POTASSIUM CHANNELS AS PHARMACOLOGICAL TARGET FOR THE TREATMENT OF CONGENITAL CENTRAL HYPOVENTILATION SYNDROME

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Introduction: 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 polyalanine triplet expansions (from 4to 13residues) (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 tracts 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 as pharmacological respiratory stimulants have proved to be ineffective. PHOX2B is one of the master transcription factor whose expression is required for the development of the autonomic visceral circuits. Consistent with its role as transcriptional regulator, it is reasonable to suppose that transcriptional dysregulation might be an important mechanism of CCHS pathogenesis. 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. 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. Stemming from the fortuitous observation that progestin Desogestrel can relief some symptoms of the disease, that the progestin-induced down-regulation of the expression of PHOX2B and of its target genes , led us to identify new PHOX2B target genes as potential pharmacological targets for alternative molecules without contraceptive effects.

Material and methods: ChIP-seq analysis in IMR32neuroblastoma cell line allowed us to identify many PHOX2B target gene candidates that are under validation by comparing wild-type and CRISPR-CAS9Knocked-down PHOX2B expressing IMR32cells. Among the newly identified PHOX2B target genes we focused on genes encoding potassium and sodium channels since they have a central role in neuronal excitability and regulation of respiratory rhythm.

Results: Here we show that PHOX2B mutant proteins have acquired a gain of function activity increasing the expression of potassium channels gene, leading to an altered electrical activity and therefore excitability of the cells.

Discussion and conclusion: Our results suggest that transcriptional dysregulation and dysfunction of ion channels activity due to the mutant proteins may contribute to the onset of respiratory problems associated with CCHS and reinforce the idea that ion channels may be promising therapeutic targets. Due to the important role that these ion channels have on neuronal physiology, restoring their expression and/ or activity to the normal physiological level can by-pass PHOX2B mutant effects, leading to amelioration of CCHS symptoms. Moreover, several drugs targeting these proteins are already used in clinics, thus prompting the idea that the potential progress toward a therapeutic intervention to treat CCHS is today more than concrete.