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EGF and the potassium channel Kv1.3 are promising pharmacological targets against neuro-degenerative diseases

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ABSTRACT

The adult mammalian brain contains neural stem cells (NSCs) that generate neurons and glial cells throughout the lifetime of an organism. NSCs reside in at least two germinal epithelium regions of the adult brain, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the hippocampus. Newborn neurons incorporate into the existing functional networks and play important innate and adaptive roles in cognition, behavior and tissue repair[1,2]. The identity of particular neural stem cells that generate different classes of neurons and glia, as well as the molecular mechanisms that governs this process in vivo, is a subject of extensive research and debate. Epidermal Growth Factor Receptor (EGFR) activation is one of the most important pathways controlling neural stem cell number and self-renewal[3,4]. On the other hand, the Shaker-type delayed rectifier K+ channel Kv1.3 functions during cell proliferation, differentiation and migration in many cell types[5]. This channel is expressed in brain progenitor cells where participates in modulating their final fate. This review summarizes the major findings concerning Kv1.3 and neural stem cell modulation, emphasizing the combination of Kv1.3 with EGFR as promising pharmacological targets against autoimmune neuro-degenerative diseases.

Kv1.3 in brain progenitor cells

Kv1.3 plays important physiological roles and participates in the onset of several pathological dysfunctions in many non-neuronal cell types, such as T-cells[6], platelets[7], microglia[8] and macrophages[9]. Kv1.3 blockade in T-cells results in depolarization, inhibition of activation and the attenuation of immune responses *in vivo*. Similarly, the inhibition of Kv1.3 in microglia reduces activation and prevents neurotoxicity[10]. In neurons, Kv1.3 is particularly important for maintaining tonic firing during sustained depolarization[11]. However, the contribution of Kv1.3 in controlling the proliferation of adult neural precursor cells remains controversial. Although some authors do not confirm the pharmacological expression of Kv1.3 in adult neural precursor cells (NPCs)[12], others identified gene and protein expression in adult rat mesencephalic-derived neurosphere NPCs[13] and oligodendrocyte (glia) progenitor cells (OPCs)[14]. The apparent discrepancy concerning Kv1.3 expression in brain progenitor cells may be associated with the developmental timing and anatomical origin of brain samples. Kv1.3 was not functionally detected in adult mouse NPCs obtained from primary neurospheres derived from the forebrain SVZ[12] but was expressed in adult rat mesencephalic-derived neurosphere NPCs[13] and OPCs from rat neonatal cerebral cortex[15,16]. Results from our lab indicated the possibility that Kv1.3-

positive cells may be more abundantly expressed in the posterior region of the SVZ (pSVZ), as primary cultured cells and neurospheres derived from this brain area contained functional Kv1.3 channels[17]. Our results are consistent with the facts that pSVZ contains progenitor cells that generate glial cells[18,19] and that Kv1.3 is functionally expressed in OPCs from the SVZ.

Large Kv currents have been described in proliferating OPCs, whereas post-mitotic oligodendrocytes do not express such currents[15, 19]. In fact, Kv1.3 is a key element in OPC proliferation, playing a crucial role in the G1/S transition[15]. Moreover, while overexpression of Kv1.3 increases OPC proliferation in the absence of mitogens, it has minor effects on the early stages of the oligodendrocyte differentiation and slightly increases the formation of $O4^+$ pro-oligodendrocytes[16]. In addition, genetic and pharmacological inhibition of Kv1.3 increases the neuronal fate among differentiated NPCs[20]. In contrast, evidence indicates that selective blockage of Kv1.3 increases adult murine mesencephalic NPC proliferation, based on the model of long-term cultured neurospheres under non-differentiating conditions[13]. Consistent with these studies, granzyme B (GrB) released by T-cells increases the expression of Kv1.3 within NPCs and hampers NPC proliferation and neuronal differentiation. Blocking Kv1.3 with margatoxin or with specific shRNAs abolishes the inhibitory effects of GrB on NPCs[21]. The above-mentioned results hindered a direct interpretation of Kv1.3 function, possibly suggesting different roles for Kv1.3 in controlling stem cell proliferation and differentiation.

