● PERSPECTIVE

Glyco-sphingo biology: a novel perspective for potential new treatments in Huntington's disease

Huntington's disease (HD) is the most common dominantly inherited neurodegenerative disorder, mainly characterized by the progressive striatal and cortical neurodegeneration and associated motor, cognitive and behavioural disturbances (Zuccato et al., 2010). The disease-causing mutation is an expansion of a CAG trinucleotide repeat (> 36 repeats) encoding a polyglutamine stretch in the N-terminal region of huntingtin (*Htt*) (Zuccato et al., 2010), a ubiquitous protein whose function is still unclear (Zuccato et al., 2010). Expansion of the polyQ stretch endows mutant *Htt* (mHtt) with toxic properties, and results in the development of a broad array of undesirable effects in both neuronal and non-neuronal cells (Zuccato et al., 2010). Among all cellular dysfunctions and biochemical imbalances classically associated with HD, perturbed metabolism of (glyco) sphingolipids appears to play a crucial role in the pathogenesis of the disease. Over the last years, we and other have extensively contributed to these findings (Desplats et al., 2007; Maglione et al., 2010; Di Pardo et al., 2014, 2016). We have demonstrated that genes involved in the synthesis of a specific class of sphingolipids, termed gangliosides, classically defined as sialic acid-containing glycosphingolipids most abundant in the nervous system and essential for many biological events (Schnaar et al., 2014), were significantly down-regulated in multiple HD pre-clinical models and importantly in fibroblasts from HD patients (Maglione et al., 2010; Di Pardo et al., 2014, 2016).

Interestingly, administration of exogenous mono-sialic acid-containing ganglioside GM1 restored its endogenous normal levels in HD multiple models and exerted important therapeutic effect on the disease-associated symptomatology in *in vivo* HD model (Di Pardo et al., 2012). Nevertheless, today it is still unknown what the neuroprotective action of this sphingolipid is attributable to. Evidence let us to suppose that sialic acid residues may be important determinants; asialo-GM1 is, in fact, devoid of any beneficial effect *in vitro* (Favaron et al., 1988). The efficacy of sialo-conjugates seems to be directly proportional to the number of contained sialic acids (Favaron et al., 1988). Along this line, we have evidence indicating that tri-sialic acid-containing ganglioside GT1b is more effective than GM1 in an *in vitro* system of HD (unpublished observation).

Sialic acids are nine-carbon acidic monosaccharides that occur naturally at the end of sugar chains attached to the surfaces of cells and soluble proteins. In the human body, the highest concentration of sialic acid (as N-acetylneuraminic acid) occurs in the brain, where it participates as an integral part of glycan structures during brain development, synaptogenesis and synaptic transmission (Schnaar et al., 2014). Sialic-acid is a key player in the brain development and in the maintenance of the neuronal and glial homeostasis (Schnaar et al., 2014), thus, even a partial reduction of sialylated molecules is likely to profoundly affect the overall function of central nervous system (CNS) at multiple levels in both embryonic and adult life. Coherently, studies in animal models demonstrated that defective metabolism of sialic acid affects neuron and myelin homeostasis ultimately leading to a number of neurodevelopmental and neurodegenerative conditions such as Salla, Tay Sachs and Sandhoff diseases (Schnaar et al., 2014). Previous findings from us and others revealed an overall alteration in the metabolism of sialo-conjugates (gangliosides and glycoproteins) also in HD, early in the pre-symptomatic stage of the disease (van der Borght and Brundin, 2007; Di Pardo et al., 2016). Further studies are now needed to test the hypothesis that such a defect may occur during brain development also in HD. Presumably, an exogenous source of sialic acid is likely to be critical under conditions of extremely rapid brain growth and function, particularly during the first months after birth. In the light of that, a precocious intervention aimed at recovering normal levels of intracellular sialic acid may hypothetically restore normal levels of glyco-conjugates and preserve HD homeostasis. This represents a very challenging and intriguing scenario we are currently exploring.

