Description for the general public

The nucleolus is the non-membranous part of the nucleus in the eukaryotic cell. The nucleolus is involved in many biological processes, e.g. the formation of ribosomes which are necessary for the production of proteins in the translation process; ageing of the cell; the regulation of the cell cycle or cell response to stress. It is a strategic point in the cell because it is responsible for maintaining intracellular balance, i.e. homeostasis. At present, there are about 4500 different proteins located in the nucleolus, either permanently or transiently, which participate in different biological pathways and those whose functions have not yet been known. Our group has recently observed FUS (*fused in sarcoma*), U7 snRNA (*small nuclear RNA*) and hnRNP UL1 (*heterogeneous nuclear ribonucleoprotein U-like 1*) in nucleoli. The FUS protein is involved in many cellular processes of RNA metabolism, moreover, it is involved in the maintenance of genome integrity and DNA recombination. The hnRNP UL1 also takes part in the repair of damaged DNA and acts as a transcription regulator. U7 snRNA in complex with 7 core proteins forms U7 snRNP – ribonucleoprotein complex that participates in the 3'end processing of histone pre-mRNA. Our group has previously shown that FUS, hnRNP UL1, and U7 snRNP interact with each other and participate in replication-dependent histone gene expression in the nucleus (Raczyńska et al., 2015). However, the function of these three factors in the nucleolus is unknown.

Interestingly, mutations in both the *FUS* and *hnRNP UL1* genes were identified in patients with hereditary form of amyotrophic lateral sclerosis (ALS). It was reported that nuclear transport of ALS-linked FUS mutants is abrogated and protein accumulates in cytoplasmic aggregates. Moreover, our group observed that U7 snRNA/snRNP is also mislocalized in these aggregates, along with FUS. The mislocalization leads to loss of nuclear functions of both factors. Therefore, I suppose that the location and function of FUS, hnRNP UL1 and U7 snRNA in the cell nucleolus may also be disturbed in the case of ALS disease, which is caused by mutations in the *FUS* gene and *hnRNP UL1*.

The aim of my project is to elucidate the function of FUS, hnRNP UL1 and U7 snRNA in the nucleolus. I also intend to determine the effect of ALS-linked mutations in the *FUS* and *hnRNP UL1* genes on the location and function of all three factors in the nucleolus. My research hypothesis assumes that:

- FUS, hnRNP UL1 or U7 snRNA participate in rDNA transcription or rDNA damage response pathway, and they can be required for nucleolus integrity;
- The nucleolus is the place of posttranscriptional modification of U7 snRNA, this could be catalyzed by snoRNP and mediated by FUS and/or hnRNP UL1;
- The nucleolus is the place for sequestration of FUS, hnRNP UL1 and U7 snRNA, which can be released or activated in particular time or phase of the cell cycle or in response to stress.

All experiments will be performed on human cell lines HeLa. I will try to identify other proteins and RNAs that interact with FUS, hnRNP UL1, and U7 snRNA in the nucleolus. This will shed further light on the biological process in which they are involved. I will check the influence of the examined factors on rDNA transcription, participation in the rDNA damage response pathway and posttranscriptional modifications of U7 snRNA. I will perform these analyses in wild cells and in cells exposed to stress and mutagenic factors. Moreover, I will check the influence of FUS and hnRNP UL1 mutations associated with ALS on the location of FUS, hnRNP UL1 and U7 snRNA and their function in the nucleolus.

The nucleolus and the cellular processes taking place in it are still not fully understood. New proteins participating in different biological pathways in the nucleolus are constantly being discovered. Some of them may perform completely different roles in different time of cell life. An important discovery was the influence of the nucleolus on cancer, which contributed to the creation of a genetic drug for breast cancer, which is currently in clinical trials. My goal is to check the biological importance of FUS, hnRNP UL1, and U7 snRNA, whether they are part of already known and/or new mechanisms in the nucleolus. My result may prove useful in research concerning treating diseases related to nucleolar dysfunction. In addition, responses to these research hypotheses may be key to understanding the molecular basis of amyotrophic lateral sclerosis (ALS).