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FUS-dependent processing of snoRNAs into sdRNAs and regulation of ribosomal RNA modifications: implications in Amyotrophic Lateral Sclerosis (ALS)

FUS is a DNA/RNA binding protein involved in many aspects of RNA metabolism. Moreover, mutations within the nuclear localization signal (NLS) of FUS result in the mislocalization of this protein into the cytoplasm, resulting in the formation of cytoplasmic aggregates, and it is associated with amyotrophic lateral sclerosis, a neurodegenerative disease. Small nucleolar RNAs (snoRNAs) are a family of small non-coding RNAs that guide site-specific 2'-O-methylation (2'-O-Me) and pseudouridylation of ribosomal RNAs (rRNAs) and small nuclear RNAs (snRNAs). These epitranscriptomic modifications provide stability and maintain the structural fidelity of the ribosomes. Additionally, contrary to the previous belief, about two-thirds of these sites on the rRNA are fractionally modified; this provides another layer of generating ribosomal heterogeneity. Not limited to only guiding rRNA and snRNA modifications, both C/D and H/ACA box types of snoRNAs can be processed into smaller, stable fragments called sdRNAs (snoRNA-derived RNAs). These sdRNAs may function as microRNAs and regulate gene expression at transcriptional and translational levels. Moreover, the role of FUS in the biogenesis of microRNAs is known and well documented, but its role in regulating snoRNA expression and processing into sdRNAs is not explored.

In this work, using high-throughput sequencing, it was identified that FUS regulates snoRNAs in SH-SY5Y (neuroblastoma) cells. Since snoRNAs are involved in guiding rRNA and snRNA modifications, quantitative, next-generation sequencing (NGS)-based techniques, RiboMeth-seq and HydraPsiSeq were used to map changes in 2'-O-Me and pseudouridine levels in wild-type and FUS-depleted cells (FUS KO). Many fractionally modified 2'-O-Me sites on ribosomal RNAs showed a higher proportion of modification in FUS-depleted cells, and a subset of guide C/D box snoRNAs were also upregulated. Furthermore, pseudouridine changes in the FUS-depleted cells were subtle, but an overall increase in the modification of rRNAs was noticeable, along with changes in guide H/ACA box snoRNAs. Next, SH-SY5Y cells carrying ALS-associated *FUS* R495X mutation that lack an NLS also displayed significant changes in snoRNAs and 2'-O-Me and pseudouridine levels compared to wild-type control. In addition, ALS-patient-derived fibroblasts with FUS mutations and age-sex-matched controls were used to explore if 2'-O-Me changes are also observed in ALS patients with FUS mutations. As expected, fibroblasts carrying 'strong' FUS P525L mutation displayed the highest number of significantly changed 2'-O-Me sites, whereas 'mild' FUS mutations R521C and R521L displayed fewer sites. These results were complemented by 2'-O-Me data from an isogenic pair of induced pluripotent stem cells, neural progenitor cells and motor neurons carrying FUS P525L mutation. Interestingly, most of the 2'-O-Me and pseudouridine sites mapped to the outer periphery of the 80S ribosome, suggesting that depending on their modification levels, these fractionally modified sites may regulate the binding of ribosomal proteins or other factors.

As mentioned above, small RNA sequencing data showed that some snoRNAs were differentially expressed in SH-SY5Y FUS KO cells and, that many sdRNAs are generated from C/D and H/ACA box snoRNAs. In the case of the C/D box snoRNAs, these sdRNAs showed conserved box C or box D motifs. Moreover, a single snoRNA produced multiple sdRNAs with varying levels of expression. The sdRNA profile was different for proliferating and differentiated SH-SY5Y cells, suggesting that external cues such as retinoic acid treatment can also influence the processing of snoRNAs into sdRNAs. These results indicate that FUS influences snoRNA expression and ribosomal RNA modification. Secondly, some snoRNAs are processed into sdRNAs in a FUS-dependent manner. However, the function of these

sdRNAs remains to be explored. Functional studies are necessary to explore the effects of individual rRNA modification sites on translation and how ALS-associated FUS mutation influences this process.

Keywords – snoRNA, ALS, FUS, 2'-O-Me, pseudouridine, sdRNAs.