**PROJECT**

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| **Supervisor:** | Irene Franco |
| Title: | Impact of ischemic damage on somatic mutation in the human kidney |
| *Curriculum*: | CMB |
| Link to the personal page of the University or relevant hospital site website: | https://research.hsr.it/en/divisions/genetics-and-cell-biology/somatic-mutation-mechanisms.html |

**Description of the Project (max 3,000 characters including spaces)**

**Background/gap of knowledge**

Understanding what makes our cells prone to develop cancer is key for identifying effective strategies of tumor prevention. Somatic mutation is a driver of cancer. However, the mechanism that promote mutation in human tissues during a lifetime are mostly unexplored. By analyzing the genome of normal cells and cancers of the kidney, we have identified a shared pattern of somatic point mutations with unique characteristics and unknown origin (Franco I et al, 2019 and lab Franco, unpublished). The study of mutational patterns and signatures is a powerful tool to uncover exposures to specific mutational processes (Helleday T, et al 2014; Franco I and Supek F, 2024) and it has been used to search for mutational processes active in the kidney, but multiple mutational signatures identified in the kidney remain unexplained (Senkin et al, 2024). The mutational signature that we have observed in the kidney (which we have named ToCCATA) is a) specific to this tissue; b) restricted to a specific population of kidney cells of the proximal tubule; c) over-represented among cancer driver variants observed in kidney cancer (lab Franco, unpublished).

**Rationale and hypothesis**

Our preliminary analyses point to adaptation to low-oxygen conditions (hypoxia) as an environmental stimulus favoring the ToCCATA signature in the kidney. We want to test this hypothesis in patients undergoing surgical procedures that include occlusion of the kidney artery, inducing transient ischemia and hypoxic stress in the kidney.

**Objectives and specific aims**

We plan to study the ToCCATA-bearing cells in human kidneys and a) follow their population dynamics; b) study their genome and transcriptome at a single cell level; c) understand the underlying causes of excessive mutation. Having already established a non-invasive assay to detect these cells in urine samples, we can analyze numerous donors, longitudinally and without biopsies. Thanks to a collaboration with the Urology Unit of San Raffaele, we can access patients that undergo a surgical procedure that includes clumping of the kidney artery, causing 10-30 minutes of kidney ischemia. We will collect cells from the urine of these patients before (basal) and after surgery, at different time points (2 days, 1 months, 6-12 months). These unique experimental setting will allow us to analyze the genome and transcriptome at single cell level, and to explore cellular dynamics in the human kidney upon ischemia.

**Expected outcomes**

Overall, this study will determine whether hypoxic stress is mutagenic and cancer-promoting. Moreover, we will collect information on kidney tissue changes upon an ischemic episode.

**Skills that the student should acquire** (max. 600 characters including spaces):

Culturing of single cell clones from human urine and kidney biopsies, DNA extraction and sequencing, RNA extraction, qPCR, RNAseq, histology. The PhD student will perform analyses of genetic and gene expression data and acquire advanced computational skills by directly interacting with bioinformaticians within and ouside the lab. 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.

**References** (max. 15)

Franco, I., et al., *Whole genome DNA sequencing provides an atlas of somatic mutagenesis in healthy human cells and identifies a tumor-prone cell type*. Genome Biol, 2019. 20(1): p. 285.

Helleday T, Eshtad S, Nik-Zainal S. *Mechanisms underlying mutational signatures in human cancers.* Nat Rev Genet. 2014;15(9):585-598

Franco I., Supek F. *Genomics reveal unknown mutation-promoting agents at global sites.* Nature 2024

Senkin S., et al. *Geographic variation of mutagenic exposures in kidney cancer genomes*. Nature 2024