EGFR stimulation *in vivo* drives proliferation in the SVZ[22, 23] modulates proliferation and migration of SVZ type-B astrocytes *in vitro*[23] and enhances migratory oligodendrocyte precursors in the rostral migratory stream[24]. Furthermore, the infusion of EGF into the lateral ventricle triggers the migration of SVZ cells from their normal route into the adjacent striatum and septum[25]. Furthermore, EGFR overexpression during early postnatal development promotes SVZ-to-lesion migration, enhancing oligodendrocyte generation and axonal myelination[3]. However, EGFR can also inhibit axon regeneration[26] by blocking Myelin Basic Protein (MBP) production into matured oligodendrocytes[27]. This apparently contradictory EGFR function could respond to different roles during recruitment-migration and differentiation-myelination phases of oligodendrocyte life. The mechanism behind this change of behavior remains unknown. In this context, integrins, interacting with extracellular matrix (ECM) components, participate on the cell adhesion and motility[28]. β1 integrin is required for propagating the EGFR signaling from the plasma membrane to the nucleus by sustaining the EGFR-dependent endocytic machinery[29]. β1 integrin expression diminishes during oligodendrocyte differentiation, concomitantly with a decrease into their migratory potential[30]. Genetic ablation of β1 integrin generates a deficit in myelination[31], suggesting that basal levels of this protein are required in oligodendrocytes to extend their myelin sheets. Kv1.3 associates with β1 integrin[32,33]. Evidence suggests that the activation of Kv1.3 triggers conformational changes into neighboring integrins. Because the Kv1.3 pharmacological blockade or integrin detachment to ECM can disassembly the supramolecular complex this issue is worth of study during oligodendrocyte life. Kv1.3 is modulated by EGF signaling pathways and, interestingly, we recently described a reinforcement of the EGF effect on NSC migration by blocking Kv1.3 in SVZ-derived explants. Via detailed analysis of the molecular mechanisms governing this effect, we demonstrated that Kv1.3 activity is reduced by EGFR activation in an unconventional dual pathway, comprising both tyrosine phosphorylation-dependent inhibition of activity and p42/44 MAPK (ERK1/2) threonine phosphorylation-dependent internalization of the channel[17]. Contrary to previous reports, the EGFR activation initiates a signaling cascade that decreases current amplitude via both covalent modification of the channel and diminishing Kv1.3 abundance at the surface. Therefore, evidence suggests that EGFR activation and downregulation of Kv1.3 acts synergistically to promote NSC proliferation and migration. It is not known whether Kv1.3 internalization affects β1 integrin activity. Whether Kv1.3 endocytosis would somehow inhibit integrin function, this could represent a potential mechanism to counteract the activity of EGFR and shorten its effect, possibly driving the cells to a more differentiated state. The activity of Kv1.3 together with β1 integrin could be associated with the maintenance of a proliferative state. The down-regulation of the channel associated with EGFR activity could act as an initial mechanism to counteract the activity of this mitogen and initiate the differentiation of oligodendrocytes. However, further research should be done in this respect.

Conclusions and future perspectives

Kv1.3 and EGFR are promising pharmacological targets for developing new therapies against inflammatory-associated neurodegenerative diseases such as multiple sclerosis (MS), brain infarction and other demyelinating disorders.

For example, lysolecithin (LPC)-induced focal unilateral demyelination of the corpus callosum upregulates VEGF and EGFR ligands (HB-EGF, TGFα) in the SVZ[34]. Similarly, focal cerebral ischemia is associated with elevated EGF levels and increased NPC proliferation in the SVZ[35]. In cultured SVZ cells, EGF induces oligodendrogenesis and subsequent myelin production[34]. Adult SVZ $GFAP^+$ type-B astrocytes exhibit a positive dose-dependent effect of EGF on proliferation and migration. These $Olig2^+$ $NG2^+$ cells are

