hnRNP A18: A New Pathway to Regulate Protein Translation in Cancer Cells

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Abstract
Intracellular changes that lead to modification of normal gene expression and protein metabolism patterns are a hallmark of cancer cells. These changes confer specific growth advantages and unlimited replicative potential that are necessary for a continuously dividing cancer cell. Targeting the refueling capacity of actively proliferating cancer cells by limiting the amount of protein synthesized provides an attractive mechanism to prevent cancer progression. New anti cancer therapies targeting the protein translation regulator mammalian Target Of Rapamycin (mTOR) highlight the importance of this signaling pathway for cancer progression. Here, we describe an alternative mechanism to regulate protein translation in cancer cells. In contrast to mTOR which predominantly targets the general translational machinery, the heterogenous Ribonucleoprotein A18 (hnRNP A18) regulates the translation of specific mRNA transcripts in response to cellular stress such as hypoxia and UV radiation. hnRNP A18 mediates this effect by binding to an hnRNP A18 signature motif in the 3'UTR of targeted transcripts important for cancer progression such as HIF-α, RPA, TRX, eIF5A and ATR. hnRNP A18 interacts with the general translational machinery by binding to eIF4G, thus bridging the 3'UTR to the 5'UTR to initiate translation. hnRNP A18 is over expressed in several human cancers and can immortalize murine primary cells. hnRNP A18 could thus, as mTOR, be targeted to prevent protein translation in cancer cells.

Keywords: Protein Translation; Cancer; hnRNP A18; Hypoxia

Protein Translation in cancer cells
Protein translation can be regulated at several levels. The mammalian target of rapamycin (mTOR) is one of the best understood signaling pathways in protein translation and an important target for anti cancer drug development. This serine/threonine kinase is a member of the PI3-related kinase (PIKK) and downstream of AKT. Ultimately mTOR stimulates protein synthesis by phosphorylating key components of the translational process such as the ribosomal protein S6 kinase 1 which activates ribosomal biogenesis and the eukaryotic initiation factor 4E binding protein 1 (eIF4E-BP1) which activates eIF4E and promote protein translation (1). Drugs targeting mTOR have shown clear benefit in some cancers such as mantle cell lymphomas, Renal Cell Carcinoma and Tuberous Sclerosis Complex-related tumors but have limited efficacy as single agents in most other cancers. This is mainly due to substrate specificity and feedback mechanisms.

Protein translation can also be regulated by RNA binding proteins (RBP). Previous reports have indicated that RBP RNA Binding Domains (RBD) binding to 3'UTR of transcripts can regulate the rate of translation initiation by interacting with 5' cap binding proteins (2). This phenomenon led to a model of circularization of mRNA (3) in which 3' and 5' ends of RNA form a “kissing” loop through the interaction between 3' RNA binding proteins and the cap binding protein eIF4F. Such end-to-end complex is thought to increase translation efficiency and permits 3'-UTR mediated translation control (4).

Protein synthesis is essential to maintain the demands of cell proliferation and normal cellular functions. Targeting protein translation is actively being pursued by both the academics and
pharmaceutical industries as a mechanism to stop cancer progression. Regions of acute/chronic hypoxia (very low oxygen concentration) found in the majority of solid tumors contribute to activate protein translation on a constitutive basis. Under hypoxic conditions the mTOR protein translation signaling pathway is regulated by multiple mechanisms that in cancer cells converge into enhanced activity even though in normal cells such conditions should suppress it (5). The loss of functions of mTOR inhibitors through mutations in cancer cells is in part responsible for the constitutive activation of the protein synthesis pathways in these cells. Cellular adaptation to growth under hypoxic conditions allows the tumor to resume its progression and correlates with a poor clinical outcome in several types of cancer (6). Modification of protein synthesis patterns that occur following exposure to cellular stress typically include an immediate arrest of general protein translation followed by an increased rate of targeted protein synthesis (7). Down regulation of protein synthesis in response to stress is thought to be an adaptive response triggered to protect the cells and conserve the resources required to survive (8). On the other hand, induction of specific proteins including several ribosomal proteins in response to stress indicate the involvement of the translational machinery in sensing and responding to cellular stress (9). The association of several ribosomal proteins with the oxidative stress response (10) is additional evidence that translational regulation is a significant component of the cellular stress response. Several types of stress, including heat shock stress and several chemicals, can induce the synthesis of stress proteins while inhibiting the rate of general protein synthesis (11). Thus, regulation of general translation as well as translation of specific transcripts that can confer growth advantages are important contributing factors to cancer cells progression.

