Transcriptional and post-transcriptional positive regulatory loops between HIF-1α and the RNA-Binding protein hnRNPA18.

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ABSTRACT

Hypoxia, the scarcity of oxygen, is a hallmark of solid tumors to which they adapt by activating the hypoxia-inducible transcription factor 1 (HIF-1). HIF activates the transcription of genes bearing hypoxia responsive elements (RE) in their promoter regions including genes related to angiogenesis, glucose metabolism, cell proliferation, survival, invasion, and metastasis. The HIF-1 pathway is thus an attractive target to prevent cancer aggressiveness and improve the effectiveness of cancer therapy. HIF-1α activity is known to be mainly regulated through post-translational modification by prolyl hydroxylase domain (PHD) enzymes, but accumulating evidence indicate that it is also regulated by other mechanisms such as transcriptional initiation, translational initiation, protein-protein interaction, post-translational modifications and post-transcriptionally, primarily through the action of trans-acting factors (noncoding RNAs and RNA-binding proteins) that interact with the HIF-1α mRNA to regulate its decay and translational rates. We recently identified the RNA binding protein hnRNP A18 as a regulator of HIF-1α translation under hypoxic conditions. hnRNP A18 is a nuclear stress responsive protein that translocates to the cytosol in response to cellular stress including hypoxia to bind to a recognition motif in the 3'UTR of its targeted transcripts including HIF-1α to stabilize the transcripts and increase their translation. Using a bicistronic reporter plasmid containing a HIF-1α internal ribosome entry site (IRES) we now show that hnRNP A18 can also regulate HIF-1α through its IRES in the 5'UTR. In fact, cells expressing hnRNP A18 significantly increase translation of a reporter CAT protein under the control of HIF-1α IRES in the presence of the hypoxia mimetic agent CoCl2 . On the other hand, deletion of hnRNP A18 prevents the translation of the CAT reporter protein even in the presence of CoCl2. Moreover, we also identified several HIF-1α REs in hnRNP A18 promoter. Two of the predicted HIF-1α REs, located at -57 and -226 upstream of the hnRNP A18 start codon were validated as bonafide HIF-1α binding sites by Electromobility Shift Assay with recombinant HIF-1α protein and by Chromatin Immunoprecipitation assay in human pancreatic cancer cells Panc 01. These data thus indicate that a positive regulatory loop between hnRNP A18 and HIF1-1α exist to amplify HIF-1α expression under cellular stress. hnRNP A18 can upregulate HIF1α protein expression by stabilizing its transcript at the 3'UTR and by binding to its IRES. On the other hand, HIF-1α can upregulate hnRNP A18 by binding to its proximal promoter. Targeting hnRNP A18 could thus provide a new mechanism to regulate HIF-1α expression and sensitize cancer cells to therapies.

BACKGROUND

- **New regulator of protein translation in cancer cells. Low or undetectable levels in normal cells. Up regulated in several human tumors**
- **Rapid induction in UV radiated CHO cells (1988). Human hnRNP A18 was cloned and characterized.**

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- **hnRNP A18 protein identified in mouse following exposure to mild cold shock and is thus also known as CIRP**
- **Under normal physiologic conditions mostly expressed in the nucleus but translocates to the cytoplasm in response to cellular stress (UV, hypoxia:GSK3-β, CK2).**

