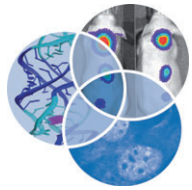
Advanced Review



Hallmarks of cancer and AU-rich elements

Khalid S. A. Khabar*

Post-transcriptional control of gene expression is aberrant in cancer cells. Sustained stabilization and enhanced translation of specific mRNAs are features of tumor cells. AU-rich elements (AREs), *cis*-acting mRNA decay determinants, play a major role in the posttranscriptional regulation of many genes involved in cancer processes. This review discusses the role of aberrant ARE-mediated posttranscriptional processes in each of the hallmarks of cancer, including sustained cellular growth, resistance to apoptosis, angiogenesis, invasion, and metastasis.

How to cite this article:
WIREs RNA 2017, 8:e1368. doi: 10.1002/wrna.1368

INTRODUCTION

Posttranscriptional Control and AU-Rich Elements

Normal cells possess well-regulated and complex posttranscriptional mechanisms of gene and protein expression to ensure cellular homeostasis. These mechanisms can occur in different multiple and interacting stages of gene expression, including 5′ capping, 3′ polyadenylation, cleavage, pre-mRNA splicing, export, mRNA decay, and translation. In tumor cells, aberrations in any of these processes are observed and can participate in the maintenance and progression of cancer. Both *cis*-acting sequences and transacting factors comprise the regulatory features of mRNA decay, a focus of this review. The 3′-untranslated region (3′UTR) at the 3′ end of the mRNA transcript is the main hub for the most important sequences that influence mRNA decay. Among the most studied sequence in the 3′UTR is AU-rich elements (AREs), which are characterized not only by an enrichment in A and U but, importantly, by their patterns, repeats, and context as well (Box 1). The minimum ARE is not simply AUUUA but rather is the pentamer in AU context; an example is UAUUUAU or UUAUUUAU or two or more of overlapping repeats of the pentamer. AREs can be classified in two ways, namely, class clustering (classes: I, II, and III) and pentamer cluster-based classification (five clusters based on the number of repeats). Currently, more than 4000 ARE-containing transcripts are found in the human genome, but only a fraction has been experimentally validated. Other *cis*-acting regulatory sequences, such as GU-rich elements (GREs)[1,2] regulate mRNA decay, but their roles in cancer are not well defined.

In general, *cis*-acting sequences are specifically recognized and regulated by *trans*-acting factors, which can be either proteins (RNA-binding proteins, RBPs) or noncoding RNAs such as the microRNAs (miRNAs). The latter are a conserved class of small noncoding RNAs (approximately 22 nucleotides) that imperfectly base pair with their targets and cause mRNA degradation or translational repression. miRNAs themselves can cooperate with AREs in mRNA decay and can also affect RBPs and thus indirectly control ARE-mediated mRNA

*Correspondence to: khabar@kfshrc.edu.sa

King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Conflict of interest: The author has declared no conflicts of interest for this article.

BOX 1

ARE SNAPSHOT

ARE Classes
- Class classification:
  - Class I: Dispersed AUUUA in U-rich context
  - Class II: Overlapping (AUUUA)n
  - Class II: U-rich stretch
- Cluster classification (Revised):
  - Cluster 1: WWWUAUUUAUWW
  - Cluster 2: WWAUUUAUUUAWW
  - Cluster 3: AUUUAUUUAUUUA
  - Cluster 4: WAUUUAUUUAUUUAUUUAW
  - Cluster 5: WAUUUAUUUAUUUAUUUAW

Signaling regulating ARE–mRNA decay and translation
- Mitogen-activated protein kinase pathway
- Phosphatidylinositol 3 (PI3)-kinase/AKT pathway
- Wnt signaling pathway

RNA-binding proteins impacting ARE (examples)
- mRNA-stability promoting proteins
  - ELAVL1 (HuR) RRM domain
  - AUF1 RRM domain
- mRNA-decay promoting proteins
  - ZFP36 (TTP) CCCH domain
  - ZFP36L1 CCCH domain
  - ZFP36L2 CCCH domain
  - KSRP K homology domain
  - AUF1 RRM domain

Animal models or RNA-binding proteins
- See Box 3

Resources and databases

AU-rich element-containing mRNA database (ARED): http://brp.kfshrc.edu.sa/ARED/
AREsite: http://nibiru.tbi.univie.ac.at/AREsite2/welcome
AREscore: http://arescore.dkfz.de/arescore.pl
Atlas for 3′UTR regulatory activity (AURA): http://aura.science.unitn.it/cite/
Scan for Motifs: http://bioanalysis.otago.ac.nz/sfm/sfm_main.pl
CLIPdb: http://lulab.life.tsinghua.edu.cn/clipdb/
UTRsite and UTRdb: http://utrsite.ba.itb.cnr.it/ http://utrdb.ba.itb.cnr.it/
RNA-binding proteins database: http://rbpdb.ccbr.utoronto.ca/

decay and translation.[3] A recent and thorough account of the interactions of miRNAs and RBPs is available.[4]

RBPS: EXPRESSION AND ACTIVITY IN NORMAL AND CANCER CELLS

Estimates indicate that RBPs can be in thousands, and a manually curated list of 1542 RBPs has been created.[5] Several distinct domains distinguish RBPs; the most characterized ones are the RNA-recognition motif (RRM), K homology domain, and the Cys–Cys–Cys–His zinc finger domain. Several ARE–RBPs have been explored in detail. Among the RRM family is the embryonic lethal abnormal vision-like protein group that comprises four members (HuB/Hel-N, HuC, HuD, and HuR). HuR is the most ubiquitously expressed and largely studied member and has been a focus for many years, particularly in cancer research. It is more of a universal RNA stability factor that binds loosely defined AU/U-rich sequences, both in the 3′UTR and in introns, and in numerous transcripts.[6,7] HuR has been thought to bind first to its pre-mRNA, and both are transported to the cytoplasm, where HuR-bound and stabilized mRNA can be efficiently translated.[8] The CCCH ZFP36 family consists of the following members: ZFP36 (TTP, TIS11, GOS24, and Nup475), ZFP36L1 (BRF-1, Tis11b, Berg36, and ERF-1), and ZFP36L2 (BRF2, Tis11d, and ERF-2). ZFP36/TTP is the most widely studied ARE–mRNA decay-promoting RBP, particularly in the context of its strong anti-inflammatory role; in recent years, it has also been studied for a tumor suppressor role. The AU-rich-binding factor 1 (AUF1), also called heterogeneous nuclear ribonucleoprotein D (HNRNPD), has two quasi RRM domains and comprises the following four isoforms: p37, p40, p42, and p45.[9] These isoforms differ in their ability to modulate mRNA decay or stability on the basis of the specific isoform, cell type, and, most probably, interactions with other RBPs, namely, HuR and TTP.[10,11] T-cell intracellular antigen-1 (TIA-1) and TIA-related (TIAR) proteins recognize AREs and cause translational inhibition. These and other RBPs have been covered by several reviews and can be found elsewhere.

The specificity of RBPs toward AREs and other RNA decay sequence determinants depends on different factors, including type of ARE, its length and context, secondary structure microenvironment, post-transcriptional modifications of the RBPs themselves, and interactions with other RBPs. RBP–RBP interactions are complex and can involve either competitive roles toward AREs, such as in the case of TTP and HuR or cooperative modes, e.g., TTP and KSRP affecting the affinity and stereochemistry to AREs. An important interaction observed in healthy cells as

opposed to cancer cells and is frequently mentioned in this review is the TTP–HuR axis and balance. In this process, TTP binds and controls HuR mRNA through competition for the binding of HuR itself (auto-regulation) to its 3′UTR.[12,13] ARE–RBPs can be subjected to a number of modifications that affect their function, such as phosphorylation in response to signals or cellular changes. Several signaling pathways, such as mitogen-activated protein kinase and PI3 kinase pathways, control ARE and RBP interactions (Box 1).

