Repulsive Guidance Molecule A Regulates Adult Neurogenesis Via the Neogenin Receptor

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ABSTRACT: Repulsive guidance molecule A (RGMa) exhibits repulsive guidance of axonal growth and regulates neuronal differentiation during development of the mammalian brain. In this commentary, we describe the findings of our recent paper, "Repulsive Guidance Molecule A Suppresses Adult Neurogenesis," and discuss a possible model for RGMa suppression of newborn neurons that fail to properly migrate into the granular cell layer. In the study, we provided evidence that RGMa suppressed neurite growth and survival of newborn neurons in the adult dentate gyrus. This effect depends on the multifunctional Neogenin receptor expressed in adult neural stem cells through activation of the Rho-associated protein kinase leading to neurite growth inhibition and ultimately cell death. It should be noted that both RGMa and Neogenin interact with several well-described molecules, including bone morphogenetic proteins, that regulate neuronal development. Thus, it is likely that RGMa interacts with other intricate molecular networks that regulate adult neurogenesis.

KEYWORDS: Neurogenesis, RGM, Neogenin, Rho

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Background

The development of the mammal brain is facilitated by a concert of biological processes. A major event during brain development is the differentiation of neurons from neural stem cell precursors and the subsequent outgrowth of axons and dendrites. The neurite outgrowth is strictly guided toward synaptic partners by a complex pattern of attractive and repulsive chemical cues. One such cue is repulsive guidance molecule A (RGMa), a glycosylphosphatidylinositol-anchored glycoprotein essential for neural development. During brain development, RGMa exhibits strong repulsive guidance of growing axons and while also acting as a regulator of neuronal differentiation and neuronal survival.[1,2]

Compared with the developing brain, the adult brain is highly static. Nevertheless, important plastic changes in established neuronal networks still occur and are important for the formation of memory and adaptation to new situations. These plastic changes in the adult brain range from minor molecular changes in synapse strength to the generation of adult-born neurons derived from adult neural stem cells (aNSCs).[3] Two strictly defined aNSC niches exist in the adult mammalian brain: the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus. In both niches, self-renewing, multipotent aNCSs reside that differentiate into neurons and neuroglia throughout adulthood.[3] After initiation of differentiation, derived adult-born neurons in the subgranular zone start to migrate, first tangentially and then radially into the granular cell layer.[4] Meanwhile, an outgrowth of axons and dendrites and formation of synapses begins, concluding with a fully functional integrated adult-born granular neuron.[3,5] While only a subpopulation of the aNSCs-derived neurons survive and become fully integrated, these adult-born neurons are nonetheless considered to be important for memory formation and learning.[3] Therefore, to understand brain plasticity, it is important to elucidate the intricate molecular systems that regulate adult neurogenesis. Before our study, it was shown that the multifunctional receptor Neogenin is important for several aspects of adult neurogenesis.[6,7] Loss of Neogenin in the hippocampus led to deficits in aNSCs proliferation, neurogenesis, and neuronal activity of adult-born neurons.[7] In the developing brain, Neogenin is the main target for RGMa activity and RGMa/Neogenin signaling has been shown to trigger several downstream cytoskeleton-related signal pathways resulting in inhibition of neurite outgrowth.[8] Thus, we hypothesized that RGMa signaling might be involved in regulating adult neurogenesis via Neogenin.

RGMa Suppresses Adult Neurogenesis Through the Neogenin Receptor

To test our hypothesis, AAV-mediated overexpression and knockdown of RGMa was performed locally in the dentate gyrus of adult mice. This resulted in a significant decrease and increase, respectively, of mature NeuN positive adult-born neurons, whereas the number of adult-born GFAP-positive astrocytes was not affected. Importantly, no changes to the proliferation rate of aNSCs or to the total number of

