Unravelling stem cell destiny: calcineurin-NFAT axis in early stem cell commitment and immune exclusion

Stefano Cozzi[1], Laura Marongiu[1], Marco Galli[1], Francesca Colnaghi[1], Giuseppe Rocca[1], Anna Celant[1], Giulia Stucchi[1], Metello Innocenti[1], Francesca Granucci[1]

E-mail: s.cozzi28@campus.unimib.it

[1] Department of Biotechnology and Biosciences, University of Milano Bicocca, Milan, Italy

Keywords: Calcineurin, NFAT, stem cells, tissue resistance, differentiation, immune modulation

Abstract:
The Nuclear Factor of Activated T Cells (NFAT) is a family of transcription factors composed of 5 members, four of which (NFATc1-4) are regulated by calcineurin (CN), a calcium-dependent phosphatase. Our group shed light on additional non-inflammatory roles of this transcription factor in dendritic cells. The aim of this work is to unveil how NFAT activation modulates early stem cell commitment and differentiation while exploring new tissue-dependent mechanisms of immune system alertness towards cell proliferation.

We investigated two tissues with distinct regenerative capabilities: the brain, characterized by slow regeneration, and the small intestine/colon, known to be highly proliferative. *In vitro*, we infected neural stem cells with a lentivirus carrying a CN inhibitor (iCN), while *in vivo*, we conducted intracranial injections targeting the ventricular-subventricular zone (V-SVZ) with the same virus. To assess if this mechanism extends to the small intestine/colon system, we utilized mice with inducible inhibition of NFAT in LGR5+ stem cells, predominantly found in intestinal and colon crypts. We also employed 2 models of disease: Parkinson's disease (PD), for the brain system, and dextran sulfate sodium (DSS)-induced colitis.

When NFAT-CN interaction is inhibited, there is an expansion of the neural stem cells in resting condition both *in vitro* and *in vivo*. Furthermore, we showed that this expansion can ameliorate a damage induced by PD. This phenotype is also conserved in the small intestine and colon where there is an expansion of LGR5+ cells leading to tissue elongation. Consistent with our previous findings, this expansion of stem cells can mitigate colitis damage induced by DSS. We also found that NFAT regulates a checkpoint phase mediated by the immune system.

In summary, we propose that NFAT is involved in an early phase of stem cell differentiation, controlling directly or indirectly adult stem cell differentiation and immune system surveillance on activated progenitor cells. Whole-genome CRISPR/Cas9 knockout screening will be utilized to identify and analyze additional critical targets and interactors within this pathway that are involved in the differentiation process.