RBPs can exist either in the nucleus, cytoplasm, or both as shuttling proteins; when in the nucleus, they exist as heterogeneous ribonucleoprotein particles (hnRNPs) as a complex with the pre-mRNA. This compartment localization is subject to tight regulation in normal cells and can be affected by different stimuli. For example, under unstimulated conditions in normal cells, HuR largely resides within the nucleus, whereas in cancer cells or cells under stress, its preferred localization is in the cytoplasm. The nucleocytoplasmic translocation of HuR appears to be controlled by several posttranslational modifications, such as HuR phosphorylation, methylation, ubiquitination, isomerization, and cleavage. In case of phosphorylation, several kinases have been shown to mediate this process, including the protein kinase C, AMP-activated protein kinase, the mitogen-activated protein kinase p38 MAPK, cyclin-dependent kinases 1 and 5 (CDK1 and CDK5), checkpoint kinase 2, Janus kinase 3, SRC, and Abl-1 tyrosine kinases.[14–18] Cytoplasmic translocation appears to be necessary for the ability of HuR and other RBPs to regulate mRNA stability or translation through binding to their 3′UTR. HuR may modulate translation of several ARE–mRNAs by a process that is independent of ARE or 3′UTR, which involves binding of HuR to IRES in the 5′UTR.[19–21]

AREs mediate their effects on mRNA decay by first enhancing deadenylation (polyA shortening), a first step in mRNA decay facilitated by CCR4/NOT and PAN2/PAN complexes. Through their cognate RBPs, AREs recruit the degradation machinery, the exosome, which is a complex of exonucleases that degrade the mRNA at 3′ to 5′. Also, the removal of 5′ cap decapping enzymes, followed by the 5′–3′ decay-mediated process, is another mRNA decay pathway. Many of the ARE–mRNAs are localized to processing (P)-bodies which are cytoplasmic RNA granules and they are the sites in which 5′ ARE–mRNA degradation and translational repression occur. P-bodies can also occur as part of the stress granules that contain additional proteins and translationally inactive mRNAs. Several ARE–RBPs are associated with either P-bodies or stress granules. For example, TIA-1 and TIAR are a part of stress granules, whereas HuR and TTP can be recruited to both. A different player in cellular ARE–mRNA decay, ribonuclease L (RNase L) has been recently identified; it is an endoribocuclease that usually targets UU/UA ribonucleotides in viral mRNAs. RNase L promotes the decay of HuR and other cell cycle mRNAs, probably through interactions with TTP; cellular growth is suppressed as a result.[22–24] The cellular mRNA substrates for RNase L appear to be also specified by cooperation with specific miRNA signatures, specifically miR-17/miR-29/miR-200.[25]

Several players and mechanisms, such as ZFP36 family members and KSRP, participate in the translational repression of ARE–mRNA by RBPs. In normal cells, particularly when not undergoing exposure to stress or external stimuli, AREs ensures that the mRNA is translationally silent. Deadenylation, which causes mRNA decay, can also attenuate ARE–mRNA translation by reducing the occupancy of the poly-A tail-binding protein (along its interacting partners); this process can be either promoted or attenuated by TTP or HuR, respectively.[26] Furthermore, TTP promotes 5′-decapping, which can also affect translation. TTP promotes the binding of eukaryotic initiation factor 4E2 (eIF4E2), a translational inhibitor during normal conditions, to ARE–mRNAs to block its translation.[27] Another mechanism of TTP-mediated protein repression is interacting with translational repressor RCK to impart ARE–mRNA translational inhibition.[28] Another interacting co-factor in TTP-mediated translational repression is the cap-binding translation repression 4EHP–GYF2 complex in which GYF2 interacts with the conserved tetraproline motifs of TTP.[29]

The Perturbed Balance and Activity of RBPs in Cancer

One can envision that any aberrations in many of the processes that comprise mRNA decay and translational repression can lead to the overproduction of gene products and thus their activity in cancer. ARE–mRNA expression is overrepresented in multiple cancer types.[35] In cancer, several processes that change one or more of the following traits of ARE–RBPs occur: expression levels, compartment localization, and activity. In general, under-expression or loss of the activity of mRNA decay-promoting proteins, such as TTP or ZFP3L, will lead to the overexpression of cancer ARE genes. By contrast, overexpression or an increased activity of mRNA stability-promoting proteins, such as HuR, is seen in

cancer states. HuR expression and/or cytoplasmic preference are increased in many cancer types both in cell lines and in tumors, such as brain, colon, breast, and liver.[30–33] A higher HuR expression correlates with reduced patient survival in several cancers.[34] However, TTP is deficient or inactive in cancer, and different reasons are outlined in Box 2. TTP deficiency leads to not only increased mRNA stabilization but also translation de-repression, which results in the prolongation or overproduction of cancer genes that participate in different hallmarks of cancer. Thus, a perturbed balance of the levels or activity of mRNA stability- and mRNA decay-promoting proteins is found in cancer. TTP controls HuR mRNA,[12,32] so the TTP–HuR axis is reversed in cancer because of both TTP deficiency and HuR overexpression/cytoplasmic preference. A large set of overexpressed cancer ARE genes was recently uncovered; this set correlated with the TTP/HuR mRNA ratio and was shown to be enriched in several processes, including mitotic cell cycle, cell motility, cell adhesion, and response to external stimuli.[35]

Aberrant TTP–HuR axis in cancer cells can also occur as the result of changes in the phosphorylation status of each RPB (See Box 2 on TTP phosphorylation). Phosphorylated TTP becomes inactive and has reduced affinity toward AREs, and thus increased levels of ARE–mRNAs, including HuR mRNA, are observed in tumor cells. The function of HuR is highly dependent on the key phosphorylation site in the basic hinge region that harbors the nucleocytoplasmic shuttling sequence,[17] which connects RRM2 and RRM3 domains.[57] Since there are many kinases in which their activities are altered in cancer, they can affect the phosphorylation status of HuR and thus participate in the aberrant TTP–HuR axis. However, HuR phosphorylation is complex and dependent on the kinase and the phosphorylation site on HuR, meaning that they lead to either increased or decreased affinity of HuR to its mRNA targets. For example, in case of MK2, HuR phosphorylation shifts HuR toward cytoplasmic compartment and increase ARE–mRNA stability and translation.[45] In contrast, cdk5, which is higher in cancer cells, can phosphorylate HuR, but leads to decreased ARE–mRNA stability.[17] Thus, the role of HuR phosphorylation in cancer cells as opposed to normal cells remains elusive.

The deregulated expression of miRNAs is another important means through which cancer cells try to avoid mRNA decay-promoting RBPs, or possibly; RBPs antagonize miRNA-mediated mRNA decay and translation. A notable example is miR-29a, which targets TTP 3′UTR and leads to its

BOX 2

TTP DEFICIENCY IS A HALLMARK OF CANCER

TTP repression is observed in many cancer types due to multiple processes that control different stages of TTP gene and protein expression. For the purpose of this review, the term TTP deficiency is applicable for both expression and activity of the protein. At transcriptional levels, no definite answer exists for the epigenetic silencing of the TTP promoter. However, methylation of the TTP promoter, specifically in a single CpG site located within a TGB1-responsive region, has been pinpointed in liver cancer cells and tumor tissues where TTP is downregulated.[36] Overexpression of histone deacetylases (HDACs), which leads to transcriptional repression through chromatin remodeling in cancer, may repress TTP promoter activity in colon cancer.[37,38] EGR1 can act as a transcriptional factor not only for TTP[38] but also for other tumor suppressor genes, including PTEN, p53, and TGFβ1;[39] thus, reduced levels of EGR1 in cancer[40] may contribute to the deficiency of TTP and other tumor suppressors. Further studies are needed to establish the link between EGR1 and TTP in cancer. Another transcriptional mechanism for TTP deficiency is the failure of mutant p53, a frequent variant in cancer, to induce TTP transcription, particularly during the response of cancer cells to chemotherapeutic agents, such as doxorubicin.[41] Cells with mutant or absent p53 have a lower TTP transcriptional activity than do p53-proficient cells.[41]

At the mRNA level, miR-29a, which is overexpressed in pancreatic and breast cancer, targets TTP 3′UTR for the mRNA degradation leading to decreased levels of TTP mRNA and protein.[32,42,43] At the protein level, deficiency has been observed in breast cancer with a synonymous polymorphism (rs3746083) in the open reading frame of TTP, and as a result, TTP mRNA translation is reduced, but TTP mRNA stability is unaffected; this has been demonstrated in HER2-positive breast cancer patients' tumors and associated with a lack of response to the anti-HER trastuzumab.[44] How widespread this interesting mutation is in other types of cancers remains unknown but deserves in-depth examination.