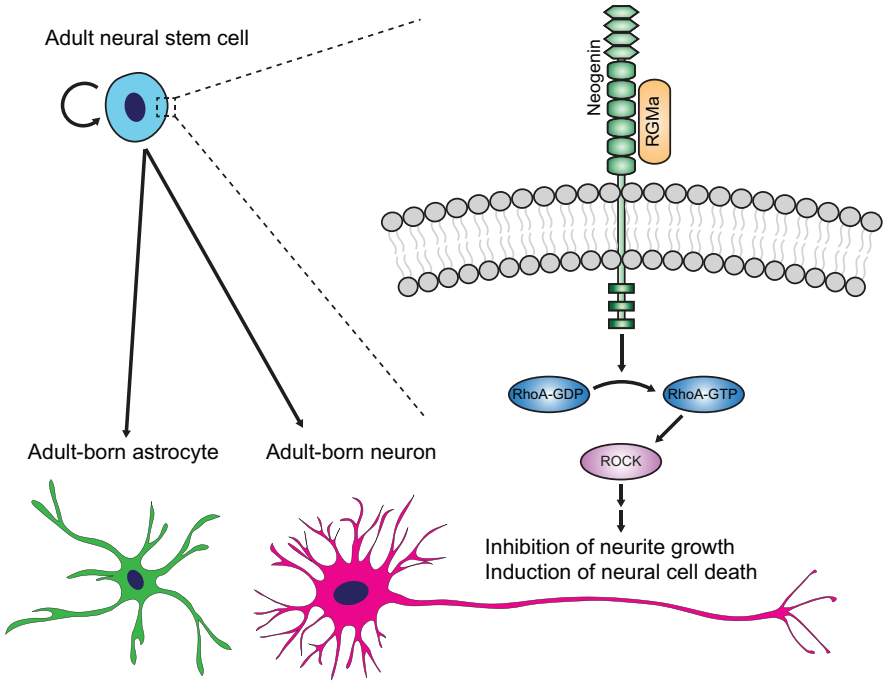

Figure 1. RGMa signaling in aNSCs. Adult neural stem cells residing in the subgranular zone of the dentate gyrus are self-renewing, multipotent cells that differentiate into neurons and neuroglia throughout adulthood. We found that RGMa activates the RhoA/ROCK pathway in aNSCs via the Neogenin receptor. This activation causes inhibition of neurite growth and induction of cell death, resulting in RGMa-mediated suppression of adult neurogenesis. aNSCs indicates adult neural stem cells; RGMa, repulsive guidance molecule A; RhoA, ras homolog gene family member A; ROCK, Rho-associated protein kinase.

Sox2-positive aNSCs were observed in the dentate gyrus following RGMa knockdown. Overall, this implicated RGMa as a selective regulator of neural differentiation and/or neural development in the adult brain.

In support of the in vivo findings, stimulating cultured aNSCs with recombinant RGMa led to no changes in proliferation rates. Conversely, when stimulating differentiating cells, a significant decrease in derived neurons, but not derived astrocytes, was observed. As embryonic developing neurons exhibit strong axonal growth inhibition by RGMa,[2] we tested for this effect on the aNSCs-derived neurons. Repulsive guidance molecule A stimulation led to a significant decrease in length of both Map2-labeled dendrites and Tuj1-labeled axons. This neurite growth inhibition by RGMa resulted in a rise of apoptotic cleaved Caspase-3-positive cells, likely explaining the overall reduction of derived neurons observed after RGMa stimulation of differentiating aNSCs.

Our study also revealed some of the molecular pathways of RGMa signaling in aNSCs (Figure 1). Initially, siRNA-mediated knockdown of Neogenin in cultured differentiating aNSCs prevented RGMa-induced suppression of neurogenesis, demonstrating the dependency of RGMa on its recognized receptor Neogenin in aNSCs. Downstream of RGMa/Neogenin, we found that the ras homolog gene family member A (RhoA) was highly activated after RGMa stimulation. The Rho-associated protein kinase (ROCK) is the main effector of the Rho family of GTPases including RhoA.[9] Thus, we tested the effect of the selective ROCK inhibitor Y-27632 together with RGMa stimulation. ROCK inhibition completely prevented RGMa-induced neurite growth inhibition and neural cell death. Notably, this dependency on ROCK inactivity for neurite outgrowth is also essential in embryonic developing neurons.[10]

Finally, as RGMa primarily was observed to be expressed within the hilus area of the dentate gyrus, we hypothesized that RGMa could be involved in eliminating adult-born neurons that fail to properly migrate radially into the granular cell layer. Indeed, when analyzing the migration of newborn adult neurons after RGMa knockdown, we found that while RMGa knockdown increased the number of mature adult-born neurons, many of these neurons failed to migrate into the granular cell layer.

Our findings provide evidence for a function of RGMa in adult neurogenesis and consequently in adult brain plasticity. Taken together, our data could support a model where RGMa expressed in hilar cells forms a gradient toward the granular cell layer. Newborn neurons derived from aNSCs residing in the subgranular zone will experience less RGMa suppression as they migrate into the granular cell layer, thereby allowing the outgrowth of their axon and dendrites. Conversely, the neurite outgrowth of newborn neurons that fail to migrate into the granular cell layer will be inhibited by RGMa activity. Exactly how RGMa inhibition of neurite growth in the adult-born neurons leads to cell death is still unclear; however, it is possible that an intracellular feedback system induces apoptosis based on the inability to outgrow the neurites and thus form meaningful synaptic connections.[11] Neogenin has been shown to affect aNSCs migration, as Netrin-1 was shown to affect aNSCs migration

from the subventricular zone via Neogenin.[6] Netrin-1 and RGMa exhibit competitive effects via Neogenin, with Netrin-1 exhibiting chemoattractant activity for growing axons, while RGMa exhibiting repulsive activity.[12] Whether Netrin-1 also affects aNSC migration in the subgranular zone of the dentate gyrus remains to be investigated. However, upregulation of Netrin-1 in the hilar region following epileptic seizures led to an increase in adult-born neurons within the hilar region.[13] In light of our study, this effect could be attributed to Netrin-1's previously shown chemoattractant activity or to the competitive effect of inhibiting RGMa's repulsive effect within the hilar region. Finally, RGMa and Neogenin also interact with bone morphogenetic protein (BMP) signaling.[8,14] Several BMPs are important for inducement of aNSCs differentiation, and it has been shown that Noggin inhibition of BMP2 and BMP4 signaling is critical for maintaining an aNSC population within the stem cell niche.[15] Repulsive guidance molecule A acts as a co-receptor for both BMP2 and BMP4, regulating their binding to the BMP type II receptor.[14] Thus, it is likely that RGMa and Neogenin interact with several intricate molecular networks to regulate adult neurogenesis. Further research will hopefully shed light on this interaction.

Author Contributions

TJI and TY wrote the manuscript.

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