A new view of Ewing's sarcoma : testing the provocative hypothesis that post-transcriptional mechanisms of mRNA processing contribute to the oncogenic properties of EWS-Fli1 fusion

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I. Introduction :

The FET/TET proteins, which include TLS/FUS, EWS, and TAF15 are RNA- and DNA-binding proteins, with functions in transcription and RNA processing. Each of these proteins harbors a potent N-terminal transcriptional activation domain (the EAD) that is revealed in the context of oncogenic EWS-fusions and a C-terminal RNA-Binding Domain (RBD) (fig.1) implicated in different aspects of RNA biogenesis and being the most conserved region within the TET protein family (fig.2).

The FET members are involved in chromosomal translocations in which the 5' part of human FET genes is fused to the 3' region of genes encoding potent transcription factors, including members of the Erg transcription factor family (ERG, Fli1 and FEV). FET-Erg fusions have been associated with Ewing's sarcoma (ES) family tumors and acute myeloid leukemia and a plethora of evidence leaves little doubt that these fusions are essential to the development of these malignancies. Because they include the C-terminal half of Erg proteins, which contains the ETS DNA-binding domain, fused to the amino-terminal portion of FET proteins, which behaves as a potent transcription activation domain, FET-Erg fusions have mostly been studied as oncogenic transcription factors. Among these fusions, EWS-Fli1 is found in ~85% of the Ewing sarcomas (fig.3).

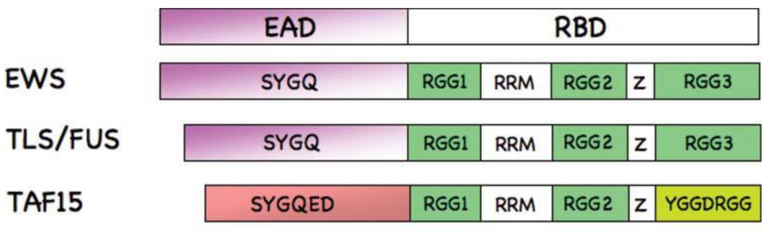

Figure 1. The TET/FET protein family contains three members TAF15, EWS and TLS/FUS. TETs are containing an N-terminal region referred to here as EWS-activation domain (EAD, purple boxes) and a C-terminal RNA-binding domain (RBD). The TET RBD contains two elements (an RRM and Arg/Gly rich RGG boxes) and a C2–C2 zinc finger (Z).

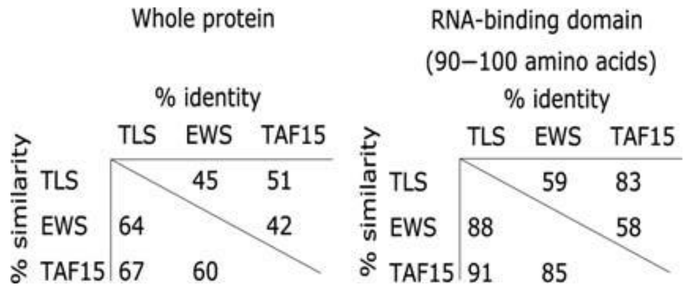

Figure 2. TET/FET proteins are highly related. Amino acid similarity and identity compared over the whole protein and within the RBD for TLS, EWS and TAF15.

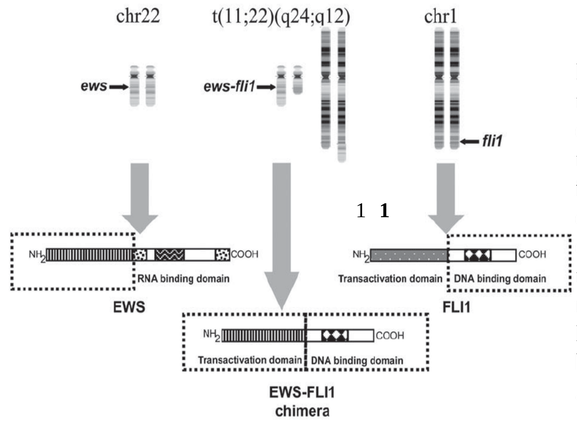

Figure 3. The reciprocal translocation between chromosomes 11 and 22 results in the formation of an EWS-Fli1 fusion gene on the abnormal chromosome 22 that codes for a chimeric transcription factor with the N-terminal transcriptional regulatory domain deriving from EWS and the ETS -specific DNA-binding domain derived from Fli1 .

II. Objectives :

In this project, we will take advantage of recent findings from our laboratory and will assess the provocative hypothesis that important post-transcriptional functions of EWS protein, in particular in translation, might be deregulated in EWS-Fli1 fusion and contribute to its roles in cancer initiation and progression.

