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·Minireview·

Slit2/Robo1 signaling in glioma migration and invasion

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Abstract: Slit2/Robo1 is a conserved ligand-receptor system, which greatly affects the distribution, migration, axon guidance and branching of neuron cells. Slit2 and its transmembrane receptor Robo1 have different distribution patterns in gliomas. The expression of Slit2 is at very low levels in pilocytic astrocytoma, fibrillary astrocytoma and glioblastoma, while Robo1 is highly expressed in different grades of gliomas at both mRNA and protein levels. Acquisition of insidious invasiveness by malignant glioma cells involves multiple genetic alterations in signaling pathways. Although the specific mechanisms of tumor-suppressive effect of Slit2/Robo1 have not been elucidated, it has been proved that Slit2/Robo1 signaling inhibits glioma cell migration and invasion by inactivation of Cdc42-GTP. With the research development on the molecular mechanisms of Slit2/Robo1 signaling in glioma invasion and migration, Slit2/Robo1 signaling may become a potential target for glioma prevention and treatment.

Keywords: Slit2/Robo1; glioma; invasion; migration

1 Introduction

Human gliomas originate from neural mesenchymal cells and account for 4%-50% of the nervous system tumors. They are subdivided into 4 grades based on their histology and prognosis[1]. The grade IV malignant glioma, glioblastoma multiforme (GBM), is diffuse, highly invasive and often multifocal, having a dismal prognosis with a median survival of only 1 year for GBM patients[2]. Patients with grade III gliomas survive for 2-3 years, and those with grade II gliomas survive for 10-15 years[3,4]. One of the major obstacles to the effective treatment of gliomas is the infiltrative nature of gliomas, resulting in incomplete surgical removal, and the intrinsic ability of single tumor cell infiltration would extend tendrils of the tumors several centimeters away from the main tumor mass, inducing a high frequency of tumor recurrence. Malignant glioma development is a complex multi-step and coordinated biological process, most likely controlled by distinct genes and signaling pathways in different steps[5,6]. The present review mainly focused on the relationship between Slit2/Robo1 signaling and glioma migration and invasion.

2 The structure and function of Slit2/Robo1

The Slit family consists of large extracellular matrix-secreted and membrane-associated glycoproteins, expressed and secreted by the midline glia. The Slits are ligands for the repulsive guidance Robo receptors and play important roles in axon guidance and neuronal migration during central nervous system (CNS) development[7,8]. In vertebrates, the Slit

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protein contains an N-terminus signal peptide, 4 tandem leonine-rich repeats (LRR), 9 epidermal growth factor (EGF)-like motifs, an Agrin-Laminin-Perlecan-Slit (ALPS) spacer between EGF6 and EGF7, and a cysteine-knot that is usually found in secreted growth factors[9]. Humans, mice and other vertebrates possess 3 *Slit* genes, including *Slit1*, *Slit2* and *Slit3*. *Slit1* is specifically expressed in adult brain and is located at chromosome 10q24.1. *Slit2* is expressed in a variety of adult tissues and located at chromosome 4p15.2. *Slit3* has a similar expression pattern as that of *Slit1*, and occupies approximately 600 kb (chromosome 5q34-5q35.1)[10,11].

The Roundabout (Robo) gene was initially identified in Drosophila in a large scale mutant screen for the genes that control midline crossing of axons. In vertebrates, the Robo family consists of 4 members (Robo1, Robo2, Robo3/Rig-1 and Robo4/Magic Roundabout), each having 5 immunoglobulin (Ig)-like domains and 3 fibronectin type III repeats in the extracellular domain and 4 conserved cytoplasmic motifs (CC1-4)[12,13]. Both *Robo1* and *Robo2* genes reside at chromosome 3p12.3. *Robo3* and *Robo4* genes are mapped to chromosome 11p22.4[14-16]. The 5 Ig domains of Robo can bind the LRR domain of Slit protein which is necessary for the exclusion effect of Slit[9]. Abelson kinases can phosphorate tyrosine residues in CC1 motif, and then down-regulate the Robo-mediated signal transduction. The CC2 motif contains the binding site for cytoskeleton protein Enabled (Ena). The CC3 motif serves as a binding site for Slit-Robo GTPase activating protein (srGAPs), regulating the activity of the Rho family of small guanosine triphosphotases (GTPases)[8,17]. Huminiecki *et al.* have found that Robo4 is a vascular-specific receptor that inhibits endothelial cell migration and tumor angiogenesis[18].

The Slit/Robo system represents an evolutionarily conserved chemo-repulsive ligand-receptor system which is involved in axon guidance, axonal branching as well as regulation of neural cell migration. The Slit/Robo interaction is able to prevent commissural axons from recrossing the *CNS* midline. Furthermore, it participates in guidance and motility of neural and non-neural cells such as neuronal precursor cells, Langerhans cells and vascular smooth muscle cells[19,20].

