Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. ALS is typically of adult onset and is characterized by paralysis of voluntary muscles due to the progressive death of motor neurons in the brain and spinal cord. About 90% of all the cases are sporadic with no family history while the remaining 10% are familial, with mutations in several genes, including SOD1, TDP43, and FUS/TLS. FUS/TLS, EWS and TAF15 belong to the FET family of DNA and RNA binding proteins. FET proteins are involved in multiple cellular functions including DNA damage and repair, transcription, mRNA splicing, RNA transport and stress response.

In addition to FUS/TLS, recent reports identified mutations causative of familial ALS in the other genes encoding FET proteins (EWSR1 and TAF15). While significant progress has been made in understanding the disease mechanism and in the identification of new therapeutic strategies, several questions still remain largely unknown. The work presented here aims at understanding the normal functions of FET proteins, in particular EWS, as well as the pathogenic mechanisms by which they lead to disease.

In this work I have performed a characterization of FET expression and localization during mouse brain development, in neural stem cells (NSCs) commitment and differentiation and in retinoic acid-induced neuronal differentiation of SHSY5Y cells. Interestingly, I observed that EWS protein display different localization in brain compartments. Moreover, my data document a fine-tuned regulation of EWS protein and RNA expression during brain development and during neural stem cell differentiation. In particular, EWS protein expression decreased during differentiation, while the EWS mRNA was only slightly affected. In addition, EWS knockdown in neuroblastoma cells was sufficient to induce
neurite outgrowth. Therefore, downregulation of EWS expression could play a role in normal neural human and mouse differentiation. Interestingly, these experiments provide important tools to improve the current understanding of EWS role in neural differentiation and its involvement in ALS disease.

It has been shown that ALS-associated FET mutations cause FET protein relocalization into cytoplasmic aggregates, thus impairing their normal function. Protein aggregation has been suggested as a co-opting factor during the disease pathogenesis. Cytoplasmic mislocalization of FET proteins contributes to the formation of cytoplasmic aggregates that may alter RNA processing and initiate motor neuron degeneration. Interestingly, oxidative stress, which is implicated in the pathogenesis of ALS, triggers the accumulation of mutant FUS in cytoplasmic stress granules where it binds and sequester wild-type FUS. In order to evaluate the role of FET proteins in ALS and their involvement in the response to oxidative stress, FET protein expression and localization was analyzed in neuroblastoma cell lines upon different oxidative stress conditions. Furthermore, signal transduction pathways induced upon imposed oxidative stress were characterized in neuroblastoma cell lines and cell survival was monitored. Interestingly, I found that FET proteins display a different localization upon oxidative stress condition and that none of them translocate into stress granules upon the imposed oxidative stress treatment.

Collectively, our data provide a new link between the oxidative stress response and RNA metabolism in ALS disease.

Taken together, these data provide new evidence on the role of FET proteins in normal neuronal development, with potential implication in the onset of neurodegenerative disease, in particular ALS disease.