UNIVERSITÀ
DEGLI STUDI
DI PADOVA

EpiG4Mito - The mitochondrial epigenome: the role of DNA secondary structures and base modifications in the pathophysiology of mtDNA replication

Mitochondria are key organelles as they are responsible for supplying the proper form of energy necessary to eukaryotic cells to exert their functions. Conversely to other cellular compartments, they possess their own DNA (mtDNA). Qualitative and/or quantitative loss of the genetic information harboured in the mtDNA is detrimental for the cell and, in humans, is associated with the occurrence of severe multisystemic disorders named mtDNA maintenance defects (MDMDs). Non-canonical secondary DNA structures, called G-quadruplexes (G4s), can potentially form in the mtDNA. Strikingly, putative G4 forming sequences are enriched at disease-associated mtDNA deletion breakpoints. In addition, due to its localization in the mitochondrial matrix, the mtDNA is expected to be prone to oxidative damage and to accumulate base modifications such as 8-oxo,7,8-dihydroguanine (8-oxoG). In the nDNA, 8-oxoGs destabilize G4s at promoter regions and impact gene transcription. Whether these modifications affect the formation of G4s in the mtDNA is still unknown. With this proposal, I aim to shed light on the effects of non-canonical secondary structures and epigenetic modifications on mtDNA stability and to clarify the mechanisms leading to loss of mtDNA integrity. Within the H2020-MSCA-IF MITOQUAD project, I developed mtG4-ChIP, an innovative method that allowed to map G4s in the mtDNA and to link for the first time the occurrence of G4s with mtDNA instability. I found that mtDNA-G4 structures can form within the cellular context particularly in conditions of impaired mtDNA replication and that the increases in mitochondrial G4s, by the use of a mitochondrial specific G4-stabilizer, lead to a rapid loss of mtDNA, indicating that elevated levels of G4s block mtDNA replication. In here, I will exploit the mtG4-ChIP tool and develop novel approaches to study the interplay between DNA secondary structures and base modifications and their role in mtDNA maintenance. I will combine genomic, proteomic and single molecules methodologies to dissect how mtDNA G4s form and are removed as well as identify the factors participating in these processes. In parallel, I will analyse the metabolism of G4s in MDMDs-patients' derived cells, in order to understand the role of G4s in the pathogenesis of these disorders. Finally, I will investigate the interplay between G4s and 8-oxoG in the mtDNA. I plan to develop a novel method to detect 8-oxoGs and to combine it with mtG4-ChIP to understand the cross-talk between 8-oxoGs and G4s in the regulation of mtDNA replication. Overall, this project will lead to a deeper comprehension of how mtDNA integrity is maintained. Beside the clarification of a key process in the biology of eukaryotic cells, this will be relevant to understanding the early development and progression of several human mitochondrial disorders, in order to treat or prevent their occurrence.