Another important cause of TTP deficiency is the aberrant signaling that occurs in cancer, such as during growth factor receptor over-

activity. Growth factor/cytokines bind their receptors to activate a series of signaling events leading to the phosphorylation of MEK3/MEK6 kinases that subsequently phosphorylate p38 MAP kinase and then MK2 kinase, which in turn phosphorylates TTP. As a result, its complex with the mRNA is sequestered by 14–3–3 binding proteins, and the phosphorylated TTP loses its ability to bind the AREs and recruit deadenylases and other effectors that cause ARE–mRNA stabilization and translational de-repression.[45] Other signals that are abnormal in cancer, such as Ras and the PI3K signaling pathway, may also participate in TTP phosphorylation and loss of its activity, which leads to ARE–mRNA stabilization.[46]

TTP becomes phosphorylated by several putative kinases and at multiple serine residues.[47] High levels of phosphorylated, and thus inactive TTP has been demonstrated in several types of cancer cell lines and tissues such as those of head and neck and brain.[48,49] Normally, TTP protein is unstable and rapidly degraded by proteasome. Thus, a feature of the phosphorylated inactive form of TTP is that it becomes stable, during certain conditions such as p38/MK2 activation,[50,51] which further sustain the aberrant TTP–HuR axis. A recently studied player of TTP suppression is the pyruvate kinase M2 (PKM2), which is overexpressed in many cancers and participates in the increased glycolysis (Warbug effect). It is thought that PKM2 physically interacts with TTP and leads to its phosphorylation; however, it promotes its degradation via proteasome.[52] The discrepancy in the relationship between phosphorylation and protein stability of TTP has been noted in literature. For example, the multiple eight serines mutant TTP form (thus unrphosphorylatable) was found stable when expressed in glioblastoma cells but still demonstrates increased ARE–mRNA instability compared to wild-type TTP.[48] Thus, TTP stability as affected by phosphorylation is dependent on the kinase type itself, the site stoichiometry, and probably the nature of interactions with 14–3–3 or other relevant proteins. Regardless of the protein stability outcome, phosphorylation of TTP in cancer states led to reduced mRNA decay promoting action of TTP and thus subsequently perturbed TTP–HuR balance.

TTP repression can lead to other abnormalities that are unrelated to its posttranscriptional function but are related to other unique functions. Specifically, TTP can act as a co-repressor for estrogen factor (ERα) transactivation activity, so TTP repression contributes to sustained proliferation in ER-positive cells.[53] Another co-repressor role is found, and it represses NF-kB transcriptional activity.[54,55] Again, TTP repression can exaggerate the production of NF-kB-dependent cytokines that participate in the tumor microenvironment and the hallmarks of activating invasion and metastasis. In general, the NF-kB pathway has been recognized as an important pathway in cancer maintenance.[56]

Many of the types of cancer involve TTP deficiency, and each type or subtype appears to have more than one cause of TTP repression. The outcome of TTP deficiency occurs in almost every hallmark of cancer, as discussed throughout this review.

mRNA degradation (Box 2). HuR competes with certain miRNAs for nearly the same binding site on specific messages; a recent example is the ability of HuR to prevent miR-21-mediated repression of the tumor suppressor gene, programmed cell death 4 (PDCD4), which is under-expressed in cancer. Other earlier examples of these HuR–miRNA interaction changes in cancer are miR-122 and CAT-1 mRNA, miR-16 and COX-2 mRNA, and miR-331-3p and ERBB2 (HER/neu) mRNA. The details of these examples can be found in previous reviews.[4,58]

The following is an account of the involvement of AREs in each of the hallmarks of cancer. The hallmarks of cancer refer to a concept in which the authors of the original paper narrow cancer as a disease to six features, and later, these features were upgraded with two enabling and two hallmarks of cancer.[59] To understand and appreciate the link between AREs in cancer, a brief introduction on each of the cancer hallmarks is presented in each section as well.

CANCER GROWTH AND DIVISION HALLMARKS AND AREs

Cellular growth and division are tightly regulated groups of transient processes that proceed whenever a need for them exists, such as during tissue regeneration, wound repair, blood cell formation, and immune cell expansion. When they are no longer required, various shut-off mechanisms are in place to guarantee a healthy homeostasis. The ARE-mediated

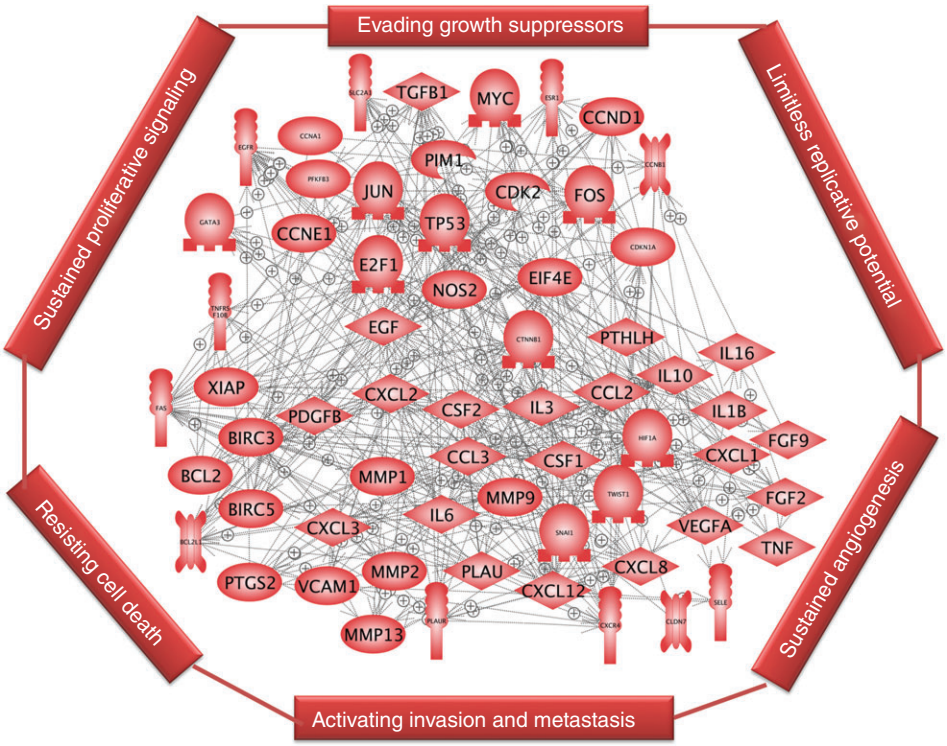


FIGURE 1 | ARE gene product interactome. Gene products coded by ARE–mRNAs that participate in the hallmarks of cancer and their interactions are shown as an interactive map. Pathway Studio program (Elsevier) was used to create the interacting map. Because genes participating in cancer hallmarks overlap, they were not connected to the individual hallmarks.

pathway is one of these mechanisms that ensure the regulated half-life of the mRNA and swift translation. An appreciable number of genes code for ARE–mRNAs that participate in cellular growth and division. In cancer cells, the following three hallmarks allow them to continue growing: sustained proliferative signaling, evading growth suppressors, and possessing a limitless replicative potential.

Sustaining Proliferative Signaling

Cancer cells tend to stimulate their own growth in a number of ways, so this hallmark is also called self-sufficiency in growth signals. Over-secretion of growth factors, abnormal mutant receptor activation, and aberrant signaling components can all promote the sustained growth of tumor cells. A number of gene products participating in this hallmark belong to the ARE transcriptome (Figure 1 and Table 1). A number of cell-cycle regulators, notably cyclins, such as cyclin A1, cyclin B, and cyclin D, as well as c-myc, c-jun, and c-fos, are coded by ARE–mRNAs and regulated by HuR; these are therefore overexpressed in cancer. MYC (class 1 ARE–mRNA), which encodes a nuclear phosphoprotein transcription factor that regulates the cell cycle, is amplified (DNA copy number) in many tumors and is one of the earliest well-studied molecules in mRNA decay.[126]

An example of an ARE–mRNA that codes for a protein involved in cancer growth is the Pim-1 proto-oncogene, serine/threonine kinase, which is targeted by TTP.[127,128] It act as an oncogene and is upregulated in several human tumors; it enables cell cycle progression and suppresses apoptosis. In cancer cells, pim-1 mRNA has been shown to be functionally correlated with TTP expression in several cell lines and tumor tissues.[112] Pim-1 kinase phosphorylates different substrates, including Myc, p21/ Cip1/ WAF1, and p27K, and thus regulates cell cycle progression. TTP-induced reduction of Pim-1 thus leads to a decrease in the phosphorylation of p21 and p27; in turn, TTP deficiency can result in increased tumor cell growth.[128] HuR overexpression, particularly with cytoplasmic localization, can increase pim-1 mRNA stability, as shown in pancreatic cancer.[113]

Several tyrosine kinase growth factor receptor oncogenes, including ARE gene receptors EGFR and stem cell factor (kit, an ARE gene), in addition to other receptors, such as VEGFR, PDGFR, and hepatocyte growth factor (met), activate the PI3-kinase/ AKT pathway. The increased PI3-kinase activity in cancer cells increases tumor growth and survival.