**hnRNP protein family**

Our early studies (12) have shown that a number of RNA binding proteins (RBP) are specifically activated in response to genotoxic stress such as UV radiation. The heterogenous ribonucleoprotein A18 (hnRNP A18) was the first RBP reported to be inducible by UV radiation (13). Heterogeneous nuclear RNAs are the most abundant RNAs synthesized in eukaryotes by RNA Polymerase II (14). The proteins that bind hnRNAs are called heterogeneous nuclear ribonucleoproteins (hnRNPs) (15). The conventional view is that hnRNPs are involved in a wide variety of processes occurring in the nucleus only. These include transcriptional regulation, maintenance of telomere length, immunoglobulin class-switch recombination, alternative pre-mRNA splicing and pre-mRNA 3’-end processing (16,17). More recent data show that some hnRNPs shuttle between the nucleus and cytoplasm. Many studies provide evidence that hnRNPs not only accompany mRNAs into the cytoplasm but also control the activities of the mRNAs in the cytoplasm by directing the assembly of multiprotein-RNA complexes thereby regulating gene expression (18). As more and more new biological functions have been discovered, hnRNPs have been recognized as a family of RNA binding protein having multifaceted roles in many important biological processes including DNA repair, telomere biogenesis, cell signaling and in regulating gene expression at both transcriptional and translational levels (19). Through these key cellular functions, hnRNPs have been shown to play important roles in tumor development and progression (19).

**Characteristics of hnRNP A18 protein structure**

The human hnRNP A18 contains 172 amino acid residues and is divided into two domains: N-terminal RNA binding domain (RBD) (amino acid from 6-84) and C-terminal RGG domain (arginine-glycine rich domain) (amino acid 92-158) (Figure 1A). According to a BLAST search, the nucleotide and amino acid sequences of hnRNP A18 in its RBD domain exhibit high homology with the hnRNPs of diverse origin, especially the human and rat hnRNP A1.

The alignment with other RBD-containing proteins is shown in Figure 1A. Like most RBD containing proteins, hnRNP A18 canonical RNP1 (hexamer) and RNP2 (octamer) motifs can be perfectly aligned with those of the other RBD containing proteins (Figure 1B). Figure 1C shows a 3D structure of hnRNP A18 RBD as predicted by the Swiss-Model software application based on the X-ray structure of hnRNP A1 (Protein data bank accession number:1u1qA ). As seen in other RBD containing hnRNPs, the βαββαβ secondary structural elements form a four-stranded antiparallel beta sheet flanked by two alpha helices.

In addition to the RBD domain, the COOH terminal domain of hnRNP A18 contains five Arg-Gly-Gly repeats that together constitute an RGG box. The RGG box was initially identified in hnRNP U protein where it was found that the RGG motif in

hnRNP U represents the entire RNA binding domain of this hnRNP protein (20). The involvement of an RGG box in specific RNA binding has not been found in hnRNPs other than hnRNP U. However it has been reported that the RGG box may increase the general RNA binding affinity (17). In fact both the RBD and the RGG are required to confer maximal binding activity to hnRNP A18 (21). Unlike the RBD of hnRNP A18, the structure of the RGG domain in hnRNP A18 is not predictable, since no homology has been found by BLAST sequence analysis. Few hnRNPs with only one RBD have been found and those hnRNP with one RBD often do not have the RGG domain. Therefore, the one-RBD-one-RGG feature of hnRNP A18 is rather uncommon among the hnRNP family.