3 Expression of Slit2/Robo1 in glioma

Epigenetic inactivation of tumor suppressor genes (TSGs) by genetic mechanisms has been well documented. The genetic alterations include hemi- or homozygous deletion and mutations. The epigenetic silencing of genes is achieved through the combination of DNA methylation and histone modifications, which may affect the chromatin structure and promoter accessibility. DNA methylation is likely to be the most important epigenetic event that inhibits gene expression in the transcriptional level. DNA methylation often occurs in the number 5 carbon of cytosine pyrimidine ring, located next to a guanine forming, which is known as a CpG dinucleotide. A typical CpG island has 200-500 base pairs with GC content higher than 50%. As revealed by some reports, the CpG island of human *SLIT2* in the promoter region is frequently hypermethylated in lung, breast, cervical cancers and colorectal tumors as well as in neuroblastoma and Wilms' tumor, and the hypermethylation correlates with loss of gene expression[1]. However, by using qRT-PCR and immunohistochemistry methods, Mertsch S *et al.* have found that Slit2 is distinctly expressed by normal cerebral neurons, but at very low levels in pilocytic astrocytomas, fibrillary astrocytoma and glioblastoma in human specimens. Moreover, with the progression of malignant glioma, the expression of Slit2 is gradually decreased or even silenced[21]. Differences in expression of Slit2 protein can be mainly attributed to hypermethylation of CpG island in the promoter region. In an earlier study, no inactivating somatic mutations of Robo1 in lung and breast cancers were found, but a CpG island in the 5' region of Robo1 was hypermethylated in breast and kidney tumors[22]. However, Robo1 is not only expressed in normal brain neurons, but also over-expressed in different grades of gliomas at both mRNA and protein levels[22]. Thus, we propose that the differences in Robo1 expression in diverse tumors are possibly related to cell types, tissue backgrounds and interactions of Robo1 with other signaling pathways.

4 Slit2/Robo1 in glioma invasion and migration

The development of tumors is a complex multi-step process, including malignant tumor invasion and metastasis.

Acquisition of the insidious invasiveness by malignant glioma cells involves multiple genetic alterations in signaling pathways. A large number of studies have demonstrated that the Slit2/Robo1 signaling channels can inhibit glioma invasion and migration. However, the specific roles of Slit2/Robo1 in cancer cell invasion *in vivo* have not yet been completely revealed.

The functions of the Slit/Robo system in repelling axons and neuronal and glial cells of the developing and adult nervous system have been well established. More recently, Slit2, a chemorepulsive factor, has also been indicated to inhibit chemotaxic migration of various types of cells such as leucocytes and dendrite cells *in vitro*[23]. This inhibition appears to be mediated by Robo1. However, this effect in cancer cell invasion has not been reported yet. Due to *Slit2* promoter methylation or the high frequency of allele loss, the expression of Slit2 is lost in malignant gliomas, and lung, breast and colorectal cancers. The loss of Slit2 expression could be restored by treatment with a demethylating agent 5-aza-2'-deoxycytidine or by exogenous expression of Slit2 *in vitro*. Therefore, *Slit2* may represent a tumor suppressor gene that inhibits migration and invasion of tumor cells. Mertsch S *et al.* have found that the Slit2/Robo1 system serves as a chemorepellent for glioma cells in a modified Boyden chamber assay, suggesting that glioblastoma cells migrate away from higher Slit2 concentrations[21]. This function prompts Robo1-positive glioma cell invasion along gray matter tracts as well as into white matter including the corpus callosum. *In vivo* studies reveal that after implantation of invasive SNB19 glioma cells stably expressing Slit2 into the brain of 8-week-old female mice, glioma cell infiltration into the brain parenchyma is markedly attenuated. Meanwhile, ectopic expression of Slit2 in SNB19 cells could attenuate cell migration and invasion, but has only minimal impacts on cell proliferation and survival, as revealed by cell viability assay[24]. These results suggest that Slit2/Robo1 can inhibit glioma invasion and migration both *in vivo* and *in vitro*.

5 Slit2/Robo1 functions in glioma by downregulating Cdc42 activity

Cdc42, a member of the small Rho GTPase family, plays important roles in cell migration by regulating the actin cytoskeleton and cytoskeletal remodeling, and is crucial for axon guidance and axonal branching. A novel family of Rho GTPase-activating proteins (srGAPs, including srGAP1, srGAP2, and srGAP3) *is* situated in the downstream of Slit2/Robo1 signaling. It has been elucidated that the repelling activity of Slit during neuronal migration requires the interaction between the intracellular domain (CC3 motif) of Robo1 and srGAP1. That is to say, this interaction specifically inhibits Cdc42. Slit increases srGAP1-Robo1 interaction and inactivates Cdc42[24]. A dominant negative srGAP1 blocks Slit inactivation of Cdc42 and Slit repulsion of migratory cells from the anterior subventricular zone (SVZa) of the forebrain. Yiin JJ *et al.* have investigated whether Slit2 expression in glioma cells regulates the activity of Cdc42. They clarify that Slit2, via its receptor Robo1, specifically inhibits Cdc42 activity and glioma cell motility *in vitro*. Simultaneously, Slit2 overexpression or treatment with recombinant Slit2 attenuates GTPase activity of Cdc42 but not the expression of Cdc42 protein in glioma cells[24].

