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Review/Praca poglądowa

The role of SLIT–ROBO pathway in crucial cell processes during physiological and pathological conditions

Znaczenie szlaku SLIT-ROBO w kluczowych dla komórki procesach w warunkach fizjologii i patologii

Aleksandra Gołos, Agnieszka Wierzbowska *

Katedra i Klinika Hematologii UM w Łodzi, Kierownik: prof. dr hab. n. med. Tadeusz Robak, Łódź, Poland

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ABSTRACT

The SLIT glycoproteins and their roundabout (ROBO) receptors were originally identified as axon guidance molecules that prevent axons from re-crossing the midline. In addition, the SLIT–ROBO interaction is involved in the regulation of cell migration, cell death and angiogenesis. Furthermore, it has a pivotal role during the morphogenesis, controlling the correct development of lung, kidney, liver and breast. The functions which the SLIT–ROBO fulfills during tissue morphogenesis are often dysregulated during cancer development. Therefore inactivation of certain SLITs and ROBOs is associated with advanced tumor formation and progression in disparate tissues. However, some studies revealed that SLIT–ROBO may promote tumor angiogenesis and, consequently, its growth. This review is focused on summarizing the current knowledge about the role of SLIT–ROBO pathway in development of disparate tissues in physiological conditions and in pathogenesis of neoplasms, including hematological malignancies.

Introduction

SLIT and their ROBO receptors are members of the axon guidance molecules family that have been identified to play a crucial role in development of nervous system of vertebrates and invertebrates (Table 1). They function as a repulsive cue with an evolutionarily conserved role in preventing axons from migrating to inappropriate locations. As the vascular and axon network shows many similarities in their structure (reviewed by Carmeliet and Tessier-Lavigne, 2005) [1], researchers started to investigate whether there exist similar signaling pathways, too. As a result, in last 20 years, the axon guidance cues have widely been

* *Corresponding author at*: Klinika Hematologii UM, ul. Ciołkowskiego 2, 93-510 Łódź, Poland. Tel.: +48 426 8951 91; fax: +48 426 8951 92.
E-mail address: agawierzbowska@wp.pl (A. Wierzbowska).

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Table I – Neuronal guidance molecules and their receptors
Tabela I – Neuronalne czynniki przewodzenia i ich receptory

Ligand	Receptor
Semaphorins: Class 3/class 4–7	Neuropilins Integrins Plexin – B/C/D
Ephrins A/B	EphA/B Receptors
Netrin 1/2/4	DCC, UNC5A-D
Delta-like (Dll-1/3/4)	Notch 1/2/3/4
Serrate-like (JAG1, JAG2)	Notch 1/2/3/4
Slit 1/2/3/	Robo 1/2/3/4

studied outside the nervous system. Their wide network of downsignalling, influencing apoptosis, cell cycle, cell migration determines its multiple roles in organogenesis and tumorigenesis. There is evidence that SLIT–ROBO signaling may take part in the pathogenesis of hematological malignancies. The aim of this article is to present the current knowledge about the role of SLIT–ROBO pathway in development of disparate tissues in both, physiological and pathological conditions.

SLIT–ROBO structure and signaling

SLIT is a secreted protein that was first described as a ligand for ROBO in 1999, by Brose et al. [2]. Invertebrates have single slit protein whereas vertebrates have three of them named slit1, slit2, and slit3. All slits remain evolutionarily conserved. It concerns their structure as well as function [2]. The slit is a glycoprotein of about 200 kDa that consists of an N-terminal signal peptide, four leucine-rich domains (LRR) termed D1–D4 followed by seven to nine endothelial growth factor-like domains (EGF-like), a laminin-G domain, and a C-terminal cystein knot (Fig. 1) [3]. Structural studies have shown that the LRR domains each contain a motif that creates a concave shape that might be important for Slit interaction with their receptors. Slits are proteolytically cleaved between two EGF-like domains [2].