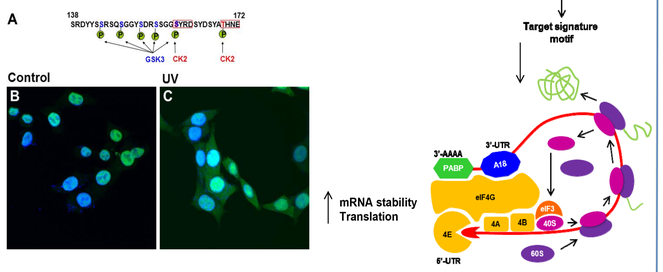

Figure 1: Down regulation of hnRNP A18 reduces tumor growth and metastasis

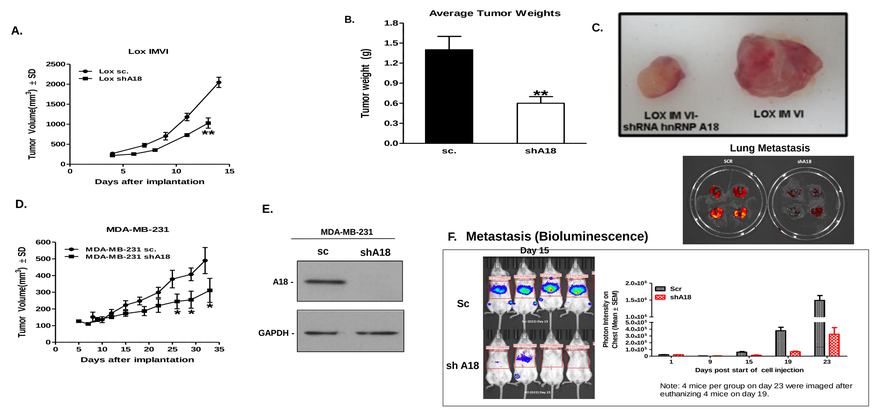

Fig.1: A-C) Downregulation of hnRNP A18 significantly reduces melanoma and breast (D) tumor growth as well as metastasis (F).

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Figure 2:hnRNPA18 regulates HIF-1α

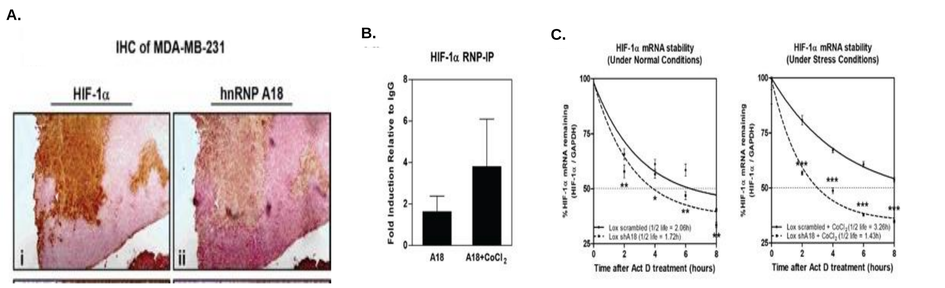

Fig.2: A) Immunohistochemistry performed on a tumor formed in a mouse xenograft injected with MDA-MB-231 cells stably transfected with scrambled RNA. HIF-1α (i) or hnRNP A18 (ii) staining. Magnification; 4X (i, ii). *p<0.05, **p<0.005. **B)** Ribonucleoprotein Immunoprecipitation (RNP-IP) on polysomes extracted from cells exposed or not to $CoCl_2$. IP was followed by RT-PCR to detect HIF-1α and GAPDH transcripts. **C).** mRNA stability assay. LOX-IMVI cells stably transfected with either scrambled RNA (solid line or shhnRNP A18 (dashed line) were treated (right panel) or not (left panel) with $CoCl_2$ for 4 hours, then Actinomycin D (10 μg/ml) was added and analyzed by Real-Time PCR.

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Figure 3: hnRNPA18 binds to HIF-1α 3'UTR and 5'UTR-IRES

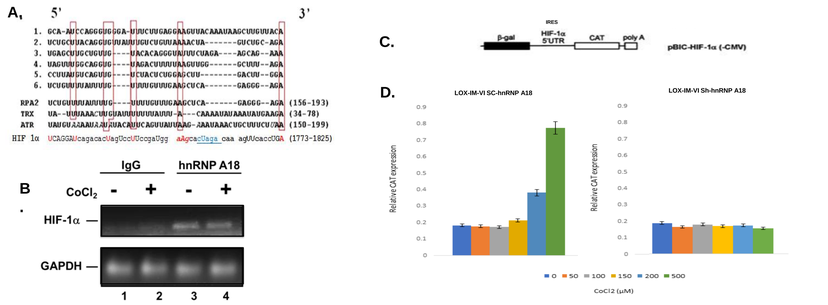


Fig.3: hnRNPA18 binds to HIF-1α 3'UTR and 5'UTR IRES. A) Consensus hnRNP A18 RNA binding motif and motif found in RPA2,TRX, ATR, HIF-1α. **B).** RNP-IP with anti-hnRNP A18 and IgG Abs followed by RT-PCR for HIF-1α and GAPDH transcripts from LOX IMVI cells exposed (+) or not (-) to $CoCl_2$ **C).** Schematic representation of the reporter construct used in this study **D).** Scramble and Sh-hnRNPA18 LOX IMVI cells were transfected with promoterless pBIC-HIF-1(-CMV), and 24 h later CAT protein levels were measured in response to $CoCl_2$