Recently we have found that sphingolipid homeostasis is also significantly affected in HD (Di Pardo et al., 2017) with metabolism of sphingosine-1-phosphate (S1P), one of the most potent signalling lipids that govern essential physiological processes underlying brain development, vascular and bone formation and regulate many of the molecular events crucial for cellular homeostasis and viability and for inflammatory response (Maceyka et al., 2012), particularly aberrant. S1P is commonly found either in the intracellular or in the extracellular compartments (Maceyka et al., 2012). When it is exported outside the cell, S1P acts as a high affinity agonist at five known G protein-coupled receptors, $S1PR_1$–$S1PR_5$, which in the brain have been shown to influence a broad array of physiological processes ranging from cell proliferation to cell differentiation and survival (Maceyka et al., 2012). S1P metabolism is a quite complex biological process, and involves the action of a number of different highly specialized enzymes. A fine balance between S1P synthesis and degradation is normally required for cellular homeostasis and functions. S1P is typically synthesized by sphingosine kinase-1 and -2 (SPHK1 and 2), and irreversible degraded by S1P lyase (SGPL1) (Maceyka et al., 2012). Under normal condition, SPHK1 activity is generally associated with cell survival (Maceyka et al., 2012), while SPHK2 is widely described as being a dual-function protein, whose activity may either guarantee the proper functioning of the physiological events or result detrimental mainly suppressing cell growth and promoting apoptosis (Maceyka et al., 2012). Uncontrolled regulation of SGPL1 may be detrimental by altering S1P availability (Maceyka et al., 2012).

Our findings demonstrated that expression of S1P-metabolizing enzymes was considerably altered in two manifested HD mouse models (R6/2 and YAC128 mice), and most importantly in brain tissues from HD patients (Di Pardo et al., 2017). From our perspective, the imbalance between increased expression of SGPL1 and reduced levels of SPHK1 may underlie the decreased bioavailability of S1P and other sphingolipids in brain tissues of HD animals (Di Pardo et al., 2017). Interestingly, our findings demonstrated that defects in sphingolipid metabolism appear very early in the disease course, supporting the hypothesis that such alteration may contribute to its pathogenesis, rather than representing a mere "epiphenomenon" associated to the progression and worsening of the pathology.

From the molecular perspective, reduced bioavailability of S1P may obviously result in suboptimal activation of signalling cascade controlled by its receptors. Thus, pharmacological interventions based on S1P mimetic molecules (receptor agonists) and/or aimed at stimulating S1P production may be beneficial. Along this line, we have previously demonstrated that stimulation of S1P receptors (S1PRs) by the $S1PR_1$, $S1PR_3$, $S1PR_4$, $S1PR_5$ receptor agonist, FTY720 (fingolimod), a FDA- approved drug for the treatment of Multiple Sclerosis, was therapeutically effective in R6/2 mice (Di Pardo et al., 2014). Although the exact underlying molecular mechanisms remain to be fully clarified, among other effects, FTY720 restored normal levels of ganglioside GM1 in treated mice. This is an intriguing finding which reveals an existing link between metabolism of sphingolipid and gangliosides and, further substantiate the idea that globally targeting (glyco)sphingolipid metabolism may be therapeutic effective.

This hypothesis is also supported by the evidence that selective stimulation of SPHK1 activity significantly mitigated the toxic effect of Huntington mutation by activating pro-survival pathways in human induced pluripotent stem cell (iPSC)-derived neurons obtained from a HD patient (Di Pardo et al., 2017). From our perspective, this is an important result which reveal a translational potential of this targeted approach, however *in vivo* studies are now needed to assess the real clinical potential.

HD is currently defined as the most "curable incurable" brain disorder, however, no disease-modifying solutions are yet available. Although several compounds have been reported to provide benefits so far, the number of effective therapeutic options remains limited with only symptomatic treatment accessible. Thus, it becomes urgent to search for new therapeutic solutions at multiple levels.

Our work strongly supports the hypothesis that (glyco) sphingolipid metabolism/pathways may represent druggable target for developing novel and alternative approach for the treatment of the disease. In our opinion, the plausible "druggability" of such pathways has huge therapeutic potential for HD and, in the case of S1P metabolism, the big competitive advantage is due to the evidence that some drugs, whose molecular targets belong to its related pathways, are already in clinical trial for different other CNS diseases (Chew et al., 2016). In this context, we believe that our findings may offer support also for a feasible repositioning of these compounds for a rare disease like HD which is currently known being orphan of effective drugs.

In the case of the sialic acid, the modulation of either its metabolism or its overall availability, even in pre-manifest stages of the disease, may provide support for a novel and unexplored therapeutic opportunities for this devastating disorder.

Finally, taking in to account the overall importance of glyco-sphingo biology in the CNS and considering the well-defined genetic nature of Huntington's disease that makes this condition a valuable study model, we believe that positive outcomes from our studies may support similar therapeutic strategy for other neurological conditions, including either neurodevelopmental disorders (*i.e.*, Rett syndrome) or more complex neurodegenerative pathologies such as Parkinson's or Alzheimer's disease.

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