As well as slit, robo was first discovered in *Drosophila melanogaster* [4]. There had been three homolog proteins described, robo1, robo2, and robo3. Subsequently, another protein, robo4 (also termed as magic roundabout), was identified in vertebrates and was initially thought to be endothelial specific [5]. Robo1, robo2 and robo3 share the same extracellular domain structure. This region contains five immunoglobulins-like (Ig) domains and three fibronectin type 3 (FN3) repeats [4]. The intracellular part of robo1–robo3 is comprised of four conserved cytoplasmic motifs (CC0, CC1, CC2, CC3). ROBO 3 lacks of CC1 domain [4]. Robo4, which shows the lowest homology with other Robos, contains only two Ig domains, a FN3 and CC2 domain [5] (Fig. 1). The D2 domain of the slit and Ig2 domain of robo are crucial for ligand–receptor interaction [6]. The slit–robo interaction leads to the actin cytoskeleton reorganization in the target cells. Slit binding to robo is involved in a great amount of cell functions, mainly concerning cytoskeletal dynamics and cell cycle (Fig. 2) [3]. To direct the motility of cells, slit–robo requires several adaptor proteins which conduct the signal to the effector proteins that subsequently can change the structure of cytoskeleton. The Rac family of small GTPases (srGAP – Slit Robo Rho GTPase activating protein) are crucial for the downstream signaling of SLIT–ROBO [7]. The most established function of slit–robo is its pro- or anti-migratory activity. Slit–robo can stimulate the interaction between E-cadherin and β-catenin and so promotes cell adhesion [8]. This was observed during mammary gland development [9]. Nevertheless, during the heart morphogenesis, slit–robo inhibits the E-cadherin/β-catenin-mediated cell to cell adhesion. This allows to form the heart lumen [10]. As well as migration and adhesion, slit–robo can regulate other processes involved in cell growth. Through direct blocking of cdc42 (cell division control protein 42) activity, the cell cycle is blocked [11]. In addition, slit and robo control these functions independently. Slit induces apoptosis through binding to netrin-1, which disables its interaction with DCC (deleted in colorectal cancer). Consequently, caspases 3 and 9 can be activated by DCC. What is more, robo binds to DCC too and in turn causes dissociation of Netrin-1 from DCC and activation of caspases [12]. Slit inhibits cell cycle by blocking SDF-1, WNT and HGF signaling [8]. Through the multiple pathways, SLIT–ROBO is involved in morphogenesis, angiogenesis and tumorigenesis.

The role of SLIT–ROBO in CNS development

Robo receptors and their ligands Slit form one of the most crucial pairings among the axon guidance molecules. *robo* gene was identified in *Drosophila* as one that controls the midline crossing of commissural axons [4]. Similarly, slit was described as a protein secreted by midline glia cells [13]. The migrating axons are chemotactically attracted to the midline. After crossing it, is started to be expressed on their surface. Consequently, the high concentration of slit in midline glia cells and the expression of robo prevent recrossing the line and so the axons find their way to the destination place [14]. In *robo*$^{-}$/*robo*$^{-}$ *Drosophila* embryos, commissural axons crossed the midline multiple times [14]. Furthermore, slit–robo functions in many developmental processes in CNS outside the midline. These include: the formation of the olfactory tract [15], optic tract and optic chiasm [16], and motor axon path finding in the hindbrain [17].

The role of SLIT–ROBO in organogenesis

In addition to axon guidance function at the CNS, slits and robos are also implicated in other developmental processes. First data about the importance of Slit and Robo in organogenesis come from experiments on mutant mice [18]. Mice with deletion of *Robo1* died shortly after its birth because of respiratory failure. Further investigations showed that these mice had severe lung defects, such as abnormal and torturous bronchiole [18]. Grieshammer et al. described kidney abnormalities in *Slit2* and *Robo2* mutant mice which consequently led them to quick death [19]. Similar findings were made in *Slit3* mutant mice. Besides kidney defects,

