CCDC96, novel ciliary protein localization and role in cilia assembly and function

Aim of research/Hypothesis

Cilia are tiny cell protrusions containing microtubular skeleton, assembled by nearly all eukaryotic cells. Based on their ultrastructure and performed functions cilia can be divided into two categories, immotile sensory cilia and motile locomotory cilia that enable motility of the unicellular organisms and shift of the mucus or particles along the surface of the ciliated epithelial cells lining internal tracks in multicellular organisms. In human, defects in cilia structure or their dysfunction result in diverse human disorders called ciliopathies.

Proper cilia assembly and function involve even several hundred of proteins. In case of many putative ciliary proteins, it was not confirmed that these are indeed ciliary proteins, while other – the precise localization and role within cilia remain unknown. It is apparent that without detailed knowledge of the proteins that build cilia it will be difficult to fully understand the function of such complicated structure. Therefore I believe that the analysis of the CCDC96 and its partners may contribute to the better understanding of the mechanisms that regulate cilia beating.

I identified CCDC96 protein as one of the proteins that co-immunoprecipitate with ciliary protein, CCDC113. Based on the preliminary data I confirmed that CCDC96 expressed as HA-tagged fusion protein under the control of its native promoter is a ciliary protein in *Tetrahymena* cells and perhaps interacts with CCDC113. Because lack of CCDC113 in *Tetrahymena* affects cilia beating, I assume that CCDC96 may also influence cilia motility.

As was showed by others, in mammalian cells CCDC96 protein co-localizes with centriolar satellites, structures that play a role in primary cilia assembly. Thus, CCDC96 can affect primary cilia assembly and / or stability.

Research methodology

To address the delineated goal I will obtain *Tetrahymena* mutant with knocked out CCDC96 gene and compare its phenotype to wild type cells (the proliferation rate (cilia motility is indispensable to separate daughter cells during final stages of cytokinesis), formation of food vacuole (oral cilia direct food particles into the oral cavity), cilia regeneration after experimental cell removal, cells motility and cilia beating pattern (frequency and amplitude)).

To identify partner proteins of CCDC96, I will obtain *Tetrahymena* cells expressing CCDC96-3HA protein or CCDC96-BirA and perform immunoprecipitation using either anti-HA antibodies or streptavidin immobilized on the agarose beads. The precipitated proteins will be identified by mass spectrometry.

To learn abort the role of CCDC96 protein in mammalian cells assembling primary cilia I will transfect mIMCD3 cells with CCDC96-HA expression plasmid and will analyze its localization. I will also investigate the effect of the elevated or reduced level (or lack) of CCDC96 protein on primary cilia assembly using commercially available antibodies.

Outcomes of the problem undertaken

Data obtained during the implementation of project focused on the analysis of the CCDC96 protein in both, motile and primary cilia, will contribute to the general basic knowledge concerning the regulation of cilia structure and function. In future, these results may help to develop new marker to diagnose PCD or help to develop appropriate therapies.

Results obtained during implementation of this project will be included into my doctoral dissertation and will be presented on the national and international conferences and will be prepared for publication in international scientific journal.