PROGETTO

Supervisore::	BOLETTA ALESSANDRA_____
Titolo/*Title*:	Refining the Molecular Mechanism of Glutamine Sensing by Primary Cilia
Curriculum:	Biologia Cellulare e Molecolare/*Cellular and Molecular Biology* _____
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/divisions/genetics-and-cell-biology/cystic-kidney-disorders.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Primary cilia are tiny solitary organelles present on the surface of most cells in our body. While they were erroneously considered as vestigial organelles for many years, work from the past two decades has defined that alterations of these organelles are associated with numerous disorders, affecting various types of tissues, collectively named the ciliopathies. The kidney is frequently and severely affected in these disorders with renal cysts formation, inflammation and fibrosis reported as hallmarks of these disorders. The first discovery that cilia are associated with disease came from the landmark finding that the polycystins, mutated in the most common of all ciliopathies in a disease called polycystic kidney disease, do localize at cilia and that removal of cilia in the renal epithelia results in cyst formation. The precise function of primary cilia is still largely elusive, although it is clear that these are sensory orgenelles protruding from the cells by several microns and functioning as antennae to capture the extracellular signals such as odorants, light, morphogens or even capable of sensing mechanical forces. We have recently found that Primary cilia are able to sense the availability of nutrients and to respond specifically to glutamine, a non-essential-aminoacid which becomes conditionally essential during metabolic stress conditions.

Rationale and hypothesis

Our data suggest that: i) cilia are essential for cells to sense and properly utilize glutamine for energy production: ii) cells lacking cilia have an impaired capability to respond to nutrient stress by utilizing glutamine; iii) the process is not dependnent on the nutrient sensor mTORC1, but it depends on one enzyme called asparagine synthetase which enables glutamine utilization under stress conditions and which we have found located at the base of cilia

Objectives and specific aims The PhD student enrolled in this project will contribute reaching a better molecular definition of the process. In particular: i) (s)he will generate cells lacking the ASNS enzyme or

expressing mutant forms of the enzyme to study its role in the ciliary response to glutamine; ii) perform a High Content automated Screening to identify pathways involved in the process (by using commercially available siRNA libraries) followed by an automated imaging of ciliary length in response to different stimuli, already established in the lab; iii) validate the above along with already identified candidate molecules (serving also as a back-up strategy in case of difficulties) involved in the response by using *in vitro* and *in vivo* systems.

Expected outcomes We expect to unravel the molecular mechanism of the ciliary response to glutamine

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

Upon completion of the project the fellow will be able to design and execute experiments independently. During the course of the project (s)he will learn how to perform IF and live imaging, western blots and qPCR analysis, cell cultures and tissues analysis (IHC).

Bibliografia (max. 15)

1. Anvarian Z, Mykytyn K, Mukhopadhyay S, Pedersen LB, Christensen ST. Cellular signalling by primary cilia in development, organ function and disease. Nat. Rev. Nephrol. 2019;15:199–219. doi: 10.1038/s41581-019-0116-9. - DOI - PMC - PubMed
2. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. Nat. Rev. Mol. Cell Biol. 2017;18:533–547. doi: 10.1038/nrm.2017.60.
3. Steidl ME, Nigro EA, Nielsen AK, Pagliarini R, Cassina L, Lampis M, Podrini C, Chiaravalli M, Mannella V, Distefano G, Yang M, Aslanyan M, Musco G, Roepman R, Frezza C, Boletta A Primary cilia sense glutamine availability and respond via asparagine synthetase. 5:385-397, 2023. doi: 10.1038/s42255-023-00754-6.