PROGETTO

Supervisore:: _Irene Franco_____

Titolo/*Title*: **Mechanisms of hypoxia-induced somatic mutation in pre-cancer kidneys**

Curriculum: ___ Biologia Cellulare e Molecolare

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/molecular-genetics-of-renal-disorders/irene-franco.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Changes in the genome sequence (somatic mutations) accumulate in the genome of normal cells during a lifetime (Franco, Helgadottir et al. 2019, Franco and Eriksson 2022) and are recognized as a driving factor in the transition from a normal to a cancer cell. The tissue environment also contributes to this transition, by i) influencing the life-long accumulation of mutations; ii) determining selection and expansion of mutated clones (Olafsson and Anderson 2021). Literature indicates that insufficient oxygen supply (hypoxia) is a common tissue stress in the kidney proximal tubule (Nourbakhsh and Singh 2014). Our preliminary data show that proximal tubule cells that adapt to hypoxia (either upon environmental exposure, or genetic inactivation of the negative regulator of hypoxia signaling) accumulate a specific signature of single base substitutions, which overall enhance mutation rates. The same signature (SBS40b) has been observed in a large analysis of clear cell renal cell carcinoma genomes and identified as a likely causative factor of oncogenic transformation (Senkin, Moody et al. 2023). However, the etiology of SBS40b is currently unknown.

Rationale and hypothesis Our data provide the first indication that hypoxic stress can induce SBS40b *in vitro* and *in vivo*. Here, we plan to expand our observations and investigate what cellular changes downstream hypoxic stress promote the accumulation of somatic mutations in normal kidney cells.

Objectives and specific aims We will eviscerate the mutagenic effect of cellular adaptation to hypoxia, *via* an *in vitro* mutagenesis assay, using 2 different and complementary approaches: i) exposure to hypoxia and ii) genetic modification of the signalling pathway (VHL/HIF) regulating adaptation to hypoxia. Finally, we will assess how metabolic changes occurring during adaptation to hypoxia affect DNA-damage repair and produce the specific mutational signature. Our experiments will expand on our preliminary results, using the same validated technology (duplex sequencing (Salk and Kennedy 2020)) and already available primary human cells and cell lines. Overarching goal is the discovery of a novel mutation mechanism and identification of a link between tissue stress and tumorigenesis in the kidneys.

Expected outcomes This project aims to define the interplay between tissue changes/stress and somatic mutation accumulation in the kidney, as a first step to identify lifestyle factors that promote kidney cancer risk in humans.

This study will:

-Characterize a specific cell subset that we have identified as the cell-of-origin of the most common kidney cancer subtype in human kidneys

-Start from analyses of human tissues and validate and clarify our findings *via* functional experiments in cellular models

-Use and develop leading edge technology for genome sequencing and somatic mutation analyses

-Dissect the molecular mechanisms that link an environmental stress (hypoxia) to enhanced mutation.

-Develop a unique research aimed to find strategies to prevent and modify the trajectory of cancer-prone cells in the kidney

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

The PhD student will be trained by the PI in all wet-lab experiments (cell culture, in vitro stimulations, WB, IF, DNA and RNA extraction, sequencing) and will perform them autonomously as soon as he/she masters the techniques. The PhD student will perform data analyses. Moreover, he/she will assist data analyses that require advanced computational skills (e.g. sequencing data analyses) by directly interacting with the bioinformatician fellow that is in

charge of analyses. The PhD student is expected to acquire presentation skills, both oral and written, and to actively participate to congresses and be in charge of manuscript preparation.

Bibliografia (max. 15)

Franco, I. and M. Eriksson (2022). "Reverting to old theories of ageing with new evidence for the role of somatic mutations." Nat Rev Genet.

Franco, I., H. T. Helgadottir, A. Moggio, M. Larsson, P. Vrtačnik, A. Johansson, N. Norgren, P. Lundin, D. Mas-Ponte, J. Nordström, T. Lundgren, P. Stenvinkel, L. Wennberg, F. Supek and M. Eriksson (2019). "Whole genome DNA sequencing provides an atlas of somatic mutagenesis in healthy human cells and identifies a tumor-prone cell type." Genome Biol 20(1): 285.

Nourbakhsh, N. and P. Singh (2014). "Role of renal oxygenation and mitochondrial function in the pathophysiology of acute kidney injury." Nephron Clin Pract 127(1-4): 149-152.

Olafsson, S. and C. A. Anderson (2021). "Somatic mutations provide important and unique insights into the biology of complex diseases." Trends Genet 37(10): 872-881.

Salk, J. J. and S. R. Kennedy (2020). "Next-Generation Genotoxicology: Using Modern Sequencing Technologies to Assess Somatic Mutagenesis and Cancer Risk." Environ Mol Mutagen 61(1): 135-151.

Senkin, S., S. Moody, M. Diaz-Gay, B. Abedi-Ardekani, T. Cattiaux, A. Ferreiro-Iglesias, L. B. Alexandrov, M. R. Stratton and P. Brennan (2023). "Geographic variation of mutagenic exposures in kidney cancer genomes." MedRxiv.