hnRNP A18 in cancer

hnRNP A18 was originally cloned by hybridization subtraction on the basis of rapid induction in UV radiated CHO cells (13). Since then, the human hnRNP A18 was cloned and characterized (22). The protein has also been identified in mouse following exposure to mild cold shock and is thus also known as CIRP for Cold Inducible RNA Binding Protein (23). Under normal physiologic conditions hnRNP A18 is mostly expressed in the nucleus but translocates to the cytoplasm in response to cellular stress such as UV or hypoxia (22, 24-26). hnRNP A18 is up regulated in several human tumors including prostate, breast and colon cancers where interestingly immunohistochemistry staining identify the protein predominantly in the cytosol (27). This is consistent with the hypoxic nature of most solid tumors (28) and the previous reports of hnRNP A18 translocation to the cytosol under stress conditions including hypoxia (25, 26). Translocation to the cytosol is mediated in part by the hypoxia inducible GSK3β kinase and CK2 (21,26). Phosphorylation by GSK3β also increases hnRNP A18 RNA binding activity (21). hnRNP A18 does not bind randomly but specifically target a 3'UTR signature motif found in stress responsive transcripts including, Thioredoxin (TRX), Replication Protein A (RPA),
UV, Hypoxia  \[\uparrow\] Cytoprotection against oxidative stress

ROS  \[\downarrow\] GSK3β

hnRNP A18 and Protein Translation

Figure 3. Schematic representation of our data and a proposed pathway for hnRNP A18 protein translation regulation in response to cellular stress. UV radiation and hypoxia activate GSK3β which phosphorylates hnRNP A18. Phosphorylation of hnRNP A18 results in its translocation to the cytoplasm where it interacts with specific target mRNAs on polysomes. hnRNP A18 increases translation by binding to 3’UTR harboring its signature motif and the initiation factor eIF4G in the translation initiation complex. Post-transcriptional regulation of the targeted transcripts by hnRNP A18 confers growth advantage to cancer cells under hypoxic and other cellular stress. TRX: thioredoxin, RPA: Replication Protein A, HIF-1α: Hypoxia Inducible Factor 1α, PABP: PolyA Binding Protein. ROS: Reactive Oxygen Species. 4A, 4B, 4E: eukaryotic Initiation Factor 4A, 4B, 4E.

Ataxia Telangiectasia and Rad3 related protein (ATR) and the Hypoxia Inducible Factor 1α (HIF-1α) (24, 21, 26). The coordinate up-regulation of these transcripts could confer growth advantage to solid tumors by inhibiting apoptosis (TRX) (29) and increasing DNA repair (ATR) (30). hnRNP A18 is also likely to play a role in melanoma progression since the hnRNP A18 signature motif is also found in the 3’UTR of transcripts associated with this disease. For example the hnRNP A18 motif is present in the 3’UTR of HDAC enzymes (Carrier F unpublished data) and over expression of HDAC1 is a contributing factor to apoptosis resistance in melanoma cells (31). Moreover, levels of HIF-1α correlate with melanoma progression (32) and inhibition of ATR has been shown to increase apoptosis in UV exposed primary human keratinocytes (33). In addition, we have found that
hnRNPA18 is not expressed in primary melanocytes but over expressed in melanoma cells under hypoxic conditions (Carrier F unpublished data). The role that hypoxia plays in hnRNPA18 up regulation is likely to be similar in most solid tumors. We have previously shown that hnRNPA18 up regulates TRX (21) and TRX increases the expression and activity of HIF-1α which is associated with poor prognosis and metastatic potential in certain cancers (34, 35). Furthermore, the hnRNPA18 signature motif is present in HIF-1α 3'UTR (Figure 2A and (26)). We have shown that the hnRNPA18 signature motif is sufficient to confer binding to hnRNPA18 (26). hnRNPA18 is thus likely to contribute to HIF-1α up regulation in response to hypoxia. On the other hand, hnRNPA18 up regulation in response to hypoxia is independent of HIF-1α (25). Nonetheless, several HIF-1α consensus binding sites are located in the sequence preceding hnRNPA18 mRNA (36). Thus the stage is set for a possible positive feedback loop whereby hnRNPA18 could up regulate HIF1-α directly by binding to its 3'UTR and indirectly through up regulating TRX (Figure 2B). HIF-1α could in turn contribute to sustain hnRNPA18 levels during prolong hypoxia.

