FET fusion oncoproteins hijack the LASR complex to rewire alternative splicing in sarcomas

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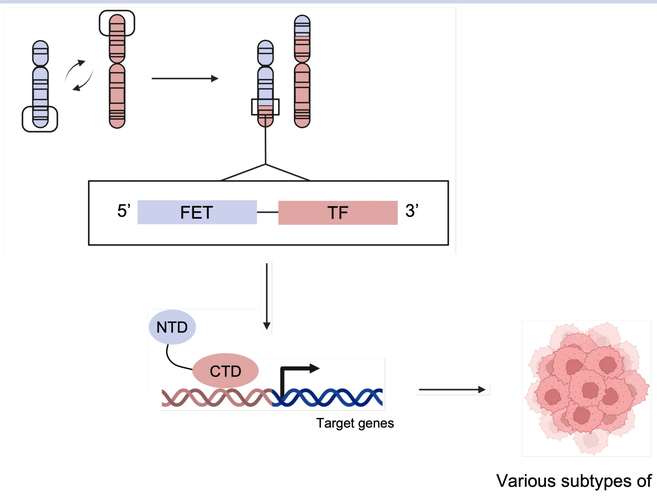
Introduction



FET (*FUS*, *EWSR1*, *TAF15*) genes are regularly involved in chromosomal translocations with diverse transcription factors (TF) genes, leading to gene fusions that drive multiple sarcoma and leukaemia subtypes. As the FET low-complexity domain provides a strong transactivation domain to the DNA-binding domain of the TF-derived moiety, FET fusion oncoproteins were initially considered as aberrant TFs modulating transcription. However, emerging evidence suggest that the oncogenic potential of FET fusions might also be explained by a post-transcriptional function in pre-mRNA splicing, as reported for the EWSR1::FLI1 fusion which drives Ewing sarcoma.

Our aim is to explore whether this post-transcriptional role can be extended to all FET fusions, and to identify a common mechanism underlying FET fusion-mediated sarcomagenesis.

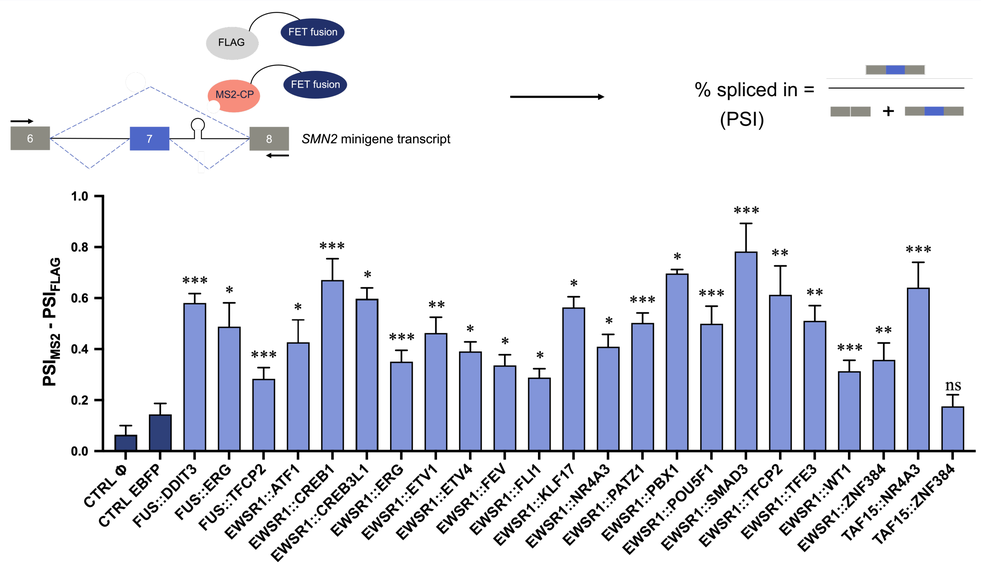
Results

1. FET fusions control the alternative splicing of hundreds of genes

Tumour type	FET fusion	Cell lines	Number of alternatively spliced genes
Clear cell sarcoma	EWSR1::ATF1	KAS	**1111**
		MP-CCS-SY	
		SU-CCS-1	
Ewing sarcoma	EWSR1::FLI1	MHH-ES1	**1059**
		A673	
		SK-N-MC	
		TC71	
Desmoplastic small round cell tumour	EWSR1::WT1	JN-DRSCT-1	**816**
		BER	
Myxoid liposarcoma	FUS::DDIT3	MLS-1765	**583**
		DL-221	