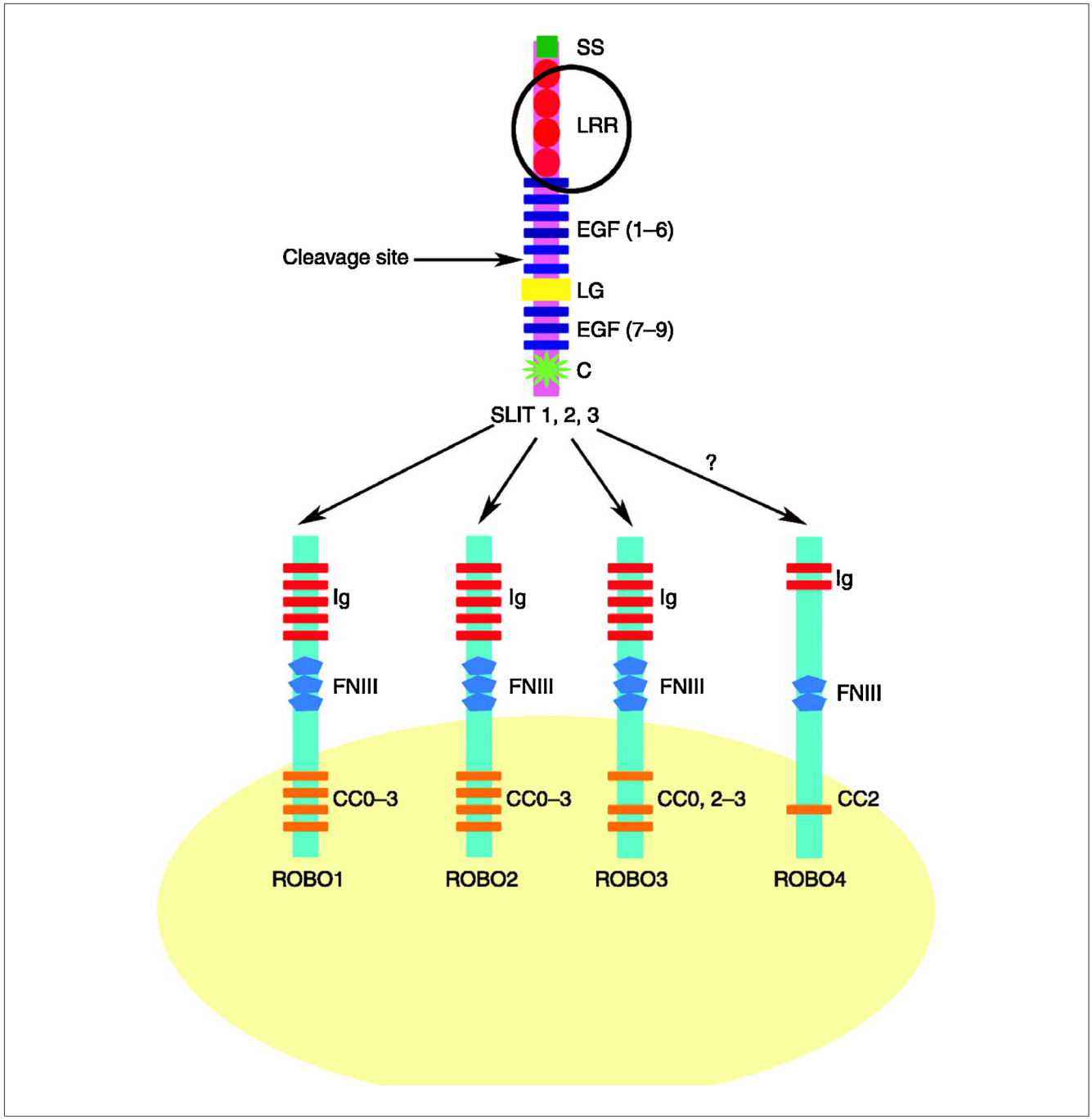

Fig. 1 – Slit and Robo structure, [3]
Ryc. 1 – Budowa Slit i Robo, [3]

