A rare Single Nucleotide Polymorphism in human WDR61 used to identify a novel complex associated with SF3A/B component of U2 spliceosome subcomplex

Genetic variation mostly strengthens the health of the general population. There are, however, instances of single or multiple gene mutations, which result in genetic diseases. Pathogenic variants can be hidden in the population, especially in an ethnically diverse one, and manifest in conditions of rapid population decline or relations with close cousins. The genetic variations is strong within the non-protein coding regions of the genome and even large deletions can have no nefarious impact on human health. However, mutations within coding regions and in particular those that code for the functional parts of proteins can result in disease development. In the course of sequencing of a individual genome many genetic variants and identified and diagnosis might be challenging if mutations occur in loci, which were not previously described to have pathogenic potential. In this case additional functional analyses are required to come to a definitive diagnosis, which could help in choosing the best treatment for the patient.

The human WDR61 protein is part of two complexes, SKIs and PAFc. While the SKI complex operates in the cytoplasm to regulate decay of aberrant mRNAs, the PAFc resides in the nucleus and regulates mRNA synthesis by the RNA polymerase II. Mutations in the two other subunits of SKI result in Trichohepatoenteric (THE) Syndrome and mutations in CDC73 subunit of PAFc is linked to Hyperparathyroidism-jaw tumor (HPT-JT) syndrome. Though the THE syndrome often results from a complete abrogation of SKI complex and the yeast WDR61 protein was shown to be essential for SKI complex integrity no clinically relevant WDR61 protein mutations were reported to this day. We initiated a functional analysis of WDR61 with the identification of a point mutant in WDR61 in a patient suffering from an undiagnosed genetic disease. In the course of our preliminary analysis we showed that the mutation disrupts WDR61 interaction with both the SKIc and PAFc and we have identified novel WDR61 interactions, which likely constitute a yet uncharacterized complex centered around two structural proteins, TTC33 and CCDC97, conserved in vertebrates. We also showed that WDR61 is essential for maintaining proper levels of subunits of SKIc and PAFc, which are essential to both complexes' function. Therefore loss of WDR61 results in inactivation of at least two and perhaps three complexes making this structural protein important for cellular physiology. Strangely, down-regulation of WDR61 in cell lines does not inhibit cell growth in contrast to loss of the scaffolding subunit of PAFc, CTR9. This might suggest that inactivation of either other WDR61-containing complexes act to compensate for PAFc loss.

Our project will aim at establishing the exact architecture of the novel complex through a series of biochemical assays and to define its function. WDR61 and the two novel structural proteins associate with factors, which play a role in splicing, regulation of PAFc activity and DNA repair. Those are important pathways, which when mutated lead to serious health issues. Control of alternative splicing has been shown to be implicated in development program activation. DNA repair is essential for preventing cancer by limiting the nefarious impact of genetoxic substances. Most of our functional assays will be directed towards testing the novel complex influence on those pathways.

Our experimental approach towards the functional characterization of the WDR61 W20C variant showed that it has pathogenic potential and led to the discovery of a new protein network. We wish to further utilize our expertise to systematically screen variants identified in patients suffering from genetic disorders. To this end we will expand our collaboration with prof. Rafał Płoski laboratory at Warsaw Medical University, who originally pinpointed the WDR61 variant. We will screen around 5 such variants as they are identified. This will hopefully allow for patient diagnosis and perhaps suggest appropriate treatment.