and protein levels (Pryzbylkowski et al., 2008). siRNA inhibition of HuR expression reduces both the steady-state and stability of ER mRNA and, thus, it has been proposed that HuR plays a critical role in the control of ER mRNA stability (Pryzbylkowski et al., 2008). However, its association

with ER resistance and hormone therapy are currently poorly understood.

Tamoxifen is a selective ER modulator, with mixed ER agonist/antagonist activities, that is thought to work through the competitive blockade of the ER, thereby inhibiting estrogen-dependent gene transcription and tumour growth (Osborne, 1998). Despite its benefit in patients with all stages of ER-positive breast cancer, the major obstacle to its use is treatment resistance, which either occurs de novo or is later acquired after initial benefit and eliminates therapeutic effectiveness (Jaiyesimi et al., 1995). While investigating how HuR alters ER mRNA stability it became clear that HuR may play a role in tamoxifen resistance (Hostetter et al., 2008). Acute treatment with tamoxifen can increase cytoplasmic localization of HuR and pp32 and such distribution may contribute to the development of resistance. Hostetter et al. also provide evidence that the subcellular distribution of HuR is a key to tamoxifen responsiveness and decreasing cytoplasmic levels of HuR increases the responsiveness in both tamoxifen sensitive and tamoxifen resistant cell lines (Hostetter et al., 2008). Although these findings indicate a role for HuR in tamoxifen resistance, elucidating the underlying mechanisms will be more challenging.

HuR regulates non-ARE c-fms

Besides specific binding to ARE-containing mRNA, recent studies also show that HuR regulates gene expression by binding non-AU-rich sequences in 3'UTR c-fms RNA in breast cancer cells. The c-fms proto-oncogene is expressed by the tumour epithelium in several human epithelial cancers (el-Kabbani et al., 1991; Ide et al., 2002). Activation or overexpression of c-fms confers invasive and metastatic properties in breast cancer (Sapi et al., 1996; Toy et al., 2005). In a large cohort breast cancer tissue array, c-fms is strongly associated with lymph node metastasis and poor survival (Kluger et al., 2004). Using a large breast-cancer tissue array, Woo et al found that HuR was co-expressed with c-fms in breast tumours. Additionally, the study showed that the over-expressing or silencing of HuR resulted in the respective significant up- or down-regulation of c-fms RNA expression and also found that the known glucocorticoid stimulation of c-fms RNA and protein was largely dependent on the presence of HuR. Based on these findings, Woo et al. describe c-fms mRNA as a direct target of HuR *in vivo* and suppose that HuR binds specifically to a 69-nt region containing 'CUU' motifs in 3'UTR c-fms RNA (Woo et al., 2009). Thus, the contribution of HuR to the progression of breast cancer may also be partially due to the regulation of non-ARE mRNA such as c-fms.

Conclusion

HuR has a central, functional role in carcinogenesis and tumour progression by binding to target mRNAs that encode proteins involved in cell division, resistance to apoptosis, maintenance of angiogenesis, invasion of tissues and metastasis and evasion of anti-tumour immune responses. As with other cancers, HuR plays a critical role in breast carcinogenesis and tumour progression. Recent studies propose that HuR may be the post transcriptional protein regulator of BRCA1 in human breast cancer. Additional evidence suggests that HuR may play a critical role in the control of ER mRNA stability and may also be involved in tamoxifen resistance. It is clear from the literature review that HuR plays a pivotal role in breast carcinogenesis and tumour progression, further supporting the feasibility of using HuR as a therapeutic target in treatment of a number of human cancers including breast cancer.

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