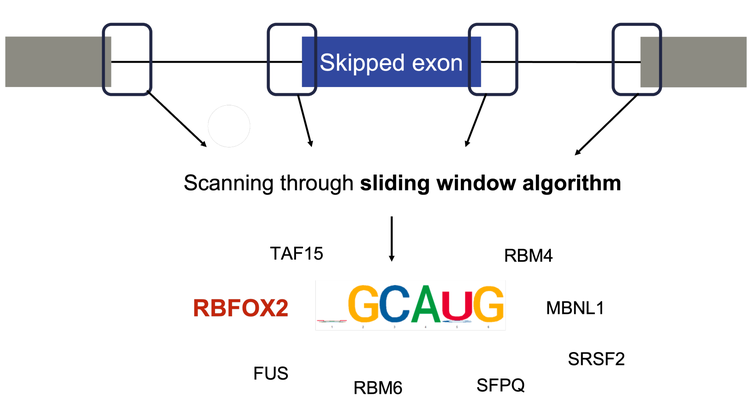
Transcriptomic analysis of sarcoma cell lines following FET fusion knockdown. The values shown represent the genes whose splicing is significatively affected in all cell lines characterized by a specific FET fusion. These results prompted us to test whether the effect of FET fusions on alternative splicing could be direct through a reporter minigene assay.

2. Recruitment of FET fusions on a reporter minigene affects its alternative splicing



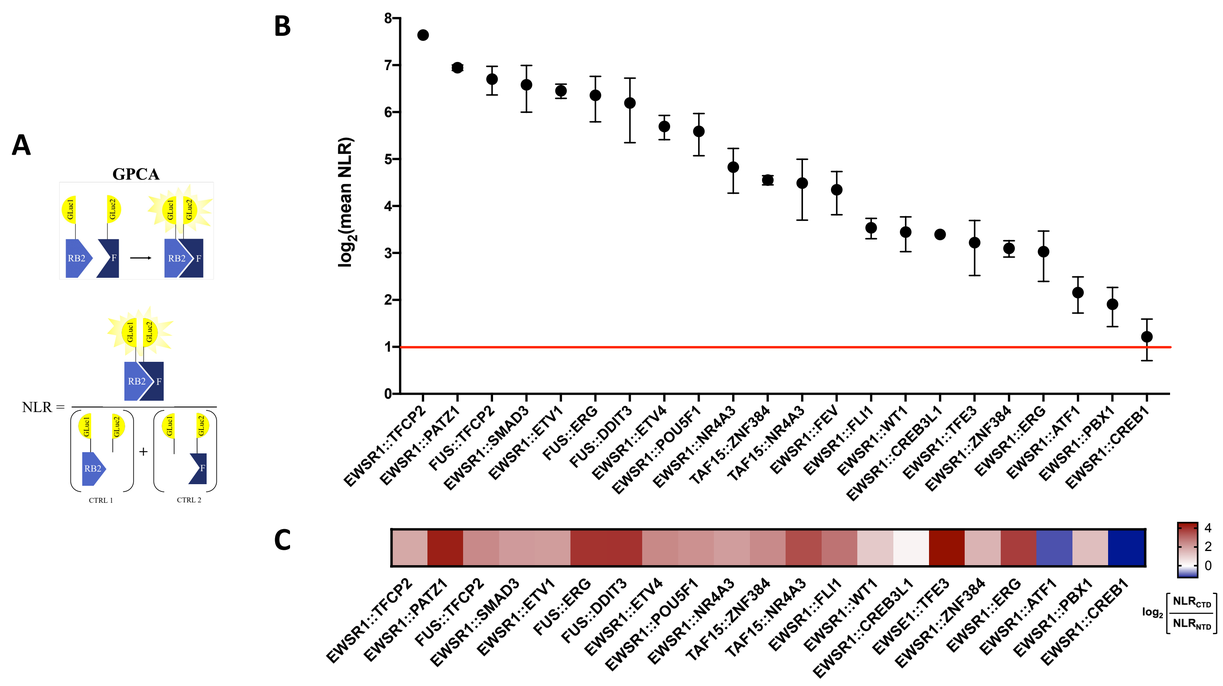
RT-PCR analysis of exon 7 inclusion in the *SMN2* minigene transcript upon FET fusion tethering. The effect of FET fusions on exon inclusion in the *SMN2* minigene transcript is strictly dependent on their recruitment to the transcript. As FET fusions are devoid of canonical RNA-binding domains, we hypothesized that they would indirectly contact pre-mRNA by interacting with an RNA-binding protein. N ≥ 3 for all.

