NEWLY IDENTIFIED PHOX2B TARGET GENES AS DRUG TARGETS IN CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

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Congenital Central Hypoventilation Syndrome (CCHS, MIM 209880), is a very rare neonatal neurological disorder characterized by a broad variety of symptoms of autonomic nervous system dysfunction including inadequate control of breathing.

In humans, heterozygous mutations, including frameshift mutations (5%) and poly-alanine triplet expansions (from 4 to 13 residues) (95%), have been detected in the coding region of the paired-like homeobox gene PHOX2B in about 90% of CCHS patients. A correlation between length of the expanded tract and the severity of the respiratory phenotype has been reported. CCHS is a life-long disorder for which the only treatment option is ventilatory support provided by tracheotomy, nasal mask or diaphragm pacing by phrenic nerve stimulation, because pharmacological respiratory stimulants have proved to be ineffective. PHOX2B is one of the master transcription factors whose expression is required for the development of the autonomic visceral circuits.

Absolute limitations to the comprehension of the pathogenesis of CCHS, and the development of new and effective treatments for this disease, is the missing knowledge of target genes regulated by PHOX2B, whose expression may be eventually dysregulated in the disease.

In vivo and in vitro studies suggest that at the basis of CCHS pathogenesis a loss of function mechanism (haplo-insufficiency), probably due to a reduction of the amount of PHOX2B wild-type protein, combined with a dominant-negative effect and/or toxic gain of function of the mutated proteins, can have an impact on the expression of PHOX2B target genes, and is responsible for the entire disease spectrum. Moreover, several mutant proteins interfere with the transcriptional activity of the wild-type protein in a promoter-specific manner, including PHOX2B expression (1, 2). This molecular mechanism may play a role in CCHS pathogenesis, as PHOX2B variants can actually negatively interfere with the expression of the normal allele, thus further reducing the amount of the normal PHOX2B protein.

Very little is known about the genes regulated by PHOX2B. Most of genes identified so far are regulatory genes that encode for transcription factors that control downstream processes involved in the survival and differentiation of specific neural structures, such as TH, DBH, PHOX2A, TLX2, ALK and PHOX2B itself.

Our general hypothesis is that impaired PHOX2B gene expression combined with more general transcriptional dysregulation plays a major pathogenic role in CCHS.

ChIP-seq analysis in IMR32 neuroblastoma cell line allowed us to identify many PHOX2B target gene candidates that are under validation by comparing wild-type and CRISPR-CAS9 Knocked-down PHOX2B expressing IMR32 cells, and in iPS-derived autonomic neurons. Gene Ontology analysis of the set of peak-associated genes identified several enriched terms, such as synaptic

transmission, regulation of embryonic development, cell-cell signaling, axonogenesis, and neuron development, consistent with PHOX2B role during Autonomic Nervous System development and maintenance.

1. Cargnin et al. (2005). J Biol Chem. 280(45), 37439-48.

2. Di Lascio et al. (2013). Neurobiol Dis 50, 187-200.