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Titre du sujet de stage : SMN as a regulator of nonsense-mediated mRNA decay

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Sujet de stage :

Spinal muscular atrophy (SMA), a leading genetic cause of infant mortality, is caused by the deletion or mutation of *Survival Motor Neuron 1* (*SMN1*) gene, and a subsequent reduced expression of SMN protein. In fact, SMN is a multifunctional protein endowed with essential roles in both the cytosol and the nucleus. It controls various aspects of the RNA metabolism, including but not restricted to transcription, snRNP and snoRNP biogenesis and assembly, pre-mRNA splicing, the 3′ end of histone mRNA processing, and mRNA transport (1). These numerous functions are dictated by the variety and the abundance of SMN interacting partners. Of particular importance, therefore, will be to elucidate the full spectrum of SMN functions in co- and post-transcriptional gene regulation and to further define the SMN-dependent RNA-processing events and downstream impeded cellular activities that contribute to the motor system dysfunction in SMA.

A recent study has shown that SMN depletion resulted in widespread retention of introns with either weak major (U2) splice sites or belonging to the minor (U12) class (2). Messenger RNA molecules retaining introns usually exhibit premature termination codons, and are therefore intercepted by the cytoplasmic surveillance mechanism called NMD (Nonsense-mediated mRNA Decay) (3). Detection of cell-poisoning nonsense mRNA species in SMA cells leads us to hypothesize that the NMD mechanism is not (or not fully) triggered in SMN deficient cells, and that accumulation of these mRNA species would cause the production of dominant-negative peptides. This would contribute to the pathophysiological effect of SMN deficiency in SMA, thus compromising motor neuron survival.

We will use SMA patient-specific induced pluripotent stem cells (iPSCs), obtained by reprogramming of skin fibroblasts. The iPSCs represent unprecedented tools for the study and elucidation of the pathogenesis of neurological disorders. They can be differentiated into human disease-specific neuronal cells in the presence of specific factors. Comparative transcriptomes obtained from SMA *vs.* control differentiated neuronal cells will be used to identify altered splicing events, and more specifically nonsense mRNA species. To provide a direct evidence of SMN-mediated regulation of NMD, we will inactivate SMN1 using inducible shRNA approach and analyze nonsense mRNA expression in treated cells.

We will next define the role in NMD regulation of each functional domain on SMN. This issue will be addressed by expressing specific domains of SMN in SMA cells. The DNA constructs encoding these domains are being tested in our team for their potential interaction with methylated histone H3 (Binda et al, unpublished).

Technologies utilisées :

Molecular and cellular biology

Mots clés : iPSC, mRNA splicing, NMD, Pathogenesis, SMN

Publications d'intérêt :

1. Singh et al., 2017 Biochim Biophys Acta *1860*, 299
2. Jangi et al., 2017 PNAS *114*, E2347
3. Lykke-Andersen and Jensen 2015 Nat Rev Mol Cell Biol *16*, 665