3. RBFOX2 binding motifs are enriched around FET fusions-regulated exons



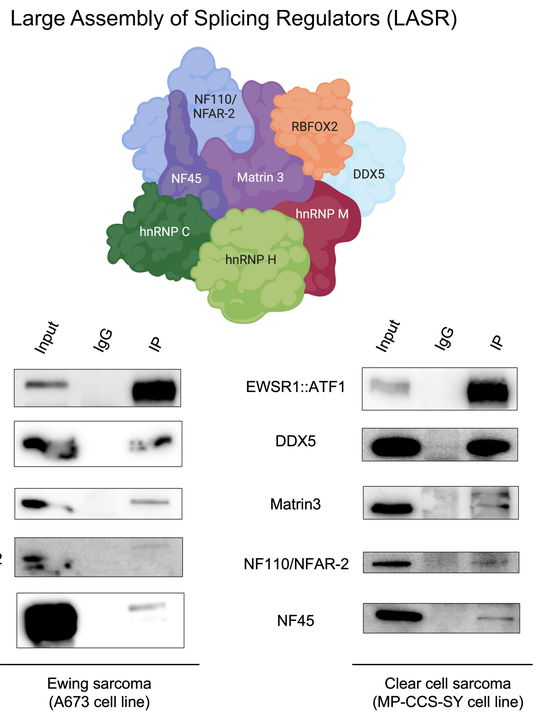
Motif enrichment analysis of our transcriptomic data using a sliding window algorithm in regulatory regions flanking significant skipped exons. The motifs of several RNA-binding proteins were highlighted by this analysis, including wild-type FET proteins and RBFOX2, a master splicing regulator known to play an important role in the splicing-regulatory function of the EWSR1::FLI1 fusion (Saulnier *et al.*, NAR, 2021). We therefore chose to test if RBFOX2 could be a common partner of all FET fusions.

4. FET fusions interact with RBFOX2 via their unrelated C-terminal domains



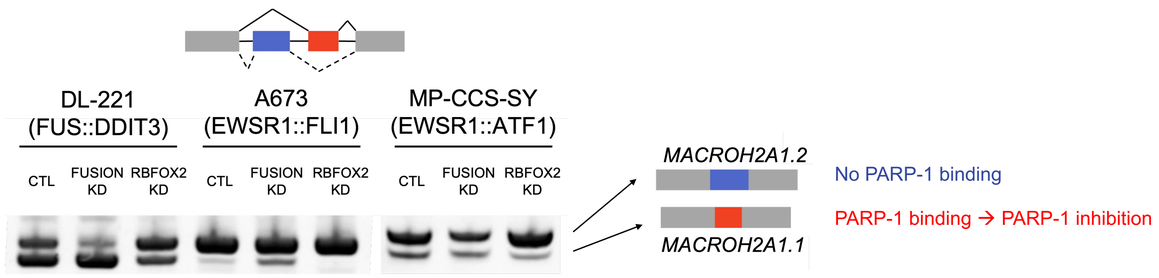
***Gaussia* luciferase Protein Complementation Assay (GPCA). A.** GPCA relies on the reconstitution of luciferase (GLuc) upon interaction of the two candidate proteins, in which case light is emitted in the presence of coelenterazine. The luminescence from each interaction is normalized with regards to the sum of the means of two negative controls, CTRL 1 and CTRL 2, which represent the background luminescence of each interactant. Interactions with a normalized luminescence ratio (NLR) above 2 were considered positive. **B.** GPCA between FET fusions and RBFOX2. The results shown are mean NLRs ± SD, in $\log_2$ scale. Most, if not all tested fusions interact with RBFOX2 **C.** GPCA between the domains of FET fusions and RBFOX2. The results shown are the mean ratios between the NLRs of C-terminal domains (CTD) and N-terminal domains (NTD) in $\log_2$ scale. The CTD is the preferred domain of interaction with RBFOX2 for almost all fusions. N ≥ 3 for all.

5. RBFOX2 partners in LASR also interact with FET fusions



Endogenous immunoprecipitations of FET fusions and members of LASR. As RBFOX2 is known to carry out its splicing-regulatory function as part of a large assembly of splicing regulators (LASR) (Damianov *et al.*, Cell, 2016) we tested the interaction of FET fusions with members of this complex. These results suggest an interaction of FET fusions with the LASR complex.

6. Coregulation of *MACROH2A1* alternative splicing by FET fusions and RBFOX2



RT-PCR analysis of *MACROH2A1* mutually exclusive exons splicing. According to our transcriptomic analysis, the alternative splicing of *MACROH2A1* is regulated by all FET fusions. Several RBFOX2 binding motifs are found in the regulatory regions flanking the mutually exclusive exons. The *MACROH2A1.1* isoform is favored in the absence of FET fusions whereas the *MACROH2A1.2* isoform prevails in absence of RBFOX2, which suggests an antagonistic coregulation of this splicing event by FET fusions and RBFOX2.

Conclusion and perspectives

We have gathered pieces of evidence supporting a shared role in alternative splicing for all FET fusions and have paved the way towards a better understanding of the mechanism underlying this function. Furthermore, FET fusions appear to be **directly involved in alternative splicing regulation**, as this post-transcriptional function can be uncoupled from their role as canonical transcription factors (**see P.9, L. Ongena**). We have yet to explore the association of FET fusions with RNA, and to thoroughly characterize the relevance of this novel function in sarcomagenesis.