increased rate of diaphragmal hernia and enlarged right ventricle of heart were observed [20]. The slit–robo interaction seems to play an important role in the development of the heart. In *Drosophila*, slit in cooperation with robo1 and robo2 directs cardioblasts migration, and through inhibition of E-cadherin, controls their adhesion. In turn, the lumen of the heart originates [10]. Mounting evidence confirms the great significance of slit–robo in reproductive system. While studying mammary gland development, Slit–Robo was considered as an adhesion molecule [21]. Dickinson et al. showed that SLIT2, SLIT3, ROBO1, ROBO2, and ROBO4 are expressed in corpus luteum (CL) in human ovary [22]. Interestingly, the concentration rate of the cues was rising with the duration time of luteal phase, reaching its top in the late phase, when CL is starting to regress [22]. Conversely, however, when hCG (human chorionic gonadotropin) is released in order to protect CL from luteolysis, the concentration of SLIT2, SLIT3, and ROBO2 decreases rapidly [22]. These data suggest that SLITs and ROBOs promote luteolysis and that their expression may be regulated

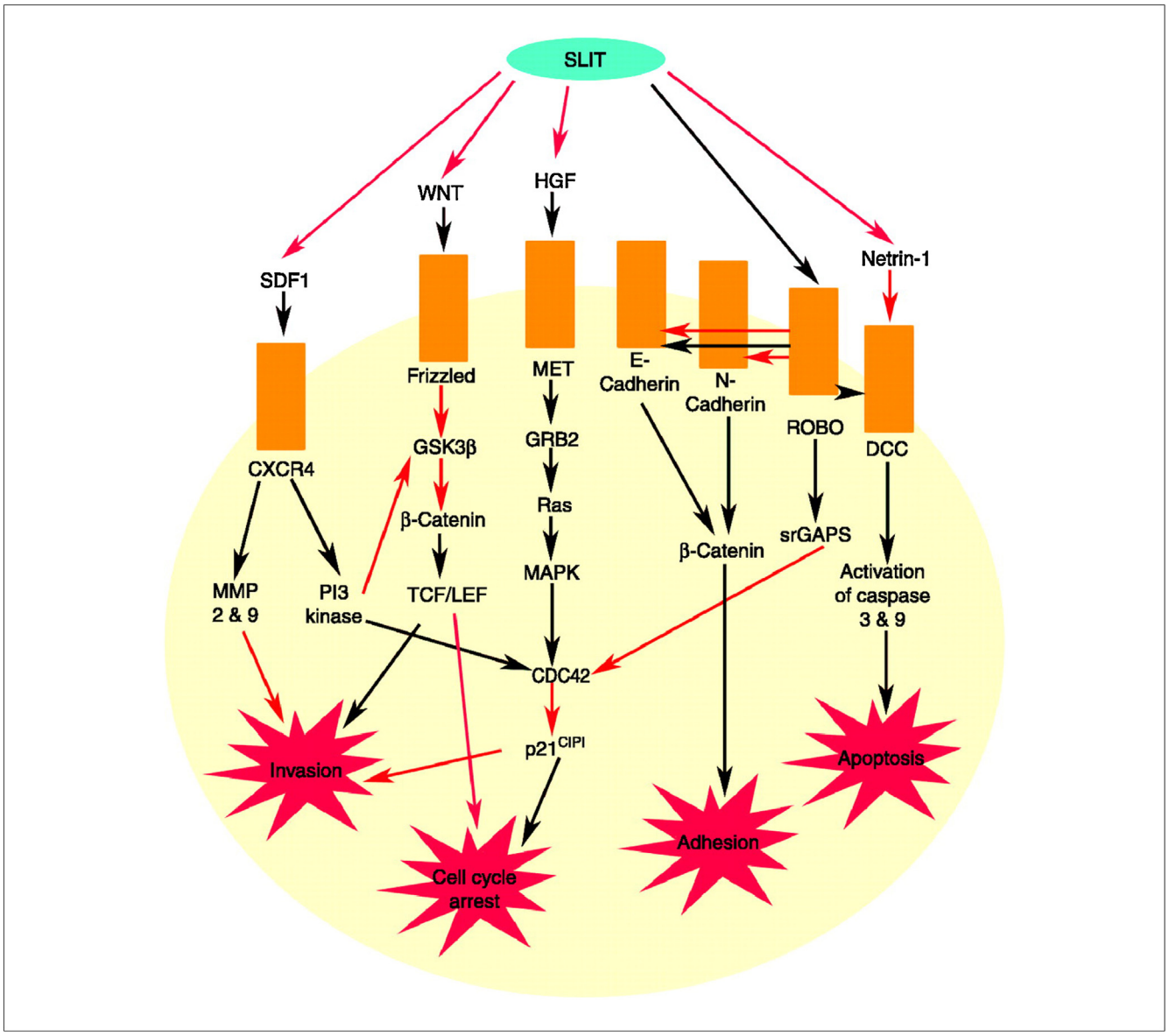

Fig. 2 – SLIT–ROBO function, [3]
Ryc. 2 – Szlaki sygnałowe SLIT-ROBO, [3]

hormonally. SLITs and ROBOs not only take part in physiological development, but their activity is also known in many pathologic circumstances. Expression of SLIT2 correlated with increased microvascular density (MVD) in endometriosis and was higher in recurrent endometriomas in comparison to non-recurrent [23]. Overall, during organogenesis, the SLIT–ROBO pathway controls numerous processes that seem to be vital in the development of different tissues.

The SLIT–ROBO interaction in angiogenesis

Blood vessels often go alongside the nerves in the body and the vascular and neural networks look similar in its structure. Indeed, there are several parallels in the development of both systems (reviewed by Carmeliet and Tessier-Lavigne [1]). One of the common factors is involving in vasculogenesis all the members of the axon