SP-09.03 - Systems Biology Approach of the Down Syndrome Critical Region 1 gene, RCAN1: implications in mitochondrial biology, cellular proliferation, and differentiation

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Down Syndrome (DS) is the product of an extra copy of chromosome 21 and is related to different neuronal and cardiac pathologies. DS patients present increased oxidative stress and, therefore, increased DNA damage; in addition to altered cell differentiation that would lead to failures in organogenesis. In humans, RCAN1, located in the critical DS region of chromosome 21, is responsible of the enlarged and over functional mitochondria observed in DS iPSC, however, the relation between these alterations and the dysfunctional cardiac organogenesis observed in DS patients is still unknown. To analyze *in silico* and *in vitro* the effect of RCAN1 on mitochondrial dynamics, proliferation, and DNA damage of DS iPSCs; and to evaluate the role of this protein in the differentiation process of iPSC-derived cardiomyocytes (iPSC-CM). Using a system biology approach, we constructed a transcriptional regulatory network for RCAN1 that shows the over-representation of processes related with organelle dynamics, cellular proliferation, and organ differentiation, all of them connected by genes related with the response to DNA damage. Moreover, *in vitro* microscopy and Western blot analysis showed that DS iPSCs present lower rates of mitochondrial fission, as well as decreased levels of PINK1. RCAN1 overexpression in DS iPSC induced an enhanced proliferation and cumulative DNA damage observed by immunofluorescence and qRT-PCR, which were dependent on the expression levels of RCAN1. Finally, DS iPSC-CM also expressed RCAN1-dependant lower levels of cardiac differentiation markers than control cells after 15 days of culture. RCAN1 overexpression regulates the increased mitochondrial fusion, proliferation and DNA damage observed in 3S iPSC; together with a decrease in the 3S iPSC differentiation ability towards a cardiomyocyte lineage. **Keywords:** Down syndrome, RCAN1, iPSC. **Funding:** This project was funded by FONDECYT 1190743 and FONDAP 15130011. U-Redes G_2018-35 and CRP-ICGEB CHL18-04.

SP-09.04. - Mechanism of rotenone inhibition of respiratory complex I

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Respiratory complex I in the inner mitochondrial membrane plays an essential role in cell metabolism. It catalyses oxidation of NADH in concert to reduction of ubiquinone (Q). This electron transfer process is coupled with translocation of protons across the membrane, creating an electrochemical gradient for ATP synthesis. Rotenone is a natural compound that strongly inhibits complex I activity and cryo-EM structures indicate it binds in three different sites inside complex I. Two of them are located in the 30 Å long Q-chamber: the first binding site (BS) is near the chamber exit and the second is the Q reactive site (RS), near the iron-sulfur cluster N2. A distant third site (PS) was found in the membrane domain. Evaluate the relative binding affinity of each site and the role of ligand internal conformation (either in straight or in bent geometry) for binding of rotenone and three derivatives with variable conformational restrictions. We applied molecular dynamics simulations and the free energy methods umbrella sampling, metadynamics and linear integration energy. We find that rotenone has similar affinities to either RS and BS sites. All derivatives have low affinity, between +4 and -3 kJ/mol, to the third PS. This result indicates that the PS may be an experimental artifact due to the high rotenone concentrations used in the cryoEM preparation. Two conformationally restricted derivatives show low affinity to the RS, suggesting that the bent rotenone conformation is stabilized in the RS, favoring complex I inhibition. Considering these, rotenone probably inhibits complex I by binding in the two sites (RS and BS) located in the Q-chamber and RS binding requires an internal flexibility to a bent geometry. We are now analysing how this internal flexibility affects rotenone transit inside the chamber. **Keywords:** Molecular dynamics, Electron transport chain, Free energy methods. **Supported by:** FAPESP