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Targets for Neural Repair Therapies after Stroke

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Abstract

Studies of neural repair after stroke have developed from a relatively small number of labs doing highly creative discovery science, to field in which reproducible evidence supports distinct pathways, processes and molecules that promote recovery. This review will focus on some emerging targets for neural repair or recovery in stroke and on their limitations.

Blockers of Axonal Growth Inhibitors after Stroke

Myelin Growth Inhibitors

Stroke induces a process of axonal sprouting in neighboring or connected cortical neurons that is associated with repair and recovery[1–3]. Adult CNS myelin or adult oligodendrocytes contain several inhibitors of axonal sprouting. These include the myelin-associated proteins Nogo, oligodendrocyte myelin glycoprotein (OMgp) and myelin associated glycoprotein (MAG)[4,5]. Nogo has emerged as a key axonal growth inhibitory protein. Pharmacological blockade of Nogo induces axonal sprouting and functional recovery in spinal cord injury[4,5] and in stroke[6]. Nogo inhibits axonal growth through Nogo receptor 1, a glycosyl-phosphinositide linked protein, and through the recently described immunoglobulin receptor PIR1[7]. NgR1 signals through the TNF family members TROY or p75 and Lingo-1[4,5]. Several groups have developed soluble Nogo antagonists, often receptor decoys or peptide antagonists[8], or Lingo-1 antagonists[9]. A Nogo blocking antibody is currently in clinical trials in spinal cord injury as delivered into the CSF intrathecally[10]. A small Nogo antagonist peptide has shown promise in pre-clinical stroke and spinal cord injury models[6,11].

MAG and OMgp clearly block axonal outgrowth in vitro, but their role in in vivo axonal growth inhibition in the adult is less clear. Genetic knockout of MAG does not promote axonal outgrowth in vivo[4,5]. OMgp knockouts do not selectively support axonal sprouting in isolation[12]. Thus therapies directed toward these two molecules do not have strong pre-clinical support in vivo. Still, an anti-MAG antibody is in clinical trial in spinal cord injury[13], perhaps reflecting interest driven by the strong in vitro action of MAG. When combined with Nogo knockout, the triple elimination of all three myelin inhibitors promotes greater axonal outgrowth and functional recovery than Nogo knockout alone[14]. This suggests a degree of compensation within myelin signaling that may provide for adjunctive therapies in stroke or spinal cord injury. A receptor decoy that consists of NgR1 and NgR2 motifs that blocks Nogo, MAG and OMgp interactions with NgR1 and NgR2 has been developed and enhances axonal outgrowth in vitro[15].

Disclosures:
None

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Myelin or oligodendrocyte axonal growth inhibitors also include Ephrin B3, semaphorins 4a, 4d and 6a, netrin 1 and RGMa[4,5,16,17]. The reactivation of these developmental axonal guidance molecules after injury, in which growth cones are again traversing regions of the CNS, suggests that they may be suitable targets to promote axonal sprouting after stroke. Netrin-1 can inhibit axonal sprouting in spinal cord injury likely through the Unc-5 receptor on neurons[18]. Antibody blockade of RGMa promotes axonal sprouting and recover after spinal cord injury[19]. However, these developmental axonal guidance molecules likely have other effects in the injured CNS. Sema4d is involved in microglial activation and oligodendrocyte differentiation after stroke or spinal cord injury[20]. Ephrins and semaphorins are important in forming tissue boundaries in the injured CNS, particularly astrocyte, Schwann cell and fibroblast zones in the spinal cord scar[21,22] and in brain trauma[23]. These findings highlight the complex interplay of cell-cell signaling systems after injury, and that axonal sprouting after stroke will not involve just the isolated interaction of myelin ligands and neuronal receptors.