guidance molecules including Slit and Robo. Robo4 was the first to be associated with angiogenesis. It was discovered by data mining for searching for new endothelial specific genes [24]. Expression of Robo4 was detected in mouse placental blood vessels [24], heart, liver, kidney, and lung [25]. However, there was no expression found in the brain, skeletal muscle, spleen, and testis [25]. Suchting et al. reported an inhibitory effect of soluble chimeric receptor Robo4 on angiogenesis *in vitro* and *in vivo* [26]. They also suggested that Robo4 does not bind to any of the Slits [26]. However, the theory was not confirmed in any of the subsequent studies.

Slit2, by activating its receptor Robo4, was reported as an inhibitor factor for vascular-endothelial growth factor (VEGF)-induced migration of mouse endothelial lung cells

[27]. Moreover, SLIT2 inhibited migration of endothelial cells of several cell lines, such as human umbilical cord vascular endothelial cell (HUVEC) [28], or human microvascular endothelial cell (HMVEC) [29]. Besides this, SLIT2 decreased migration of human aortic smooth muscle cells [30]. In another study, HEK (human embryonic kidney) cells were initially transfected with ROBO4, then placed in SLIT2-conditioned medium. This results in inhibition of migration of the HEK cells [25]. Similar effects were observed with HMVEC [25]. These results implicate SLIT as an inhibitory angiogenic factor. Nonetheless, some experiments showed discordant conclusions. HUVEC cell lines which were treated with medium containing SLIT2 presented directional migration [31]. The effect was blocked by treating the cells with an antibody against ROBO1 [31]. In addition, human malignant melanoma cells (A375), which normally induce angiogenesis, produced fewer vessels when treated with an anti-ROBO antibody [31]. Taken together, SLIT–ROBO seems to be involved in endothelial cell migration but the discordant results in studies confirm the complexity of the process. Therefore, studies on the downstream signaling of the SLIT–ROBO will be crucial to understand the differences.

