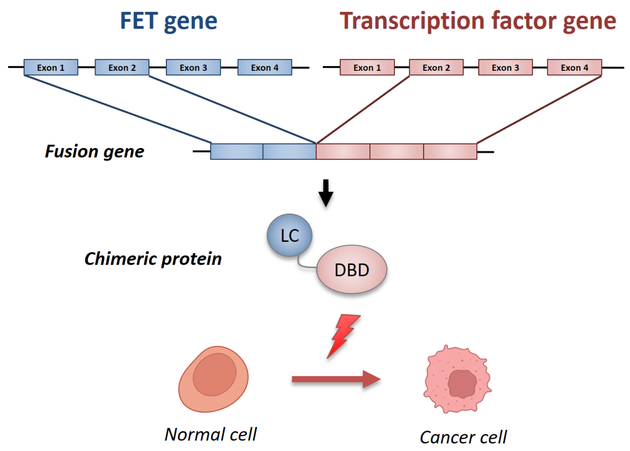
FET fusion transcription factors rewire the mRNA splicing landscape of sarcoma cells

Loïc Ongena*, **Eva Lucarelli**, Laurence Dubois, Jonathan Bruyr, Franck Dequiedt

Laboratory of Gene Expression and Cancer, GIGA-Research Institute, University of Liège (Belgium)

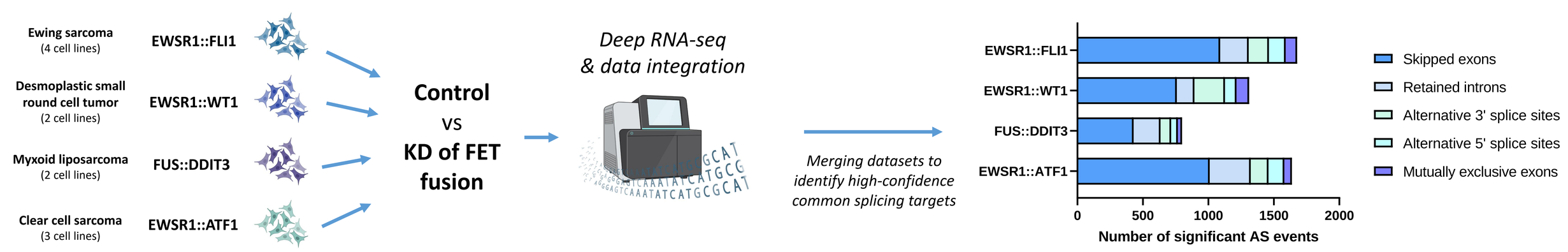
INTRODUCTION

Genes encoding the FET family of RNA-binding proteins (***F****US,* ***E****WSR1* and ***T****AF15*) are regularly involved in chromosomal translocations that result in gene fusions driving multiple neoplasms, mostly sarcomas and leukemias. These fusions systematically occur with genes encoding transcription factors (TFs) and lead to the formation of chimeric proteins harboring a disordered trans-activation N-terminal domain from the FET protein (NTD) and C-terminal DNA-binding region from the TF (CTD). This organization has led to the speculation that FET fusions act as aberrant TFs. However, evidence suggests that they carry roles beyond strictly transcriptional functions to trigger oncogenesis. **Our objective is to identify the relevance of a new post-transcriptional role for FET oncogenic fusions in alternative splicing (AS) regulation.**



RESULTS

Alternative splicing patterns are remodeled by FET fusion oncoproteins in sarcoma cells