III. Materials and Results :

1. FET proteins repress luciferase translation in HeLa and HEK293 cells

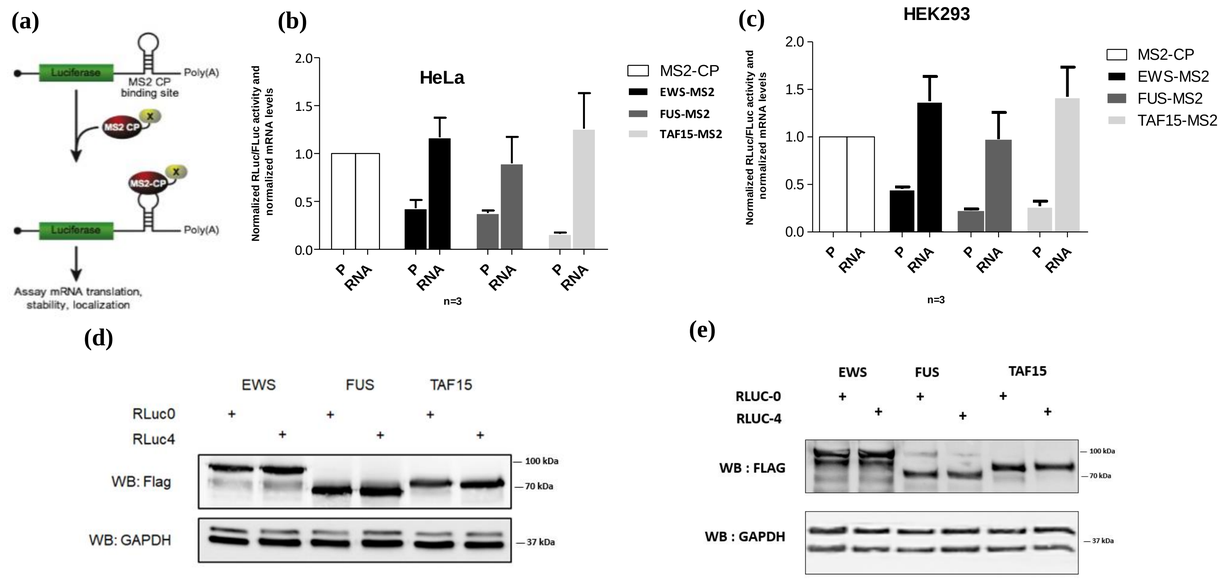

Figure 4. Tethering of FET proteins to the 3'-UTR of luciferase mRNA reporter in HeLa and HEK293 cells. (a) Principle of tethering assay: FET proteins (X = FUS, EWS or TAF15) fused to the tethering protein, MS2-Coat Protein, recognize the RNA tag sequence in the 3'-UTR of the luciferase mRNA reporter with high specificity and affinity. The function of the tethered protein is assayed using the Dual-Luciferase Reporter (DLR) Assay system. (b,c) Tethered FET proteins reduce luciferase expression (P= luciferase Protein level) and have no effect on steady-state levels of the mRNA reporter, as detected by RT-qPCR in HeLa (b) and HEK293 cells (c). (d,e) Western blot analysis of FET proteins expression in HeLa (d) and HEK293 cells (e).

2. EWS represses luciferase translation via its RBD

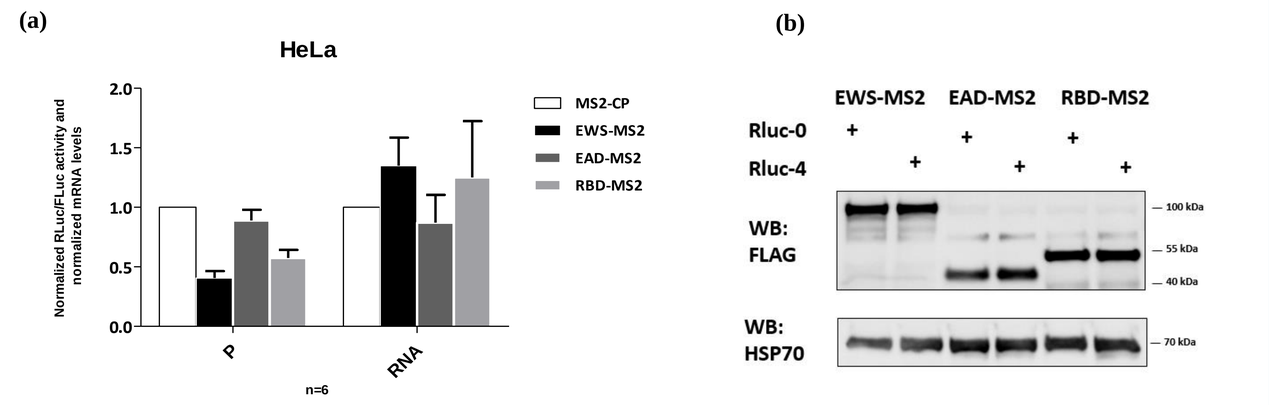

Figure 5. Tethering of EWS and its domains (EAD and RBD) to the 3'-UTR of luciferase mRNA reporter in HeLa cells. (a) Tethered EWS and its RBD reduce luciferase expression (P = luciferase Protein level) and have no effect on steady-state levels of the luciferase mRNA reporter, while tethered EAD leads to only a marginal decrease in luciferase activity. (b) Western blot analysis of EWS and its domains proteins expression in HeLa cells.