SLIT–ROBO in tumor formation

The cellular functions that are controlled by the SLIT–ROBO pathway during tissue morphogenesis are dysregulated during cancer development. Deletions or epigenetic modification of the *slit–robo* genes has recently been described in many tumors. There is mounting evidence indicating that *SLIT 1, 2, 3, ROBO1*, and *2* are candidate genes for tumor suppressor genes. The hypermethylation of these genes was described in numerous tumor types including cervical cancer, breast, non-small cell lung and ovarian [32–35]. What is more, Singh et al. suggested poorer prognosis for patients with cervical cancer patients in which the deletion of *SLIT2* locus was confirmed [32]. A decreased expression of *SLIT1, 2, 3*, and *ROBO 1* was reported in glioma, kidney, breast, and lung carcinoma [36]. In addition, another study revealed lower expression of *SLIT2, 3, ROBO1, 2* and 4 in epithelial cell ovarian carcinoma [37]. Although the findings had showed no clear correlation with tumor clinical stage, subsequent studies on many cancer cell lines revealed that the reexpression of *SLIT2* greatly inhibits the proliferation of cancer cells, which suggests the role of Slit in tumor progression [36]. The investigation of germinal cell ovarian carcinoma showed deletion of locus for *SLIT3* [38]. In one study, conducted by Xian et al., a targeted mutation of *Robo1* gene was generated in mice. The majority of *Robo1* heterozygotes developed neoplasms, including carcinomas and lymphomas [39]. Interestingly, however, these candidate genes showed no clear trend in other tumors, being upregulated in prostate carcinoma [40] and in many tumor cell lines, such as melanoma, bladder squamous carcinoma, neuroblastoma, small cell lung carcinoma [31]. Expression of SLIT3 correlated with areas of increased microvessel density in tumors and SLIT2-ROBO1 induced proangiogenic pathways [31]. The activity was blocked by an antibody to the ectodomain of ROBO–ROBO N. The ambiguous results were obtained in some independent studies concerning SLIT–ROBO activity in metastasis, too. Prasad et al. provided an evidence of anti-metastatic activity of SLIT2 in breast cancer and melanoma cells [41]. They suggested a model in which SLIT2 inhibited the SDF-1 induced chemotaxis of T-cells (for details, see Fig. 2) [41]. On the contrary, findings of Schmid et al. revealed SLIT2 as a chemoattractant for breast cancer cells that promoted brain metastases [42]. Furthermore, the expression of SLIT2 correlated with the clinical stage of endometrium carcinoma, being significantly higher in recurrent phase [43]. On the contrary, in mice bearing breast carcinoma, the injection of exogenic Slit2 reduced the tumor size by over a half [8]. SLIT–ROBO may induce apoptosis of cells and the pathway is also involved in cancer biology. In SLIT2-transfected fibrosarcomas and squamosous cell carcinoma there was a higher number of apoptotic cells and a lower rate of proliferation [44]. Additionally, SLIT–ROBO induced programmed cell death through caspase-3 activation in ovarian tumor [37]. Overall, these findings indicate that the SLIT–ROBO pathway mainly suppresses tumor formation and growth by regulating processes including invasion, migration, proliferation, and apoptosis. However, more experiments are needed to explain the differences between pro- and anti-cancerous activity of SLIT–ROBO.