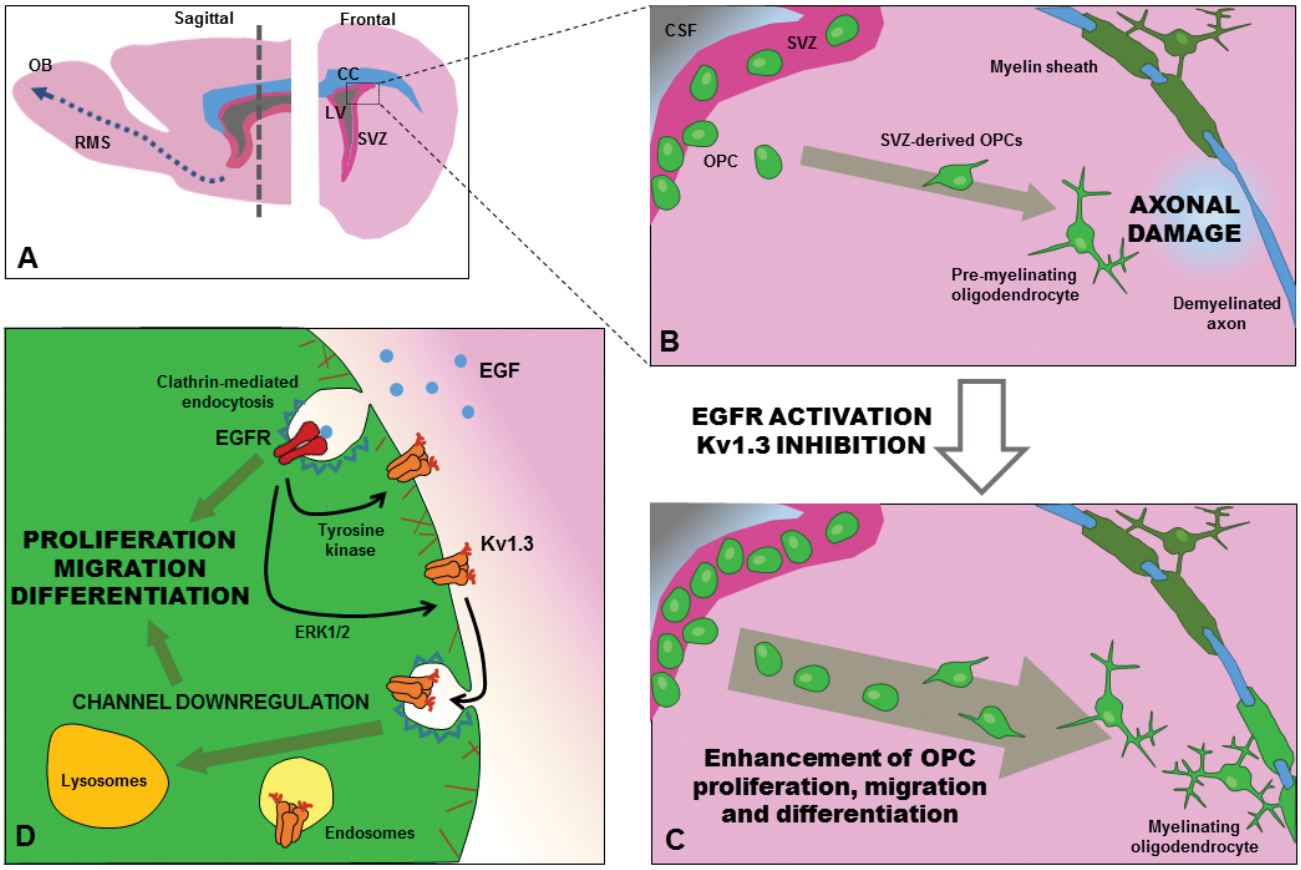

Figure 1: Schematic representation showing the action of the EGFR activation and Kv1.3 inhibition on a neuronal demyelination repair process. A) Sagittal and frontal representation of the postnatal mice brain. B) After demyelination, the inflammatory response triggers the release of factors activating proliferation, migration and differentiation of SVZ-derived OPCs. Cells differentiate into mature myelinating oligodendrocytes extending a new myelin sheath around the demyelinated axon. C) The activation of EGFR pathway and/or the inhibition of Kv1.3 would recruit SVZ-derived OPC into the damaged area, thereby improving the remyelination. Blue, axon; dark green, old myelin sheath; light green, new myelin sheath. D) Molecular mechanism of the EGFR on Kv1.3. Activation of EGFR pathway induces both tyrosine and threonine phosphorylation of the channel. Threonine phosphorylation via Ras/Raf/MEK/ERK pathway triggers clathrin-coated pits-mediated internalization of Kv1.3. Channel downregulation and EGFR activation cooperate to positively modulate proliferation, migration and differentiation of OPCs. OB, Olfactory bulb; RMS, Rostral migratory stream; CC, Corpus callosum; LV, Lateral ventricle; SVZ, Subventricular zone; CSF, Cerebrospinal fluid; OPC, Oligodendrocyte precursor cell.