Astrocyte or Extracellular Matrix Growth Inhibitors after Stroke

Reactive astrocytes produce growth inhibitory molecules, such as chondroitin sulfate proteoglycans (CSPGs)[24,25]. Within the extracellular matrix, CSPGs may be growth inhibitory by directly contacting and blocking growth cones, by presenting growth inhibitory molecules or by structurally blocking dendritic rearrangement in the perineuronal net[4,25]. Recent work has shown that a specific protein tyrosine phospatase receptor, PTPsigma[26], can selectively transduce the growth inhibitory signals of CPSGs[27] including neurocan, which is dramatically induced after stroke[24]. Digestion of CSPG side chains is one strategy to modify the CSPG matrix and improving axonal sprouting. The bacterial enzyme chondroitinase ABC has been delivered in spinal cord injury, digests inhibitory CSPG side chains, and promotes axonal sprouting and recovery[25]. Bioengineering strategies for enhancing chABC delivery, and modifications to promote temperature stability, may enable this therapy to be applied to stroke[28]. Other secreted (Wnt5a) and membrane bound (ephrin5a) astrocyte growth inhibitors have also recently been identified which limit functional recovery[29,30], suggesting additional specific astrocyte targeting approaches for neural repair in stroke.

RhoA Pathway Inhibition

Ephrins, semaphorins, Nogo, MAG, OMgp and RMGa signal through RhoA and its downstream Rho kinase (ROCK). RhoA signaling accomplishes the business end of axonal growth inhibition, by linking to the cytoskeleton and promoting microtubule depolymerization and actin contraction[4,5,31]. RhoA inhibitors mediate a powerful blockade of the axonal growth inhibition in neurite outgrowth assays in vitro for many molecules, and promote axonal sprouting in spinal cord and other CNS injury models in vivo[4,5,31]. Intracellular delivery of a Rho inactivator has been developed with tat conjugation[32]. A major problem with targeting a growth inhibitory “master switch” is that it will be active for other cellular functions in non-neuronal cells, leading to potentially widespread off-target effects. Pharmacological targets could be utilized within Rho signaling that are more tissue specific. ROCK exits in two isoforms. ROCKI is ubiquitous but ROCKII is concentrated in CNS, as well as muscle, liver and lung[31]. Recent work with ROCKII knockouts indicates that this enzyme is a viable target for promoting a more selective CNS RhoA inhibition and facilitating axonal outgrowth[33].

Axonal Growth Stimulators

Focused re-activation of a neuronal growth state after CNS injury has emerged as a key pharmacological target[34]. This is because simply blocking axonal growth inhibitors has not resulted in substantial axonal sprouting, particularly of long axonal projections such as the corticospinal tract, or in experimental injury models, the optic tract[3,35]. There is growing evidence for a specific molecular program in sprouting adult neurons after stroke[3,24,35,36].

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Several studies have uncovered pharmacological targets that promote a neuronal growth state in the adult CNS[24,26–39]. Inosine triggers a serine/threonine kinase (Mst3b) to induce greater axonal outgrowth in retinal ganglion cells, and in corticospinal neurons contralateral to the stroke site[3,39].

Interestingly, inosine induces a gene expression profile in contralateral cortex that overlaps with the gene expression profile in other sprouting neurons[36]. The phosphatase PTEN also potently controls axonal outgrowth. Blockade of PTEN after optic nerve injury promotes substantial axonal outgrowth in the optic nerve, to a degree not seen with other molecular manipulations[40]. PTEN knockout also enhances neurogenesis after stroke[41]. PTEN antagonizes the action of the PI3 kinase/Akt pathways, which mediates many of the downstream effects of neurotrophins and other growth factor receptors[40,42]. One downstream effect of PTEN is the inhibition of mTOR[40,42]. This cascade provides a target rich environment for the development of “pro-growth” approaches to promote axonal sprouting and recovery after stroke or spinal cord injury. A caveat is that PTEN is a commonly altered pathway in many cancers, such as glioblastomas[42]. Induction of a growth state in a post-mitotic cell such as neuron will require careful targeting and attention to the duration of therapy, as neighboring astrocytes, and indeed all mitotically active cells, may respond to this therapy in a deleterious “pro-growth” manner.

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