SLIT–ROBO in hematological malignancies

Although the SLIT–ROBO role in solid tumors have recently been examined extensively, the studies dedicated to hematological malignancies have started few years ago and are represented poorly. The background to research of ROBO4 expression in leukemias noticed Smith-Berdan et al. in 2011 [45]. They demonstrated the expression of the protein in hematopoietic stem cells (HSC) and described its role in hematopoietic niche reaching by HSC. Leukemia is believed to derive from leukemia stem cell (LSC) and there exists evidence that the regulation of LSC is similar to the HSC. Up to date, only two studies concerning the role of SLIT–ROBO in leukemias were conducted. The first determined the methylation status of *SLIT2* gene in 64 blood marrow samples of pediatric acute lymphoblastic leukemia (ALL), 30 blood samples of adult chronic lymphocytic leukemia (CLL), both of them obtained at the time of diagnosis, and in ten leukemia cell lines. The study also revealed the methylation status of the genes after treatment with a demethylating agent. All of leukemia cell lines had completely methylated *SLIT2*. Additionally, in pediatric ALL, 83% of T-ALL and 58% of B-ALL had methylated *SLIT2*. However, the mutational status of *SLIT2* had no impact on clinical data in ALL. Interestingly, *SLIT2* expression was restored after treating ALL lines with 5-aza-2dC (5-aza-2-deoxycytidine, decitabine). 80% of CLL samples demonstrated *SLIT2*. However, no correlation was found between the methylation status and IGVH or TP53 mutational status. Besides this, there was no association between *SLIT2* methylation and CLL progression [46]. In another study, Wellbrock et al. determined mRNA expression status of *ROBO1*, 4, and *SLIT2* genes in quantitative polymerase chain reaction (PCR) analysis in 104 patients with newly diagnosed acute myeloid leukemia (AML). The

study excluded t(15;17) samples. They found that *ROBO4* mRNA was expressed in 83% patients. *ROBO1* was detected in 27% and *SLIT2* in 18% of AML patients. The findings revealed a negative correlation of high ROBO4 expression with overall survival (OS) and event-free survival (EFS). What is more, the complete remission (CR) rate was 54% for the high ROBO4 expression group compared with 71% in the low ROBO4 expression group [47]. Interestingly, for ROBO1 and SLIT2 no significant impact on survival was observed. To conclude, these findings are promising for searching for new prognostic factors and therapeutic aims of leukemias, particularly in AML. Nevertheless, it requires more research to confirm the role of SLIT–ROBO in leukemogenesis and to determine any association with patient survival and other pathological features.

Summary

Despite the considerable body of knowledge of the role of SLIT–ROBO in axon guidance, neuronal migration and axon branching, the information on the effects of SLIT–ROBO on normal and neoplastic cells is still fragmentary. It has been suggested that the pathway seems to regulate proliferation, apoptosis, adhesion and angiogenesis. Recent studies implied its key role during organ development, tumorigenesis and physiology. Up to date, deletions or epigenetic modification of the *SLIT–ROBO* genes has been described in many tumors which might suggest their role as a tumor suppressor gene. However, some contradictory statements about its role in metastasis show that the SLIT–ROBO pathway is extremely important but highly complex. Therefore, new studies are needed to give more details about its activity and determine its role in tumor biology.

Authors' contributions/Wkład autorów

AG – study design, manuscript preparation, literature search. AW – manuscript preparation.

Conflict of interest/Konflikt interesu

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Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