### hnRNPA18 mechanism of action

Three hnRNPA18 mRNA transcripts have been described (37). The one that we referred to as hnRNPA18 has the shortest 5'UTR and is expressed at 37°C. The two other transcripts are expressed at 32°C, harbor a larger 5'UTR and have shown internal ribosome entry segment (IRES)-like activity. Functions and regulations of hnRNPA18 under cold shock are likely to be different than under more physiological conditions (37°C). The sequence and predicted structure of hnRNPA18 clearly identifies it as a member of the hnRNPA18 family (Figure 1A). To improve clarity in the literature we recommend to use the term hnRNPA18 for experiments performed at 37°C including human tissues and cells and CIRP for studies performed under mild cold shock conditions (32°C).

Although hnRNPA18 is not involved in transformation it can confer immortality to mouse embryonic fibroblasts (27). It is believed that immortalization of murine primary cells is the results of increased protein synthesis through activation of P-ERK1/2 and the phosphorylation of downstream protein synthesis initiators such as the ribosomal protein S6 kinase 1 and eIF4E-BP1 (27). In this system, hnRNPA18 thus appears to function in a manner analogous to mTOR. Nonetheless, in a more complex mammalian system hnRNPA18 had no effect on ERK1/2 activation (27). Our data suggest that hnRNPA18 increases translation in a more targeted specific manner in the mammalian system. We have shown that the predominantly nuclear hnRNPA18 is translocated to the cytosol in response to cellular stress (hypoxia, UV) (24,26). Phosphorylation by the hypoxia inducible GSK3β and CK2 is required for translocation and increases RNA binding activity (21,26). In the cytosol hnRNPA18 recognizes a specific 51 nucleotide RNA motif that is present about 11 times more often in the 3'UTR of hnRNPA18 mRNA targets than in the unigene data base (26). We identified this motif by computational analysis of primary sequences and secondary structures of hnRNPA18 mRNA targets against the unaligned sequences (26). hnRNPA18 increases the translation of the targeted transcripts by interacting with the eukaryotic initiation factors eIF4G, one component of the general translation cap-binding complex eIF-4F, on polysomes (21). Increased translation that results from hnRNPA18 binding to the 3'UTR of a transcript is thus likely to be mediated by a circularization mechanism as shown in (Figure 3).

### Conclusions

In human cancer cells it appears that hypoxia is probably the most relevant inducer for hnRNPA18 functions on protein translation. This is due to its regulation by GSK3β and its close relationships with HIF-1α and TRX. Through its signature motif in the 3'UTR of several transcripts involved in cells proliferation hnRNPA18 can confer growth advantages to cancer cells under hypoxic and other cellular stress conditions. Targeting hnRNPA18 thus offers the opportunity to shut down an entire network of genes devoted to maintain cancer cell proliferation. This is best exemplified by studies showing that down regulation of hnRNPA18 reduces cell proliferation in prostate cell lines (38) and melanoma tumor growth reduction in mouse xenograft (Carrier F unpublished data). Moreover, down regulation of hnRNPA18 enhanced chemosensitivity to cisplatin and adriamycin in prostate cancer cells (38). hnRNPA18 thus targets protein translation in cancer cells through a new mechanism different than mTOR. Solving the hnRNPA18 three dimensional structure should facilitate the identification of small molecule inhibitors through computer aided drug design to develop a new mechanism of anti cancer therapy.
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Conflicts of Interest
No potential conflicts of interest to disclose.

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