The inhibitory effects of Slit2/Robo1 interaction on migration and invasion of various cell types are mediated by several downstream effectors. Moreover, the Slit/Robo complex is known to regulate β-catenin/N-cadherin-mediated cell-cell adhesion in neuronal cells[25,26]. In glioma cells, Slit2/Robo1 halts cell migration and invasion, independently of modulation of β-catenin phosphorylation, N-cadherin and β-catenin association, or N-cadherin and β-catenin protein degradation. In Slit2-overexpressing breast cancer, Slit2/Robol system exhibits tumor suppressor capabilities through coordinated regulation of β-catenin/TCF and PI3K/Akt signaling pathways and through enhancing β-catenin/E-cadherin-mediated cell-cell adhesion[27]. In leukocytes, Slit2 inhibits chemokine/CXCR4-mediated cell migration through inhibition of ERK1/2 activation. In chick neural retinal cells, mouse fibroblasts and human embryonic kidney 293T cells, Slit/Robo prompts formation of the Robo/Abl/Cable-N-cadherin/β-catenin complex and tyrosine phosphorylation of β-catenin that moves into nucleus, resulting in disassociation of β-catenin from N-cadherin, and degradation of β-catenin and N-cadherin proteins[25].

6 Prospect

The development of gliomas is a complex process mediated by multiple genes and factors, and its pathogenesis has not been determined. More recently, the repulsive effect of Slit2/Robo1 on gliomas has attracted more attention. Robo1 is over-expressed in gliomas, but its expression is at very low levels in breast and kidney tumors and in other cancers, due to its promoter region CpG island hypermethylation. The promoter region CpG island hypermethylation and frequent allelic loss of *Slit2* closely correlate with glioma progression. Both *Slit1* and *Slit3* genes in gliomas are frequently methylated and this methylation correlates with loss of their expression. However, it has not been clear whether they function the same as Slit2/Robo1, suppressing the migration and invasion of gliomas.

The cellular signaling transduction pathways play a vital role in the onset and development of gliomas. The effect of Slit2/Robo1 signaling is prominent among those of various signal transduction ways. Slit2/Robo1 signaling inhibits glioma migration and invasion through attenuating the GTP-binding activity of Cdc42, while it has no influence on the expression of β-catenin and N-cadherin. The underlying mechanism differs from that in breast cancer cells, mouse fibroblasts or human embryonic kidney 293T cells, indicating a possible crosstalk between the Slit2/Robo1 pathway and other signaling pathways, which regulates PI3K/Akt and β-catenin in gliomas. It still needs to be further confirmed whether a relationship exists between an active chemorepulsive effect of Slit2 and Cdc42-GTP activity. Therefore, an expanding understanding of the molecular mechanisms of glioma cell migration and invasion may help find new approaches to treat the remanent glioma cells after surgical resection. The suppressive action of Slit2/Robo1 may provide new insights into the design of novel mechanism-based therapies for glioma and shed light on the important biological aspect of glioma progression. As the molecular mechanisms underlying the regulation of glioma invasion and migration by Slit2/Robo1 have been largely elucidated, the signal system has potentials to become a new target for glioma prevention and treatment.

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Slit2/Robo1 信号通路在神经胶质瘤侵袭与迁移中的作用

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摘要：Slit2/Robo1 信号通路是一个进化保守的配体受体系统。该信号通路对神经细胞的分布、迁移、轴突导向起着重要作用。Slit2及其跨膜受体Robo1蛋白在胶质瘤中的分布是不同的。Slit2在毛细胞性星形细胞瘤及胶质母细胞瘤中是低表达的，而Robo1在各级别的胶质瘤中均有高表达。恶性胶质瘤细胞的浸润侵袭机制包括多条信号通路的多种基因的改变。虽然Slit2/Robo1信号通路抑制肿瘤的分子机制尚不清楚，但已有研究报道其抑制胶质瘤细胞侵袭的作用是通过抑制Cdc42-GTP的活性来实现的。本文主要就Slit2/Robo1信号通路在胶质瘤中的作用进行详尽探讨。伴随Slit2/Robo1信号通路分子机制研究的不断深入，将会为有效治疗恶性胶质瘤提供新的策略和思路。
关键词：Slit2/Robo1 信号通路；神经胶质瘤；侵袭；迁移