UNIVERSITÀ DEGLI STUDI DI MILANO

D-MEM PhD-UNIMI | Doctorate program Milan EXPERIMENTAL MEDICINE

The role of PHOX2B in the differentiation and function of sympathetic neurons, in health and disease, by using human-derived iPSCs: the case of Congenital Central Hypoventilation Syndrome

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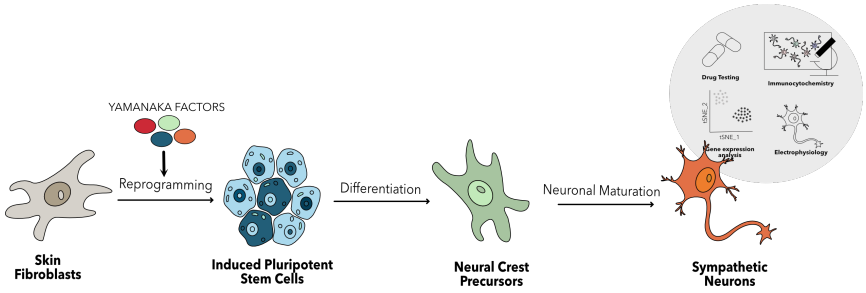
Rationale and main objectives of this project



Congenital central hypoventilation syndrome (CCHS) is a genetic disorder affecting the **Autonomic Nervous System** (ANS) and central chemosensitivity, due to heterozygous poly-alanine triplet expansions (PARM, 95%) and frameshift mutations (NPARM, 5%) in the ***PHOX2B* gene**, a transcription factor that drives the development of the autonomic visceral circuits. Alveolar hypoventilation is the hallmark of the disease, that is generally more severe during sleep than during wakefulness, which can be manifest since birth or adulthood (late-onset CHS, LO-CHS). The disease can be isolated or associated with a spectrum of non-respiratory symptoms, among which seizures and other conditions that reflect a more global ANS dysfunction, including cardiac arrhythmias, ocular disorders, Hirschsprung's disease and neural crest tumors.

In vivo and *in vitro* studies suggest that a **loss of function mechanism** and in particular **transcriptional dysregulation** may be an important mechanism in CCHS pathogenesis; moreover, poly-alanine proteins show **protein aggregation and toxic effects** and has a dominant-negative effect on the transcriptional activity of the wild-type protein.

A **natural antisense transcript (NAT)** has been mapped in the PHOX2B locus (*PHOX2B-AS1*) and data in our laboratory suggest that it positively regulates PHOX2B protein translation.

We have recently generated **iPSC lines from two CCHS patients** with *PHOX2B* PARM mutation (20/25 genotype), that differ for the disease-onset (late vs congenital), with the latter showing an ectopic expression of *PHOX2B* and *PHOX2B-AS1* already at the undifferentiated level, despite the expression of the pluripotency markers *NANOG* and *OCT4,* including both alleles, thus confirming that dysregulated *PHOX2B-AS1* and *PHOX2B* transcription at earlier developmental stages may be involved in CCHS pathogenesis.

The **aim of this project** is to study the expression and the role of *PHOX2B* and *PHOX2B-AS1* during differentiation of iPS cells into sympathetic neurons in healthy controls and understand whether the temporal deregulated expression of *PHOX2B* and *PHOX2B-AS1* can affect the differentiation program of CCHS patients-derived iPS, thus providing new insights into disease pathophysiology, and exploring the possibility of therapeutic intervention.

Most relevant publications for the project

1. Cuadros Gamboa AL, Benfante R, Nizzardo M, Bachetti T, Pelucchi P, Melzi V, Arzilli C, Peruzzi M, Reinbold RA, Cardani S, Morrone A, Guerrini R, Zucchi I, Corti S, Ceccherini I, Piumelli R, Nassi N, Di Lascio S, Fornasari D. (2022) Generation of two hiPSC lines (UMILi027-A and UMILi028-A) from early and late-onset Congenital Central hypoventilation Syndrome (CCHS) patients carrying a polyalanine expansion mutation in the PHOX2B gene. Stem Cell Res. 61:102781.

2. Di Lascio S, Benfante R, Cardani S, Fornasari D. (2021) Research Advances on Therapeutic Approaches to Congenital Central Hypoventilation Syndrome (CCHS). Front Neurosci. 14:615666.