Figure 4:HIF-1α Responsive Elements (RE) in hnRNP A18 promoter

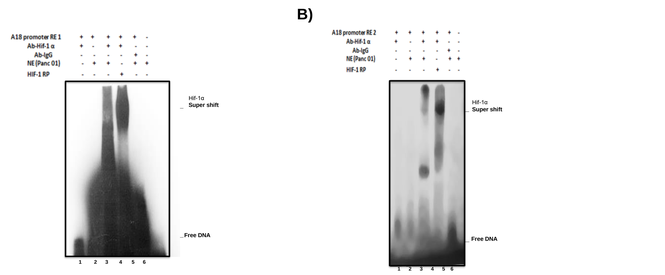


Fig.4). EMSA DNA Super Shift assay. Nuclear extract Human Pancreatic Cancer cells Panc01 (NE-P01) and HIF-1α RE in hnNRP A18 proximal promoter **A). PromotorRE1** Seq(**CAGGAGGT**). **1).** Seq(RE1)+Ab-Hif1α. **2).** RE1+ NE-P01. **3).** NE-P01+ RE1+Ab-Hif1α **4).** RecomProtein (HIF-1α RP)+ RE1+Ab-Hif1α **5).** NE-P01+ RE1+Ab-IgG **6).** NE-P01 **B).Promotor RE2** Seq(**AGGTGGTG**). **1).** Seq(RE2)+Ab-Hif1α. **2).** RE2+ NE-P01. **3).** NE-P01+ RE2+Ab-Hif1α **4).** RecomProtein (HIF-1α RP)+ RE2+Ab-Hif1α **5).** NE-P01+ RE2+Ab-IgG **6).** NE-P01.

Figure 5: HIF-1α regulates hnRNP A18 transcription

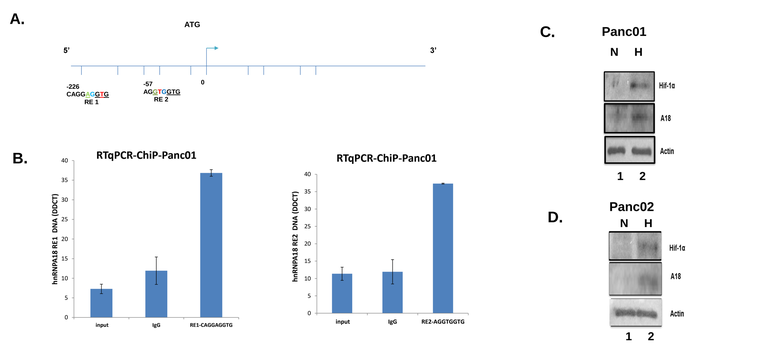
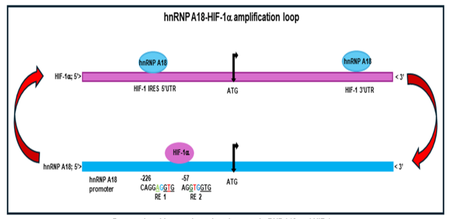


Fig.5: A). Schematic position of HIF-1α REs in hnRNPA18 proximal promoter **RE1**(**CAGGAGGTG** and **RE2** Seq-(**AGGTGGTG**. **B)** ChiP assay was performed using human pancreatic cells lines Panc01. Experiments were performed with anti-Hif-1α and control IgG antibodies. RNAs present in the immunoprecipitated complexes were quantified by RT-qPCR using specific primers. **C)** Western blot, Ab-Hif-1α and Ab-A18 on pancreatic Human Panc01 and Mouse Panc02 cell lines, treatment Hypoxia (H) Chamber 37°C (0.5 O_2 %) 24 hrs and without treatment normoxia (N) conditions.

CONCLUSIONS

- ➔ **i) hnRNP A18 can regulate HIF-1α expression by stabilizing its transcript at the 3' UTR and by binding to its IREs in the 5'UTR.**
- ➔ **ii) HIF-1α can regulate hnRNP A18 by binding to HIF-1α RE in hnRNP A18 promoter**
- ➔ **iii) The interactions between hnRP A18 and HIF-1α creates a positive feedback loop to sustain cancer cells growth under hypoxia.**
- ➔ **iii) Identification of a new class of small molecules inhibitors to specifically target hnRNPA18 would be a good alternative to regulate HIF-1α protein expression in cancer.**



Proposed positive regulatory loop between hnRNP A18 and HIF-1α.

Fig.6: Schematic representation of positive regulatory loop between hnRNPA18-HIF-1α.

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