TABLE 1 | ARE-Genes and Hallmarks of Cancer

ARE-Gene/Other Name	Hallmarks of Cancer	C	RBP	Ref
BCL2	Anti-apoptosis	III	HuR, NCL, AUF1	60,61
BCL2L1 (Bcl-X)	Anti-apoptosis	U	NCL, HuR	60,62
BIRC3 (cIAP2)	Anti-apoptosis	III	TTP	63
BIRC5 (Suvivin)	Anti-apoptosis	U	HuR, CELF1	64
CCL2	Metastasis	U	HuR, TTP	65
CCL3	Metastasis	IV	TTP	66
PLK3	Proliferation	V	TTP	67
CCL3 (MIP1α)	Metastasis	IV	TTP	68
CCNA1 (cyclin A1)	Proliferation	U	HuR, WTAP	69,70
CCNB1 (cyclin B2)	Proliferation	U	HuR	70
CCND1 (cyclin D1)	Proliferation	V	HuR, AUF1, TTP	71
CCNE1 (cyclin E)	Proliferation	U	CERP, HuR, NF90	72
CDK2	Proliferation	V	HuR	73
CDKN1A (p21)	Proliferation	IV	AUF1, HuR, TTP, PCBP4	22,74,75
CLDN7 (claudin-1)	Invasion	U	HuR, TTP	37
SLC2A1/Glut1	Proliferation	V	hnRNP A2	76,77
CSF1/M-CSF	Invasion	U	TTP, GAPDH	7879
CSF2/GMCSF	Angiogenesis	I	HuR, TTP, ZFP36, AUF1, NCL	80
CTNNB1	Metastasis	U	KSRP	81
CXCL1	Proliferation, angiogenesis	III	HuR, TTP	82
CXCL2 (MIP2a)	Metastasis	I	TTP, KSRP	83
CXCL3 (MIP-2b)	Metastasis	III	TTP, KSRP	84
CXCL12	Metastasis	V	—	85
CXCR4	Metastasis	V	TTP, HuR	86,87
EIF4E	Proliferation	U	HuR	88
EGF	Proliferation, angiogenesis, metastasis	U	HuR	58
EGFR	Proliferation	U	HuR	89
ESR1 (ERα)	Proliferation	V	TTP, HuR	90
E2F1	Migration		AUF1, TTP	71,91
FGF2	Proliferation, angiogenesis, metastasis	V	—	92
FGF9	Invasion, anti-apoptosis, angiogenesis	III	AUF1	93
FOS	Proliferation	III	AUF1, HuR, KSRP, TTP	94,95
HIF1A	Angiogenesis	III	TTP, HuR, NCL, PTB	96,97
IL1B	Metastasis	II	TTP KSRP	98
IL3	Angiogenesis	II	AUF1, HuR, TTP, ZFP36L1	99
IL6	Proliferation	IV	TTP, HuR, AUF1, KSRP	49,100–102
IL8	Angiogenesis, metastasis	III	HuR, TTP, KSRP	84, 103
IL10	Evading immunity	V	AUF1, TTP, HuR	104
IL16	Invasion, metastasis	U	TTP	105
NOS2 (iNOS)	Metastasis	V	AUF1, HuR, KSRP, TTP, TIAR, PTB	106
JUN	Proliferation	U	HuR, KSRP	107
MMP1	Invasion	U	TTP	32
MMP2	Invasion	U	TTP	49

TABLE 1 | Continued

ARE-Gene/Other Name	Hallmarks of Cancer	C	RBP	Ref
MMP9	Invasion	U	HuR, TTP	49,108
MMP13	Invasion	V	TTP	32
MYC	Proliferation, anti-apoptosis	U	TTP, HuR, AUF1, TIAR	109
PDGF	Proliferation, angiogenesis	IV	HuR	110
PFKFB3	Glucose metabolism	III	—	111
PIM1	Proliferation, anti-apoptosis	I	HuR, TTP	112,113
PTGS/COX2	Angiogenesis, anti-apoptosis	III	HuR, TTP, KSRP, TIA	31
PTHLH	Metastasis	V	KSRP	84
PLAU (uPA)	Invasion	IV	HuR, TTP,	114,115
PLAUR (uPAR)	Invasion	V	HuR, TTP	114,115
SELE	Invasion, metastasis	III	—	
SNAI1	Metastasis	U	HuR, TTP	116,117
TWIST1	Metastasis	III	TTP	117
TGFB1	Evading immunity, metastasis	U	HuR	33
TNFRSF6/FAS	Anti-apoptosis	III	HuR, NCL	30,118
TNFRSF10B (DR5)	Apoptosis	III	HuR	119
TNF	Angiogenesis, invasion, metastasis	I	TTP, HuR, AUF1, KSRP, TIA, TIAR, ZFP26L1	120
TP53 (p53)	Mutant P53 effects	U	HuR, NCL	121
VCAM1	Metastasis	III	HuR	122
VEGF	Angiogenesis	III	AUF1, HuR, TTP	123, 124
XIAP (BIRC4)	Anti-apoptosis	III	HuR, CELF1	64, 125

The PI3 kinase/AKT kinase activity stabilizes cancer ARE–mRNAs, such as TNF, β-catenin CCL2, CXCL3, CCND1, basic fibroblast growth factor (FGF2), and PLAU, by a process that involves phosphorylating and inactivating certain RPBs, including ZFP36L and KSRP modulation.[129,130] The response of EGFR to cancer-associated EGF overexpression or constitutively active EGFR members leads to the activation of multiple signaling pathways, including PI3-kinase, the Janus kinase/signal transducer and activator of transcription, and the RAS/extracellular signal-regulated kinase (ERK) pathway. These pathways can lead to ARE–mRNA stabilization by a process that involves phosphorylation of ARE–RBPs. The mRNAs of EGF and EGFR, although not harboring strong AREs, such as class II AREs, can be both bound and upregulated by HuR.[131] EGF can stabilize its own receptor, EGFR ARE–mRNA.[132,133] The alternative polyadenylation of HuR transcript has been characterized,[12,134] and changes in their patterns can affect cancer ARE–mRNA expression.[35] In general, alternative polyadenylation that aberrantly occurs in cancer can lead to the overexpression of short 3′UTR transcripts, which escape repression by AREs and miRNAs.[135–137]

Evading Growth Suppressors

Normal cells possess regulated mechanisms that aim to stop cell growth when not needed, by developing negative feedback mechanisms, such as inhibitory signals. The checkpoints that are subject to inhibitory signals, e.g. are at the G1/G_o and G_2/M phases of the cell growth cycle. The most studied growth suppressors that are attenuated or inactivated in cancer are p53, retinoblastoma protein, phosphatase and tensin homolog (PTEN), and tumor growth factor β. The loss of PTEN function because of inactivating mutations that occur in tumors, such as those of prostate cancer, endometrial cancer, and glioblastoma, amplifies PI3K/AKT signaling and can subsequently lead to further ARE–mRNA stabilization of the cancer genes. p53 is a transcriptional factor that is induced by a variety of stresses, such as DNA damage, and it regulates a multitude of functions, including cell cycle control, senescence, apoptosis, DNA repair, and stem cell maintenance, in addition to various metabolic pathways.[138] Mutations in p53 are a frequent feature in many types of cancer. Control of the p53 gene and protein expression is complex and involves both positive and negative regulators to ensure optimal levels. Not only is under-expressed or inactivated p53 an

unwanted event, but overexpressed p53 is also undesirable because it promotes aging and other abnormalities. Several mechanisms exist for the posttranscriptional control of p53, including those involving AREs, such as regulation of p53 mRNA stability and translation by a number of RBPs. The p53 3′UTR contain AU/U-rich regions, and several RBPs have been shown to modulate p53; e.g., HuR has been shown to increase p53 translation during DNA damage. The double-stranded-RNA-binding zinc finger protein, Wig1, in cooperation with hnRNP A2/B1, binds the p53 AU/U-rich region and causes mRNA stability. Mutant p53 may not be able to participate in the transcriptional activity of the TTP promoter, which is thought to contain a p53 binding site. TTP itself plus ZFP36L can be considered tumor suppressors, in which cancer cells have developed evading mechanisms (Box 2 and Figure 2).