FET fusions-modulated AS events share common features

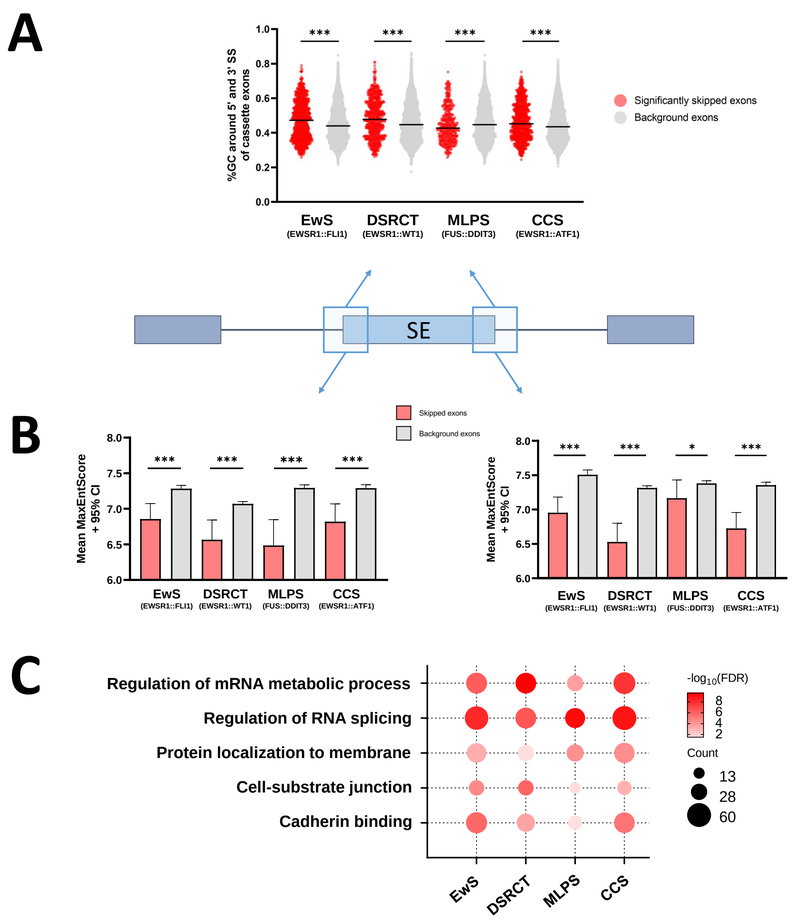


Figure 1. FET fusions control a distinct set of events characterized by specific sequence features and enrichment in common pathways. (A) Significant bias in GC content at splice junctions of cassette exons (300nt in intron/100nt in exon). **(B)** Cassette exons are surrounded by significantly weaker splice sites (lower maximum entropy scores) than background. **(C)** Enrichment of similar gene ontologies for all differentially spliced gene sets implies that FET fusions regulate common pathways via alternative splicing to promote oncogenesis.

Regulation of alternative splicing is uncoupled to transcriptional activities

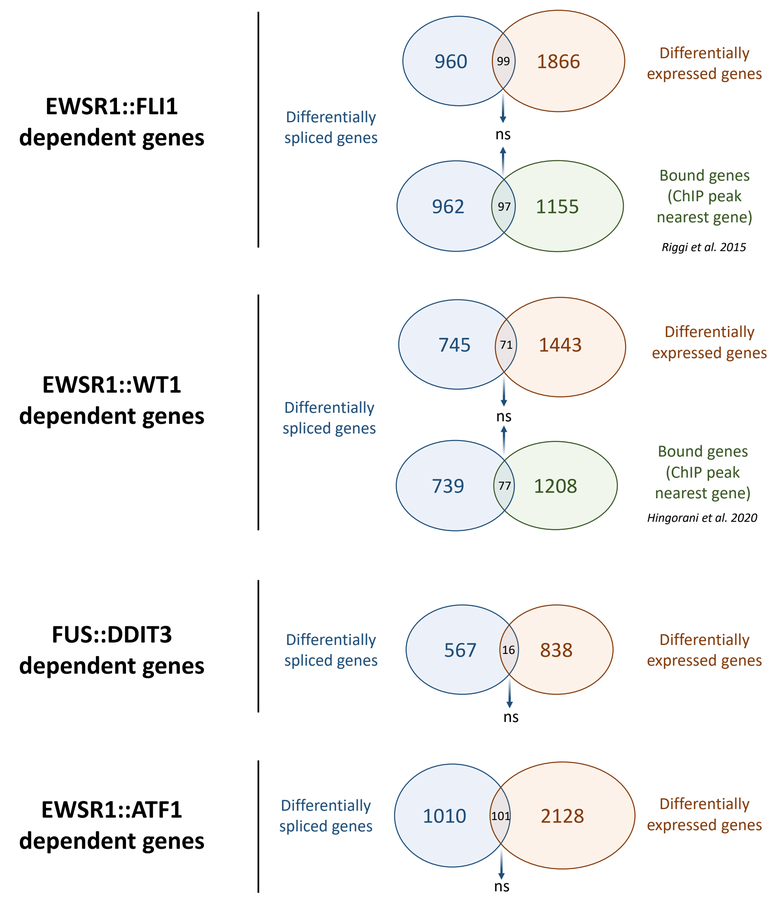


Figure 2. Genes regulated at the transcriptional level constitute a different set than differentially spliced genes regulated by FET fusions. Sets of genes modulated (this study) or bound by FET fusions (previously published datasets) were compared to genes whose splicing is affected upon fusion knockdown. This suggests that the alternative splicing and transcriptional modes of regulation are uncoupled in sarcoma cells.