3. Immunoprecipitation shows that EWS via its RBD, but not EWS-Fli1, interacts with the 40S small ribosomal subunit

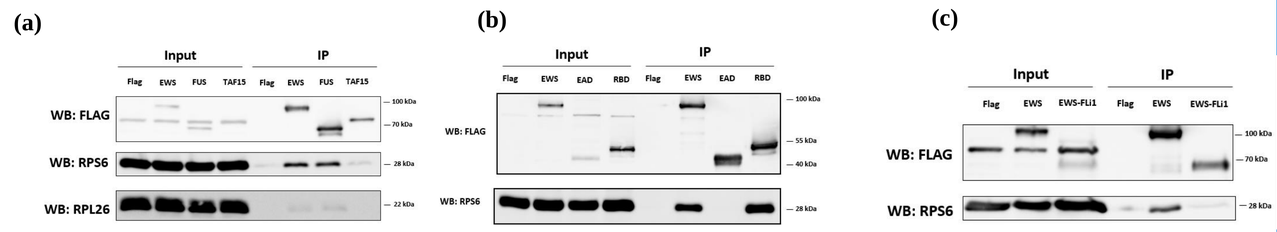

Figure 6. Immunoprecipitation (IP) of FLAG-tagged FET members (a), Flag-tagged EWS and its FLAG-tagged domains (b), Flag-tagged EWS-Fi1 fusion (c) and anti-Flag, anti-RPS6 and anti-RPL26 western blotting. Samples are lysates from HeLa cells transfected with FLAG-tagged FET members (a), Flag-tagged EWS and its Flag-tagged domains (b), Flag-tagged EWS-Fi1 fusion (c). Immunoprecipitation (IP) of FLAG-tagged EWS and anti-FLAG, anti-RPS6 and anti-RPL26 (d). Samples are non-treated (-RNase) or RNase-A treated (+RNase) lysates from HeLa cells transfected with FLAG-tagged EWS.

4. EWS-Fli1 fusion has no effect on translation

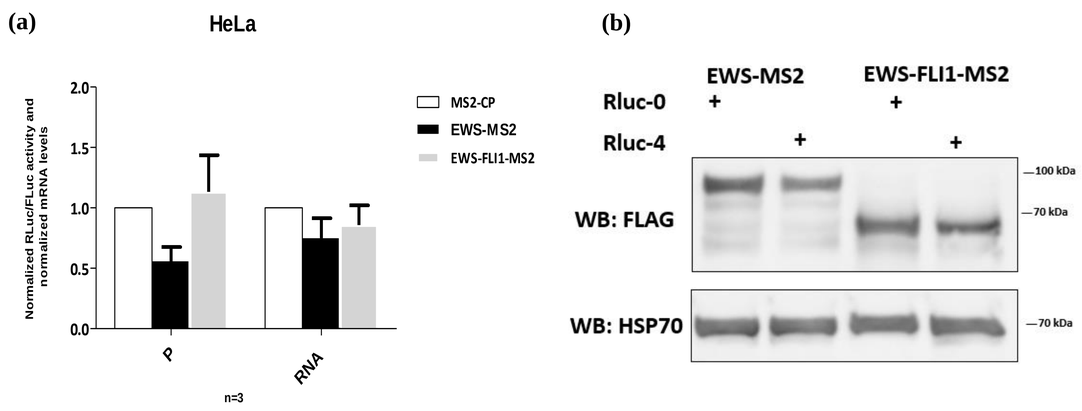

Figure 7. Tethering of EWS-Fli1 to the 3'-UTR of luciferase mRNA reporter in HeLa cells. (a) Tethered EWS-Fli1 has no effect on luciferase expression nor on steady-state levels of the mRNA reporter. (b) Western blot analysis of EWS-Fli1 protein expression in HeLa cells.

IV. Discussion and Conclusion :

A tethering approach and RT-qPCR analysis showed that FET proteins are implicated in the repression of translation. Focusing on the EWS member, we found that its RNA Binding Domain (RBD) is the domain implicated in translational repression. Our findings also suggest that repression of translation by EWS requires its interaction with the 40S small ribosomal subunit. Further experiments will further characterize the underlying molecular mechanism. Interestingly, the EWS-Fli1 fusion protein lacks the RBD and consequently has no effect on translation.

In conclusion, our observations have uncovered unsuspected functions for EWS in translation and showed that these functions are altered in the context of EWS-Fli1 oncogenic fusion.