Limitless Replicative Potential

When undergoing a response to damage, such as in the case of wound damage or a response to a growth stimulus, normal cells divide in limited rounds. This process is largely governed by the telomeres, which are specialized structures with a conserved G-rich sequence located at chromosomal ends. They consequentially become shortened as they divide until they become too short to support further division. Cancer cells have the potential to divide indefinitely by a process that either: reactivates the telomerase, the terminal transferase reverse transcriptase that adds 'TTAGGG,' or that maintains the telomere length by other activities.[139]

A posttranscriptional role in telomere maintenance has been observed, in which AUF1 binds to the G-rich strand; this process leads to the destabilization of telomeric G-rich tails and thus facilitates telomere extension.[140] Although the telomerase catalytic subunit gene, TERT, does not code for ARE in its mRNA, it can be transcriptionally activated by AUF1. This finding was discovered from the observation that AUF1-knockout mice exhibited accelerated aging and excessive telomere shortening.[141] The work demonstrated that AUF1 is required for telomere maintenance in mouse through binding to and activation of TERT transcription.[142]

AVOID AREs, AVOID DEATH

Tumors need to resist apoptosis imparted by different stresses, such as chemotherapeutic cytotoxic drugs,

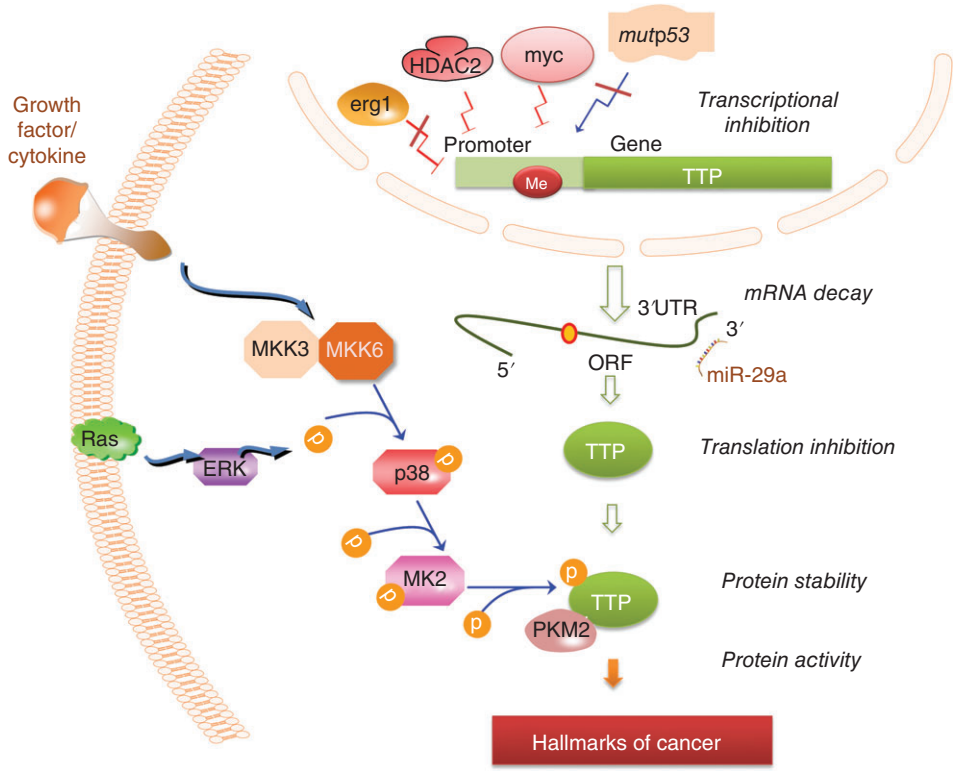


FIGURE 2 | TTP repression in cancer. The various causes that lead to TTP repression in cancer cells: The details are found in Box 2 and throughout the review.

so that these tumors have preferential growth. Morphologically, apoptosis or programmed cell death leads to a reduced cell size, followed by cell fragmentation and engulfment by phagocytic cells. Two main apoptotic pathways exist: one is the mitochondrial caspase (intrinsic) pathway, which is triggered by intracellular stress signals, such as DNA damage, and the extrinsic pathway, which is initiated by cell surface receptor engagement with their ligands. In the intrinsic pathway, the mitochondrial apoptosome consists of cytochrome c, dATP, and Apaf-1, which recruit and activate the cysteinyl aspartate-specific proteinase, caspase-9; this process leads to the activation of other effector caspases and, finally, apoptosis.[143]

Several gene products that allow cancer cells to resist their programmed cell death are coded by ARE–mRNAs, such as the BCL2 family. The key B-cell lymphoma 2 (BCL2) is an anti-apoptotic protein that blocks cytochrome c release. It is overexpressed not only in certain types of lymphomas/leukemias because of chromosomal rearrangement but also in selected tumor types because of posttranscriptional mechanisms. BCL2 mRNA has a short half-life due to the presence of cluster 3 ARE, which is a target for several RBPs, such as HuR, nucleolin (NCL), Ebp1, and AUF1. HuR and NCL synergistically stabilize BCL2 mRNA and enhance its translation by binding concurrently to BCL2 ARE, in which the NCL binding site is upstream from the HuR-binding site.[144] As a result of HuR overexpression or cytoplasmic active HuR, BCL2 and other anti-apoptotic proteins in which their mRNAs harbor AREs, including BIRC5 (survivin) and the X-linked inhibitor of apoptosis XIAP, contribute to resistance to apoptosis. The effect of HuR on BIRC5 appears to be conditional on p53 loss,[64] which is encountered in most cancers by the mutant p53 status. The other anti-apoptotic players in cancer are mutated p53 and amplified c-myc, which can both be modulated by HuR because HuR stabilizes c-myc and p53 mRNA and thus further amplifies the process.

The TTP/ZFP36 family members induce apoptosis through the intrinsic mitochondria pathway.[145–147] Both TTP and ZFP36L1 interact with BCL2 ARE and cause mRNA destabilization, which leads to apoptosis; TTP also sensitizes cells to TNF-induced apoptosis.[145,146] TTP sensitizes leukemic cells to anti-CD20-induced apoptosis in B-cell lymphocytic cells and to cisplatin-induced apoptosis in head and neck cancer cells.[148,149] Thus, TTP deficiency, which occurs in many tumor types, can lead to resistance to cellular death. Similarly, resistance to cell death by cytotoxic drugs can be affected by HuR overexpression or its cytoplasmic preference.

At least two gene members of the death receptor extrinsic pathway harbor AREs in their mRNA, including death receptor 5 (DR5) and the apoptosis-mediating surface antigen FAS, which contains cluster 3 AREs in their 3′UTR. One key RBP that has been linked to this apoptotic pathway is HuR, and unlike its universal mRNA stabilization/translational role in other hallmarks of cancer, it has opposite functions in FAS-mediated apoptosis. HuR has been shown to interact with ARE regions in the 3′UTR of FAS and thus inhibit its mRNA translation without affecting mRNA stability in hepatocellular carcinoma cell lines and clinically aggressive HCC; decreased resistance to Fas-mediated apoptosis occurs as a result.[30] Another interesting role of HuR is FAS exon 6 skipping, which leads to the synthesis of a soluble decoy Fas that inhibits apoptosis.[150]

SUSTAINED ANGIOGENESIS AND ARE–mRNA STABILITY

As the tumor grows, it consumes nutrients and oxygen leading to a state of oxygen deprivation (hypoxia) and nutrient deprivation. As a result, the malignant tumor induces new vessel formation (angiogenesis) and vascular hyper-permeability to allow nutrients to the tumor microenvironment. Hypoxia triggers HIF1A, which is the master transcriptional factor for many genes that participate in angiogenesis and also in other processes needed for tumor growth and maintenance, such as the glycolytic pathway. HIF1A is regulated appreciably by the translational modification of its α subunit, which becomes stabilized during hypoxia, and HIF1A protein levels increase as a result. Additionally, with HIF1A being an ARE gene, mRNA stabilization and translation of HIF1A are augmented in cancer cells.[151] HIF1A harbors cluster 3 (class II) AREs, which account for its labile mRNA behavior in a normal oxygen level environment. A number of RBPs, including TTP, HuR, and polypyrimidine tract-binding protein (PTB), bind to U/AU-rich sites in the HIF1A 3′UTR. Both HuR and BTP were shown to promote the translation of HIF1A mRNA.[96] TTP has been shown to bind the AREs in the 3′UTR and accelerate HIF1A mRNA decay, whereas TTP deficiency (Box 2) leads to increased mRNA stabilization and protein levels of HIF1A, particularly during hypoxic conditions.[97,152] Because several cancer types are deficient in TTP, cancer cells have increased levels of HIF1A mRNA and protein, and subsequently increased

levels of HIF1A target mRNAs in which their gene products participate in several hallmarks. Whether tumor endothelial cells per se are deficient in TTP or its function, it is unknown, but it is a possibility;[152] in this case, as HIF1A increases, VEGF and VEGF receptors are upregulated, and, thus, the angiogenesis loop is enhanced.[153] HIF1α also has a VEGF-independent role in the glycolytic pathway in myeloid cells and their differentiation into macrophages and their survival in the tumor microenvironment.[154]

