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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2020)

c-Jun and RBM39 co-regulate alternative splicing induced by chemotherapy compounds

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ABSTRACT

By generating multiple messenger RNAs from a single gene, alternative splicing (AS) controls and enlarges the diversity of the transcriptome. In human cells, 90% of the genes can undergo alternative splicing making this mechanism a general process. AS participates to stress responses as it allows a rapid modification of the transcriptome. In this work, we are investigating the global regulation of splicing following chemotherapy.

Previously, we performed a NGS sequencing of MCF7 cells treated by cisplatin and reported the modifications of both mRNA steady state levels and splicing. We began to study the molecular mechanism leading to spliceosome control after addition of cisplatin and demonstrated the importance of SRSF4 (1).

In this work, we selected a strong cisplatin-induced exon skipping event and used it as reporter to investigate the molecular regulation of the spliceosome by cisplatin. First, an siRNA screening targeting 57 RNA binding proteins was conducted. It was noted that the depletion of SF3A1, SF3B4, U2AF1 and RBM39 induced AS similar to cisplatin while the depletion of others was neutral or prevented it. Since cisplatin did not reduce the level of these 4 proteins, the AS regulation resided elsewhere. Second, as RBM39 was shown to interact with c-Jun by GST pull-down and yeast 2 hybrid(2), we investigated this interaction by immunoprecipitation and NanoLuc 2 hybrid in our experimental settings and determined that the interaction was cisplatin-dependent and RNA- and DNA-independent. Interestingly, while the depletion of RBM39 favored the studied skipping event, the depletion of c-Jun prevented it. The presence of RBM39 on the transcript intron adjacent to the skipped exon was confirmed by RNA immunoprecipitation. To investigate the role of c-Jun and RBM39 in AS on a global scale, we repeated the transcriptome analysis of MCF7 cells depleted in RBM39 and c-Jun, treated or not with cisplatin, and compared their transcriptomes.

Acknowledgments:

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hnRNPA1 promotes cancer progression in response to FGF2 signalling

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ABSTRACT

Increase in cap-independent translation of antiapoptotic proteins, such as BCL-XL and XIAP, is involved in the development of drug resistance in cancer. We previously demonstrated the role of Fibroblast Growth Factor 2 (FGF2) signalling and S6 kinase 2 (S6K2) activity in this process 1, but the downstream mediator(s) of this translational response remained unknown. We used tandem affinity purification using S6K2 as bait as well as quantitative phospho-proteomics in the presence and absence of FGF2 stimulation in small cell lung cancer and HEK 293 cells to identify new S6K2 interactors and downstream mediators of the FGF2 pathway. We showed that S6K2 interacts with and phosphorylates the heteronuclear ribonuclear protein, hnRNPA1, on Ser 4 and 6 2. This increases the association of this protein with BCLXL and XIAP mRNAs to promote their nuclear export while de-repressing their translation. A nonphosphorylatible S4/6A hnRNPA1 mutant prevented this process from occurring and impaired the prosurvival activity of FGF2/S6K2 signalling. Following phosphorylation and transfer to the cytoplasm in complex with mRNAs, phospho-hnRNPA1 associates with 14-3-3 to be sumoylated on K189 within a multi-protein complex involving UBC9. This targets hnRNPA1 for re-import into the nucleus in a caryopherin-dependent manner, a step that is essential for translational derepression of target mRNAs. The importance of this pathway for the translation of BCL-XL and XIAP in vivo was suggested by immunohistochemical staining of lung cancer tissue microarrays showing that increased S6K2 expression correlated with decreased cytoplasmic hnRNPA1 and increased BCL-XL levels. We subsequently wanted to determine large-scale changes in hnRNPA1/mRNA binding that occur in response to FGF2 and performed an RNA-IP coupled to RNA-Seq. Analysis of the interactome changes revealed several biological processes controlled by hnRNPA1 in response to FGF2, including several inflammatory pathways. In particular, this revealed the involvement of hnRNPA1 in regulating IFNγ response, with hnRNPA1 downregulation leading to sensitisation of sarcoma cells to this cytokine. We therefore initiated a drug discovery project to identify hnRNPA1 inhibitors and performed an X-Chem screen that revealed several chemical fragments specifically binding to areas of functional significance to hnRNPA1 biological activity. In conclusion, hnRNPA1 signalling promotes cancer progression and small-molecule inhibitors that hinder the activity of this protein may provide new tools to target various types of cancer.

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Muscleblind-like 2 controls the hypoxia response of cancer cells

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ABSTRACT

Hypoxia is a common feature in solid tumors as the rapidly proliferating tumor cells outgrow their blood supply. Gene expression changes in response to hypoxia add to the genetic instability of cancer cells and promote invasion and metastasis. Thus, tumor hypoxia is an indicator of more aggressive tumors and poor patient prognosis. Moreover, tumor hypoxia counteracts radio-, chemo- and immunotherapy, highlighting the importance of developing therapies effective in oxygen depleted conditions. On top of the well-studied transcriptional response exerted by the hypoxia inducible factor (HIF) family of transcription factors, hypoxia has a major effect on alternative splicing and the expression of splicing factors.