FET fusions promote the splicing of target minigenes

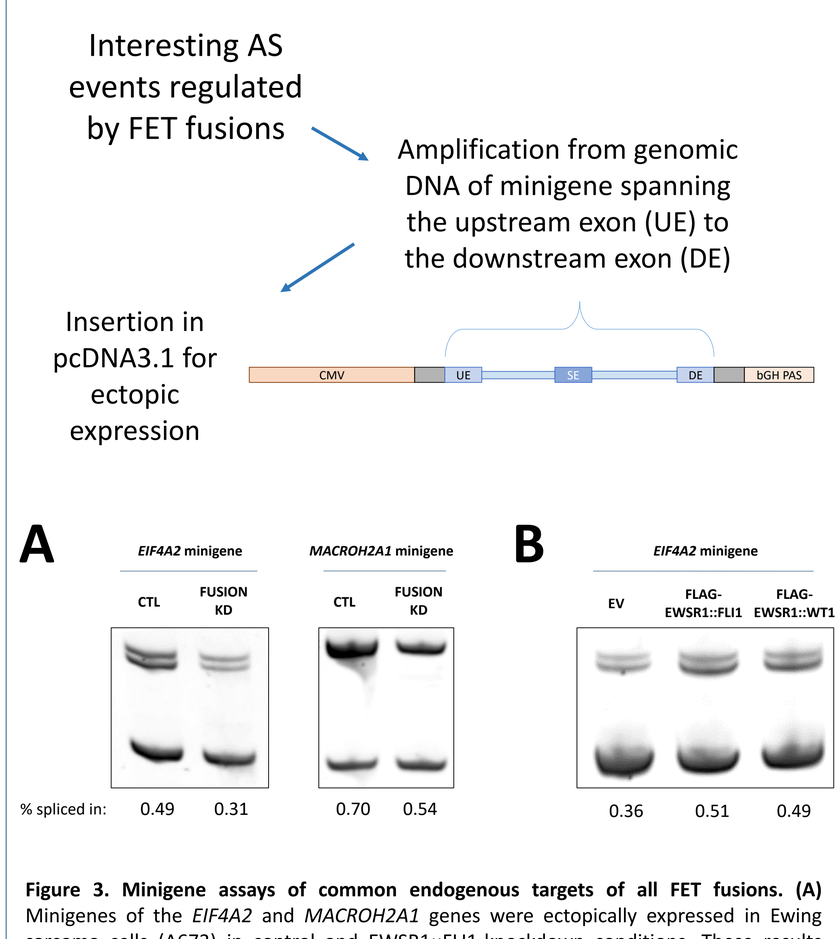


Figure 3. Minigene assays of common endogenous targets of all FET fusions. (A) Minigenes of the *EIF4A2* and *MACROH2A1* genes were ectopically expressed in Ewing sarcoma cells (A673) in control and EWSR1::FLI1-knockdown conditions. These results suggest that splicing regulation is independent of transcriptional processing, promoter identity and chromatin environment. **(B)** The *EIF4A2* minigene was co-transfected in HEK-293T cells with either empty vectors (EV) or FLAG-tagged FET fusions. These results suggest that FET fusion introduction is sufficient to hijack the splicing regulation of its target genes, even in unrelated cellular contexts. Isoform proportions were measured by RT-PCR with primers annealing to the minigene backbone and the downstream exon.

CONCLUSION

Our study provides the first direct evidence that FET fusion oncoproteins rewire the transcriptome of sarcoma cells not solely by regulating transcription, but also via the modulation of specific isoforms. In parallel to this work, we identified protein-protein interactions of mechanistic relevance, suggesting a direct implication of FET fusions on the pre-mRNA of its splicing targets (**see E. Lucarelli poster P.2**). Our objective is now to map comprehensively the transcripts bound and targeted by FET fusions and their protein partners. Deciphering the precise role of this new function in sarcomagenesis could pave the way towards the identification of novel therapeutic targets in multiple neoplasms.

télévie
fnrs LA LIBERTÉ DE CHERCHER
Fondation Léon Fredericq
LIÈGE université GIGA Doctoral School
LIÈGE université GIGA Molecular Biology of Diseases

* Correspondence: loic.ongena@uliege.be