Hypoxia-independent HIF1A can also be seen in other aberrant ARE-mediated states; e.g., the myc oncoprotein, which is overexpressed in several cancers (Figure 2), can directly suppress TTP transcription[155] and may therefore lead to HIF1A mRNA stability and high levels of HIF1A protein. During a screening of mediators for HIF1A-mediated hypoxic response, USP52/PAN2, a component of P-bodies, was found to promote the stability of HIF1A mRNA in 3′UTR-dependent manner, as documented with the use of knockdown experiments.[156] However, the direct binding of this protein to AREs was not studied, and interactions with de facto RPBs, such as TTP, can possibly happen in P-bodies.

As HIF1A is triggered, in turn, it induces many HIF-responsive genes, including those encoding for other ARE–mRNAs, notably, VEGFA, plus other growth factors, including PIGF, PDGF, COX2 (PTGS2), CXCL12, FGF2, HGF, and TGFA. The key angiogenesis mediator, VEGF, triggers a state called 'angiogenic switch,' which refers to the initiation from a dormant anti-angiogenic state to an active phase of vascular growth to sustain tumor growth and maintenance.[59,157] VEGF can be released from the infiltrating immune cells in the microenvironment, and this contributes further to the sustained angiogenesis state.[158] VEGF contains multiple U-rich/ARE elements including cluster 3 (class II) AREs that cause strong mRNA destabilization under normal oxygen levels. Several RBPs, such as HuR, PTB, poly(A)-binding protein-interacting protein 2, AUF1, NF90, ZFP36L1, and TTP, bind to U-rich/AREs in the VEGF 3′UTR and modulates its mRNA decay.[159,160] Hypoxia leads to significant VEGF mRNA stabilization independent of HIF1A, and it involves different assemblies of RBPs than those during normoxia.[161] Another HIF1A-indepdent mechanism could be the various kinases, such as Jun amino-terminal kinase (JAK) and the p38 MAPK pathway,[162] which are known to inactivate TTP by phosphorylation and thus leads to VEGF mRNA stabilization.

In cancer cells, HuR promotes the stability and translation of VEGF mRNA in several solid tumors, including colon, brain, and breast cancers.[33,163] In general, angiogenesis is associated with increased HuR expression and cytoplasmic localization. Hypoxia causes translocation of HuR from the nucleus to the cytoplasm[164] to stabilize ARE–mRNAs and/or enhance their translation. On the other hand, as TTP destabilizes VEGF mRNAs,[165] the aberrant TTP–HuR axis that is encountered in a number of cancers in principle contributes to sustained angiogenesis. Mice studies showed reduced microvessel density in transformed cell- derived xenografts in nude mice as a result of inducible TTP.[166] The loss of the tumor suppressor Von Hippel-Lindau (VHL), which is encountered in certain tumor types, such as renal carcinomas, can increase VEGF mRNA stability, which contributes to the sustained angiogenesis.[167] This effect can be mediated by either the RRM1 domain of HuR, which interacts with VHL[168] leading to the inhibition of its function, or by the fact that VHL decreases TTP expression.[169] Another notable HIF1A-inducible gene is FGF2, which is largely regulated by posttranscriptional mechanisms that involve alternative polyadenylation, 5′UTR-mediated translation, and AREs.[92] Thus, the aberrant TTP–HuR axis amplifies a network of angiogenesis through its direct effect on HIF1A mRNA, whose product is a transcriptional inducer of many ARE-coding genes, including VEGF. VEGF itself can trigger the expression of other ARE genes, such as IL-8 and other factors that can promote angiogenesis in a number of cancers.

ACTIVATING INVASION AND METASTASIS: THE ARE INTERACTOME

This hallmark of cancer is a prominent cause of cancer mortality. The process of metastatic cancer starts off with local invasion of the tumor tissues to the stroma, followed by intravasation, migration through the blood and lymphatic system, colonization, and growth at a distant organ. Many key pro-invasion and pro-metastasis genes code for ARE–mRNAs (see Table 1). Because of the importance of AREs in this cancer hallmark, the discussion in this section has been divided into several subsections.

Epithelial–Mesenchymal Transition

Epithelial–mesenchymal transition (EMT) is a multifactorial process in which cancer epithelial cells

undergo further changes to acquire a mesenchymal phenotype that will allow them to invade local tissues. The cells undergo a cytoskeletal rearrangement and lose cell–cell adhesion structures and polarity. EMT is programmed through a number of specific transcriptional factors, notably the zinc finger Snail (SNAI1) and the basic helix-loop-helix factor, Twist1 (TWIST1). These factors inhibit epithelial cell features by repressing E-cadherin transcription while activating N-cadherin, a marker for mesenchymal cells. Cadherins, named after calcium-dependent adhesion, are transmembrane proteins that form adherent junctions binding cells together. TTP has been recently shown to bind the 3′UTR, and promote the mRNA decay, of SNAIL and TWIST1 in an ARE-dependent manner[116,117] and to inhibit EMT.[117] Thus, TTP deficiency that occurs in cancer can be a plausible EMT factor, while the HuR overexpression encountered in cancer may promote EMT via enhancement of SNAIL mRNA stability or translation. This observation is evident from a work showing that loss of the tumor suppressor scribble, a component of a complex that participates in the polarity of epithelial cell structures, leads to HuR translocation to the cytoplasm and enhancement of SNAIL translation.[116]

An important mediator of EMT linked to AREs is the miR-29a. Although it has been shown to have anti-tumor activities, other evidence exists to show that miR-29a promotes EMT and the invasion and metastatic potential of a number of cancers, including breast, colon, pancreatic, and nasopharyngeal cancers.[32,42,43,170,171] Elevated levels of miR-29a are found in these tumors and are associated with TTP suppression. miR-29a suppresses TTP by targeting both mouse[43] and human TTP 3′UTR in breast cancer[32] and in pancreatic cancer.[42] The ARE found within 100 bases downstream of the miR-29a target sequence may cooperate in the accelerated mRNA decay of TTP.[32] The TTP-modulated effect of miR-29a on EMT has been shown in breast cancer and also in pancreatic cells.[42,43] In a cell line model, TTP can induce EMT and in cooperation with oncogenic Ras signaling.[43]

Several pro-tumor growth factors, including TGF-β, FGF2, and PDGF, as well as signaling pathways, such as Notch and Hedgehog, and NF-kB, participate in EMT. TGFB, a key regulator of EMT, is itself coded by an ARE–mRNA that is subject to HuR-mediated mRNA stabilization.[172] Another plausible player of EMT is AUF1. It is linked to tumorigenesis, as shown by studies on AUF1 p37 transgenic mice that developed sarcomas also involving high levels of cyclin D1.[173] AUF1 may be overexpressed in cancer tissues compared with normal ones[174] and promotes mesenchymal features in osteosarcoma cells.[175] Notably, AUF1 can also act as either a destabilizing or stabilizing factor that interacts or acts in concert with other RBPs. For example, results from a global analysis of mRNA targets by AUF1 showed that AUF1 and HuR cooperate to promote the stabilization and translation of certain mRNAs.[176] More studies are needed to further understand the role of AUF1 in cancer.