Comparison of the transcriptomic changes in response to chronic hypoxia of lung and breast cancer cells showed highly concordant changes in transcript abundance, but divergent, cell type-specific changes in alternative splicing. The levels of splicing factors are predominantly reduced in response to chronic hypoxia. One specifically induced splicing factor is muscle-blind like 2 (MBNL2), known as a regulator of tissue-specific alternative splicing. Transcriptomic changes after knockdown of MBNL2 in hypoxic cancer cells show that it promotes adaptation to hypoxia by increasing HIF response genes, such as VEGFA, at the mRNA and protein level. In addition, MBNL2 reverses hypoxia-dependent changes in alternative splicing. Consistent with its contribution to hypoxia adaptation, MBNL2 promotes cell viability and migration of hypoxic cancer cells. In summary, MBNL2 positively contributes to cancer progression through the activation of hypoxia response genes.

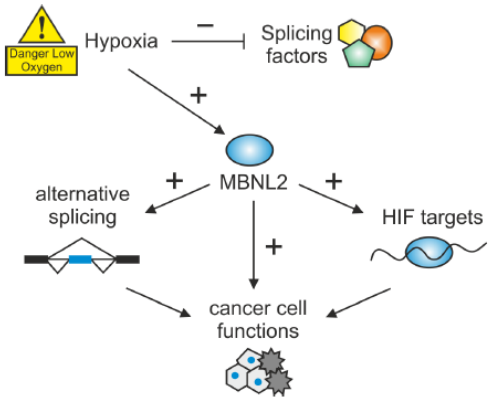

Figure 1 | MBNL2 governs the adaptation of cancer cells to hypoxia

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Expression of proline-rich proteins, P-B1, P-B, and BPLP, the parent proteins of opiorphin family, in human saliva

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ABSTRACT

The NCBI data base demonstrated that multiple transcripts of the genes, OPRPN (alias PROL1) coding for the precursor of basic proline-rich lacrimal protein BPLP (UniProt/Swiss-Prot, Q99935, 248 amino acids) [1], PBI (alias SMR3A or PROL5) for the precursor of P-B1 (Q99954, 134 a. a.) and PBII (alias SMR3B or PROL3) for the precursor of P-B (P02814, 79 a. a.) on chromosome 4q13.3 are synthesized by alternative splicing, as shown in Fig. 1. The mRNA (NCBI, AK312075) and two mRNAs (BC144529 and BC015327) code for the precursor of P-B1 and P-B, respectively. Recently, we have identified the P-B variant (Q504X8, 106 a. a.) encoded by BC094707-mRNA [2]. The three proteins are carrying an identical signal peptide of 22 a. a., an opiorphin-homolog (QRGPR), and an anti-breast cancer peptide (RGPYPPGPLAPP). In case of the OPRPN gene, the mRNA (NM_021225.5) for BPLP and the mRNA (Genbank, S83198.1/HC924356.1) for a homolog of BPLP (201 a. a.) are stored in the database. BPLP and BPLP-homolog share the signal peptide and the N-terminal 144 a. a. sequence, and the both are carrying opiorphin (QRFSR) having a painkilling effect. In addition, the mRNA (NM_001302807.2) for a nonproline- rich protein (127 a. a.) without opiorphin can also be found in the data base. At present, BPLP is known to be expressed in exosome II of human whole saliva, tears, submandibular-sublingual saliva, and parotid saliva by MALDI-TOF-MS. In this study, we further analyzed multiple fragments separated from the tryptic digest of human salivary proteins fractionated by two-dimensional chromatography. As the result, two tryptic fragments (-21MK-LTFFGLLALISCFTPSESQ+2R and -20KLTFFGLLALISCFTPSESQ+2R) covering the signal peptides of BPLP and BPLP-homolog including the fragment (+22WVPPSPPPPYDSRLNSPLSLPFVPG+48R) covering the sequence near the N-termini of the both. Surprisingly, the tryptic fragment covering the C-terminal portion of BPLP-homolog (+166ACHLHISSNP+176R) was also detected by MALDI-TOF-MS, suggesting that mRNA for BPLP-homolog is generated by the insertion of "G" between "386G" and "387C" in the fourth exon of the OPRPN gene. Western blot with commercially available anti-PROL1 antibody (Abcam co., Japan) showed two sets of positive signals; one is a set of positive signals raging in 20 -22K, another 25-27K. This suggests the possibility that microheterogeneity due to N-glycosylation occurs in the two proteins. However, the nonproline- rich protein described above and P-B1 could not be identified in the present study. In conclusion, our data demonstrated that BPLP and BPLP-homolog are present in human whole saliva. This finding suggests the possibility that mRNA for BPLP-homolog may be originated from pre-mRNA for BPLP by a mechanism of RNA editing.

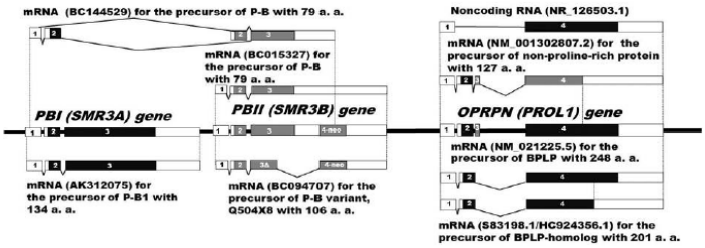

Figure 1 | Summary of alternative splicing of the genes, PBI, PBII, and OPRPN.

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