highly migratory and proliferative and differentiate into S100b$^+$/O4$^+$ oligodendrocytes upon EGF withdrawal[23]. Thus, EGF may induce SVZ-oligodendrocyte progenitors to migrate, differentiate into oligodendrocytes and finally remyelinate injured white matter[36]. Conversely, reduction of EGFR signaling in NG2-expressing progenitors decreases SVZ-to-lesion migration of NG2$^+$ cells and the subsequent oligodendrogenesis and remyelination rates. This demonstrates that the NG2$^+$ cell response in the SVZ and the subsequent differentiation of these cells after focal demyelination are dependent upon EGFR signaling[34]. Accordingly, EGFR overexpression in NPCs *in vivo* expands and accelerates oligodendrogenesis, axonal remyelination and functional recovery in the LPC-induced model of demyelination. Injured areas are repopulated by NG2$^+$ Mash1$^+$ Olig2$^+$ progenitor cells[3]. The elevated EGFR signaling reduces neurogenesis in favor of oligodendrogenesis. This is due to an induced expansion of the SVZ-NPC pool and the concomitant reduction of NSC number and self-renewal through an EGFR-mediated regulation of Notch signaling[4]. Altogether, these results demonstrate that EGFR signaling *in vivo* is involved in oligodendrocyte development and remyelination. In this context, EGF administration has been used as a therapeutic approach to counteract demyelination. The cerebrospinal fluid of MS patients in the relapsing–remitting or secondary-progressive clinical courses is characterized by a deficiency in the myelinotrophic factor EGF[37]. This deregulation in the synthesis of growth factors in MS CNS appears to be involved in the inhibition of remyelination[38]. Concomitantly, EGF administration positively affects myelin repair in the rat spinal cord white matter[39]. Co-administration of EGF and growth hormone releasing peptide-6 reduced inflammatory infiltration and microvascular damage associated with EAE, thereby

improving the clinical recovery[40]. Intranasal heparin-binding EGF (HB-EGF) administration increases SVZ cell proliferation and mobilization towards LPC-demyelinated injured areas and a subsequent differentiation shift towards the astrocytic lineage[41]. Similarly, immediately after injury of neonatal mouse brain, intranasal HB-EGF infusion decreases oligodendroglial death, enhances generation of new oligodendrocytes from progenitor cells and promotes functional recovery by diminishing ultrastructural abnormalities[42].

In this scenario, our results[17] support a dual role for EGF-signaling and Kv1.3 function in controlling multiple and complementary relevant aspects of the progression of demyelinating disorders (Fig. 1). Therefore, the manipulation of either and preferably both of these elements might ameliorate the neurodegenerative progression. EGF administration would enhance remyelination from SVZ-derived OPCs. Inhibition of Kv1.3 with therapeutically usable compounds, such as analogs of a sea anemone toxin[43,44], or psoralene dirivatives[45], would hamper the cytotoxic effect of inflammatory infiltrates, acting simultaneously on activated lymphocytes and proliferating OPCs. Thus, EGF supplementation might be a useful adjunctive for Kv1.3 inhibition in treatment of MS, brain infarction and other demyelinating disorders. In addition to the pharmacological targeting of specific host cells, stem cell therapy represents a promising alternative in the treatment of neurodegenerative processes (e.g., MS) and brain tissue damage (e.g., after hypoxia). Thus, simultaneous control of EGF and Kv1.3 might provide more effective ways to control growth, proliferation and differentiation of stem cells used for the treatment of neurodegenerative disorders or for CNS regeneration.

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