The Microenvironment

The tumor cells and stroma constitute an active microenvironment that promotes the processes underlying the hallmarks of cancer, particularly invasion. The tumor stroma comprises the basement membrane, extracellular matrix, and vasculature, together with a number of different cell types, including fibroblasts, pericyctes, immune cells, and endothelial cells. Two important and largely studied cells are the tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAF). CAFs themselves have proinflammatory properties and secrete cytokines that promote TAM activity and macrophage infiltration. TAMs are key players of cancer-related inflammation (an enabling hallmark of cancer) and constitute a large proportion of the inflammatory infiltrate in which tumor cells attract.[177] They produce many cytokines, chemokines, and growth factors, which include many ARE-coding gene products, such as EGF, CSF1, IL-8 (CXCL8), CXL12, FGF, VEGF, PDGF, TGFB, and MMPs. TAMs assist in many of the hallmarks of cancers, including tumor growth, angiogenesis, and invasion. Together with myeloid-derived suppressor cells, they can also suppress cytotoxic immune cells from killing the tumor cells, such as by secreting the immunosuppressive IL-10.[178]

Cancer cells themselves also produce CSF-1, which further amplifies the invasiveness of these cancer cells.[179] Both CAFs and TAMs secrete large amounts of VEGF and FGF that contribute to the sustained angiogenesis hallmark. Furthermore, tumor-associated endothelial cells overproduce VEGF receptors to activate angiogenesis. All these gene products are a part of the multiple growth factors that code ARE–mRNAs. In fact, cytokines and growth factors are overrepresented as a functional class in ARED. The aberrant TTP–HuR axis in the cells of the microenvironment, such as CAFs, should lead to the overproduction of these growth factors that feed the tumor.

Invasiveness

Invasion involves degradation of the extra-cellular matrix, an important entry to surrounding tissues in cancer cells way to distant organs. Among the key players are gene products that are coded by ARE–mRNAs, such as matrix metalloproteases MMP1 and MMP13, in addition to serine proteases PLAU and PLAUR. The increased activities of these mediators have been associated with invasiveness in a number of tumors. uPA converts plasminogen to plasmin, which directly (or together with MMPs) degrades the extra-cellular matrix. uPA has a well-characterized ARE that binds to both TTP and HuR, and its expression has been linked to TTP deficiency and/or HuR overexpression.[32,114] The constitutive p38 MAPK activation that can occur in a number of cancers, such as breast, stomach cancer, and multiple myeloma,[180,181] has been linked to uPA and uPAR overexpression.[114] The p38 MAP kinase pathway is activated during growth factor response, and this activation leads to the transient phosphorylation of TTP by the p38 MAPK-activated protein kinase 2 (MK2); loss of binding to AREs results, and ARE–mRNA stability and translation are subsequently enhanced.[50] These phosphorylation events decrease the affinity of TTP to the ARE and inhibit the ability to compete with HuR interaction with HuR 3′UTR; as a result, the TTP/HuR balance is switched toward ARE–mRNA stabilization and translation.[45,182] Thus, the pro-invasive factors that are overproduced because of constitutive p38 MAPK are those regulated by TTP, such as uPA, uPAR, and MMP13.[32] The pro-invasion activity of miR-29a has been shown to be mediated by the suppression of TTP, and it subsequently increases the HuR mRNA and protein levels because of the TTP–HuR axis.[32]

Migration and Metastasis

Metastasis is the major cause of mortality and morbidity in human cancer. Despite this fact, little progress has been achieved to considerably address this serious disease. One is expected to understand the intricate details of metastasis to thoroughly address the therapeutic possibilities of intervention. It is a complex process that allows cancer cells to seek refuge at distant organs, and it comprises several steps beginning with intravasation into the circulatory system, migration through circulation, and then seeding into the organs. Intravasation is the movement of cancer cells through the basal membrane into the circulatory system, both blood and lymphatic vessels, so that they can migrate to distant organs (metastasis). TAMs can help in the initial stages of metastasis by chemoattracting cancer cells toward blood vessels through secretion of pro-cancer cytokines, such EGF and IL-8. EGFR mRNA contains a 260 nt AU-rich region which causes mRNA decay and becomes stabilized upon EGF response in breast cancer cell lines.[133] Tumor cells also secrete TNF (cluster 1/class II ARE), which induces endothelial junction rearrangements that promote cancer cell transendothelial migration.[183]

In general, chemokine and specific growth factors facilitate metastatic processes. Important ARE–mRNAs that code for such factors are the components of the stromal cell-derived factor-1 (SDF-1, CXCL12)–CXCR4 axis. CXCR4 is a G-protein-coupled chemokine receptor that promotes the chemotactic migration of breast cancer cells to distant organs along a gradient of its ligand.[184] High levels of CXCR4 are associated with tumor aggressiveness in patients' tissues.[185] Recently, our work showed that CXCR4 3′UTR contains functional AREs that caused mRNA decay and bound both TTP and HuR.[86] In cancer cells, where the TTP–HuR axis is aberrant, such as in invasive breast cancer cells, CXCR4 mRNA and protein are abundant and help cells migrate to their ligand CXCL12, which itself is coded by an ARE–mRNA. Several studies confirmed the role of TTP deficiency and HuR in promoting both the invasion and migration of cancer cells.[32,86,115,186] TTP and HuR regulate various ARE–mRNAs that participate in the ability of cancer cells to migrate (Table 1 and Figure 1). Mice models using xenografted tumors with altered expression in TTP or HuR demonstrate the vivo effects on invasion and metastasis. A notable example is tumor xenografts generated from TTP-knockdown EMT cell line model, RasXT, that displays fewer metastases in the lung when compared to TTP intact cells.[43] Silencing of HuR in a mouse model of glioblastoma using human xenograft cells proves attenuation of tumor invasion and migration as assessed with GFP-labeled tumor cells.[60] In patients, HuR overexpression and/or cytoplasmic localization have been shown to be associated with lymph node metastatic disease in several cancer types, such as breast and lung cancers.[110,187] An excellent coverage of the role of HuR in different cancer pathologies was given in this reference.[188]

CONCLUSIONS: RESTORING THE AREs ORDER

The TTP–HuR axis is aberrant in many cancer cells largely because of TTP deficiency, which causes

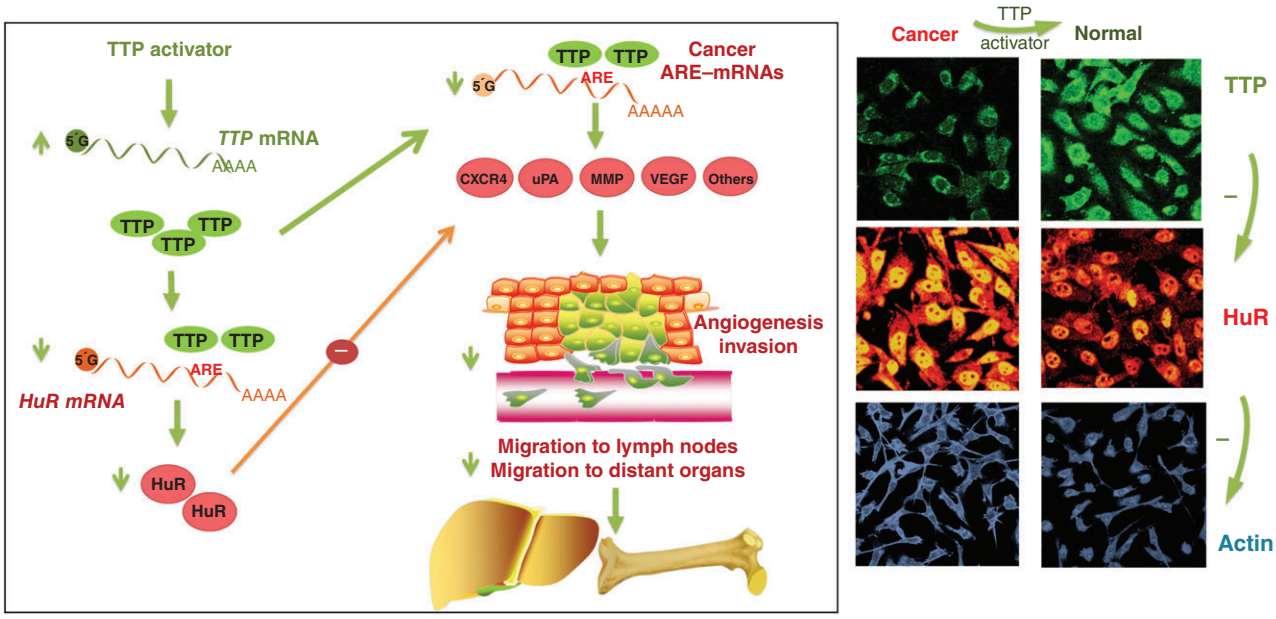


FIGURE 3 | Restoring the perturbed ARE-mediated pathway by a TTP activator. A graphical representation of how the aberrant TTP–HuR axis can be normalized by a TTP activator, which leads to a reduction of cancer ARE–mRNAs that participate in the hallmarks of cancer. The right panel shows confocal microscopy images for monitoring the changes in the expression levels of TTP and HuR, along with actin cytoskeleton visualization in normal and cancer cells.[32] Further details are found under the various sections of this review. The images on the right were adopted from our previous work[32] and reproduced from the Journal of Pathology by permission of the Pathological Society of Great Britain and Ireland.

ARE–mRNA stabilization and translational enhancement (Box 2 and Figure 2). Thus, a TTP activator would be a potential therapeutic option and may constitute a small molecule drug target that leads to upregulation or an increase in TTP activity (e.g., by dephosphorylation). Figure 3 depicts the process of normalization of the aberrant ARE-mediated pathway in cancer cells if a TTP activator is used. Indeed, this 'restoration' has been experimentally validated in a highly invasive breast cancer cell line model with the use of a cell-permeable peptide-nucleic acid against miR-29a. The inhibitor counteracted the miR-29a repression of TTP mRNA, and the TTP–HuR axis normalized as a result, along with the mRNA levels for uPA, uPAR, MMP1, and CXCR4; the breast cancer cells became less invasive and displayed normalized actin cytoskeletal polymerization[32,86] (Figure 3, right panel).

Several groups have been attempting to find drugs that either stimulate TTP or repress HuR in cancer cells. Drugs that can activate TTP include the p38 MAP kinase inhibitor, SB 203580, which inhibits TTP phosphorylation and thus leads to active TTP[189] that competes or ousts HuR in the quest for the cancer ARE–mRNA targets. Laboratory and mice studies have shown the benefits of using the p38 MAPK inhibitor in cancer models.[190,191] HDAC inhibitors, such as trichostatin, led to TTP de-repression and increased its binding capacity to ARE–mRNAs, such as COX-2 mRNA in colon cancer cells.[37,38] Because of the multifactorial nature of TTP repression, using a combination therapy to address the cancer may be necessary in the future. For HuR-targeting drugs, drug screening approaches such as using HuR/ARE interaction assay identified several small molecule drugs were found to inhibit HuR mRNA stability-promoting function through competitive binding to HuR.[192–194]

The restoration of the aberrant TTP–HuR axis by potential activators of TTP and the subsequent effects on the hallmarks of cancer in the laboratory prove the aberration of ARE-mediated pathways in cancer. Several cancer genes that code for ARE–mRNAs were studied independent of ARE involvement or posttranscriptional control; or, several ARE–mRNAs were investigated for their posttranscriptional control but not in a cancer context. Thus, many opportunities exist for further investigating the role of AREs in the hallmarks of cancer. Importantly, in vivo work and preclinical models are now much needed to address the benefit of restoring the perturbed ARE-mediated pathways (Box 3). Further work is required to find and assess kinase inhibitors or other small molecule drugs that override the various abnormalities encountered in cancer because of ARE dysregulation.

BOX 3

MOUSE MODELS AND OPPORTUNITIES IN CANCER RESEACH

Although mouse models of TTP and HuR are generated in order to understand largely the immune and inflammatory modulating role of these RBPs, they constitute a significant and potential resource for cancer studies in future. Currently, the animal studies are immune-deficient animals (e.g., nude mice) implanted with xenografted human tumors in which they were derived from cell lines with altered RBPs expression. In general, they confirm the in vivo activities of specific RBPs on the gross tumor growth. The increase or decrease of tumor volume is a result of the combined outcome of several hallmarks of cancer. Examples are xenograft tumors generated from TTP overexpressing tumor cell lines of pancreatic, gastric, breast, and lymphoma cancer types showing reduced volume of the xenografts.[42,105,155,165,166,195,196] Silencing of HuR in several types of cancer cells such as those of brain, pancreatic and renal types, in tumor xenografts leads to significant reduction in the tumor volume in mice.[60,197–199] These results confirm the in vivo role of TTP and HuR as tumor suppressing and promoting factor, respectively. Other examples were mentioned throughout the review.

Because of the tremendous efforts of establishing TTP and other mouse models, it worth listing them here in order to stimulate the interest for their utilization in cancer. Being somewhat immunocompetent, they may not be entirely suitable with established human tumor xenograft models due to transplant rejection; however, syngeneic tumors can be used instead. Additionally, these models can be used to study the role of the RBP in the tumor microenvironment and for the emergence of spontaneous and/or carcinogen inducible tumors.

- *ZFP36 (TTP)-knockout mice*

This is first TTP mouse model, where TTP is knockdown by homologous recombination-directed deletion in C57BL mice. Although appear normal at birth, they later develop arthritis, cachexia, dermatitis, autoimmunity, and myeloid hyperplasia in a manner that is largely dependent on increased TNF levels.[200] This model can be used, e.g., to study carcinogen-induced tumor development.

- *Myeloid-specific ZFP36 knockout mice*

The mice were created by taking the advantage of the M lysozyme promoter, specific for cells of the myeloid lineage, to express *cre* recombinase. The mice did not display the same systematic inflammatory patterns as the former. However, the monocytes secrete excessive TNF production in response to low doses of the gram-negative bacterial endotoxin resulting in toxic shock and organ failure.[201] Because the tumor associated macrophages play an important role in tumor maintenance (see *Activating Invasion and Metastasis: The ARE Interactome* section), this mouse model can be valuable in assessing the specific role of TTP in TAM-assisted tumorigenesis.

- *ZFP36ΔARE mice*

This is a latest laboratory variant in TTP models in which 136 bases of TTP 3′UTR, which harbors AREs, were deleted in the endogenous locus.[202] TTP AREs are important in autoregulation of TTP mRNA stability.[22,203] Cells derived from the *TPΔARE* mice have stable TTP mRNA and higher levels of TTP protein levels, and they resist the typical inflammatory diseases seen in TTP-knockout mice.[202]

- *$ZFP36_{AA}$ mice*

These mice, instead of wild-type TTP, express TTP that has alanine mutations in two prominent serine sites (ser52 and ser178 of murine ORF), so they will not be phosphorylated at these sites. The mice are normal and ARE–mRNAs such as TNF IL1, IL-12, CXCL1, CXCL2, and PIM-1 are reduced in the myeloid cells. Like the former model, they resist the systematic inflammatory response due to endotoxin.[204] The previous two models may find benefit in cancer research to study the effect of TTP on cancer ARE–mRNAs in general particularly when using syngeneic (C57BL/6J) tumor xenograft or inducible tumors.

Both of the previous two models offer dual benefit, transgenic expression of TTP and elevated expression or increased activity of TTP, mimicking putative TTP-inducing therapeutic drugs. This should allow, at least partially, assessment of the usefulness of TTP enhancing drugs in cancer.

- *Eμ-ZFP36 and Eu-ZFP36L1 mice*

These are transgenic mice for either TTP or ZFP36L1 (Tis11b) which were utilized by creating double transgenics with Eμ-myc transgenics, a mouse model of human lymphoma in which c-myc is overexpressed from Ig enhancer

promoter (thus, B cells). It was demonstrated that Eμ-TTP/Eμ-myc mice dramatically survived more than Eμ-myc mice due to resistance to the development of aggressive lymphomas.[155]

- *ZFP36L1Δ/ ZFP36L2 Δ mice*

The lymphocyte conditional Zfp36l1 and Zfp36l2 double knockout developed T-lymphoblastic leukemia T-ALL while the single knockouts were normal.[205]

- *Myeloid-specific ZFP36L1 Δ mice*

Unlike myeloid *ZFP36/TTP* knockout mice, they do not appear to participate in the inflammatory response to endotoxin, possibly due to compensation from the TTP effect.[206]

- *ELAVL1 HuR mouse models*

ELAVL1-knockout mice display embryonic lethality, thus, a number of conditional HuR-knockout or conditional HuR-transgenic mice have been developed and largely for the study of the immune system.[207–210] These models have not been evaluated in cancer studies and may offer opportunities to study the effect specific immune cells as affected by HuR on cancer. It may be preferable to use the conditional knockout models as opposed to transgenic models to avoid opposite effects of HuR due to overexpression since HuR is abundantly expressed ubiquitous gene.

- *Other mouse models*

Both KSRP- and AUF1-knockout model are available in addition to AUF1-transgenic mice.[173,211] Although the in vivo role of KSRP in cancer is not established, the latter model demonstrates that AUF1 overexpression promote development of sarcoma.[173]

ACKNOWLEDGMENT

The open access charge is supported by the intramural funding from King Faisal Specialist Hospital and Research Centre.

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