REFERENCES/PIŚMIENNICTWO

[1] Carmeliet P, Tessier-Lavigne M. Common mechanisms of nerve and blood vessel wiring. Nature 2005;436:193–200.
[2] Brose K, Bland KS, Wang KH, et al. Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell 1999;96:795–806.
[3] Dickinson RE, Duncan WC. The SLIT–ROBO pathway: a regulator of cell function with implications for the reproductive system. Reproduction 2010;139:697–704.
[4] Seeger M, Tear G, Ferres-Marco D, Goodman CS. Mutations affecting growth cone guidance in Drosophila: genes necessary for guidance toward or away from the midline. Neuron 1993;10:409–426.
[5] Huminiecki L, Gorn M, Suchting S, Poulsom R, Bicknell R. Magic roundabout is a new member of the roundabout receptor family that is endothelial specific and expressed at sites of active angiogenesis. Genomics 2002;79:547–552.
[6] Nguyen Ba-Charvet KT, Brose K, Ma L, et al. Diversity and specificity of actions of Slit2 proteolytic fragments in axon guidance. J Neurosci 2001;21:4281–4289.
[7] Huber AB, Kolodkin AL, Ginty DD, Cloutier JF. Signaling at the growth cone: ligand–receptor complexes and the control of axon growth and guidance. Annu Rev Neurosci 2003;26:509–563.
[8] Prasad A, Paruchuri V, Preet A, Latif F, Ganju RK. Slit-2 induces a tumor-suppressive effect by regulating beta-catenin in breast cancer cells. J Biol Chem 2008;283:26624–26633.
[9] Stella MC, Trusolino L, Comoglio PM. The Slit/Robo system suppresses hepatocyte growth factor-dependent invasion and morphogenesis. Mol Biol Cell 2009;20:642–657.
[10] Santiago-Martinez E, Soplop NH, Kramer SG. Lateral positioning at the dorsal midline: Slit and Roundabout receptors guide Drosophila heart cell migration. Proc Natl Acad Sci U S A 2006;103:12441–12446.
[11] Wong K, Ren XR, Huang YZ, et al. Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit–Robo pathway. Cell 2001;107:209–221.
[12] Chedotal A, Kerjan G, Moreau-Fauvarque C. The brain within the tumor: new roles for axon guidance molecules in cancers. Cell Death Differ 2005;12:1044–1056.
[13] Rothberg JM, Jacobs JR, Goodman CS, Artavanis-Tsakonas S. Slit: an extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. Genes Dev 1990;4:2169–2187.
[14] Kidd T, Brose K, Mitchell KJ, et al. Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. Cell 1998;92:205–215.
[15] Nguyen-Ba-Charvet KT, Plump AS, Tessier-Lavigne M, Chedotal A. Slit1 and slit2 proteins control the development of the lateral olfactory tract. J Neurosci 2002;22:5473–5480.
[16] Plump AS, Erskine L, Sabatier C, Brose K, Epstein CJ, Goodman CS, et al. Slit1 and Slit2 cooperate to prevent premature midline crossing of retinal axons in the mouse visual system. Neuron 2002;33:219–232.
[17] Hammond R, Vivancos V, Naeem A, et al. Slit-mediated repulsion is a key regulator of motor axon pathfinding in the hindbrain. Development 2005;132:4483–4495.
[18] Xian J, Clark KJ, Fordham R, et al. Inadequate lung development and bronchial hyperplasia in mice with a targeted deletion in the Dutt1/Robo1 gene. Proc Natl Acad Sci U S A 2001;98:15062–15066.
[19] Grieshammer U, Le M, Plump AS, et al. SLIT2-mediated ROBO2 signaling restricts kidney induction to a single site. Dev Cell 2004;6:709–717.

[20] Liu J, Zhang L, Wang D, et al. Congenital diaphragmatic hernia, kidney agenesis and cardiac defects associated with Slit3-deficiency in mice. Mech Dev 2003;120:1059–1070.
[21] Strickland P, Shin GC, Plump A, Tessier-Lavigne M, Hinck L. Slit2 and netrin 1 act synergistically as adhesive cues to generate tubular bi-layers during ductal morphogenesis. Development 2006;133:823–832.
[22] Dickinson RE, Myers M, Duncan WC. Novel regulated expression of the SLIT/ROBO pathway in the ovary: possible role during luteolysis in women. Endocrinology 2008;149:5024–5034.
[23] Shen F, Liu X, Geng JG, Guo SW. Increased immunoreactivity to SLIT/ROBO1 in ovarian endometriomas: a likely constituent biomarker for recurrence. Am J Pathol 2009;175:479–488.
[24] Huminiecki L, Bicknell R. In silico cloning of novel endothelial-specific genes. Genome Res 2000;10: 1796–1806.
[25] Park KW, Morrison CM, Sorensen LK, et al. Robo4 is a vascular-specific receptor that inhibits endothelial migration. Dev Biol 2003;261:251–267.
[26] Suchting S, Heal P, Tahtis K, et al. Soluble Robo4 receptor inhibits in vivo angiogenesis and endothelial cell migration. FASEB J 2005;19:121–123.
[27] Jones CA, London NR, Chen H, et al. Robo4 stabilizes the vascular network by inhibiting pathologic angiogenesis and endothelial hyperpermeability. Nat Med 2008;14:448–453.
[28] Liu D, Hou J, Hu X, et al. Neuronal chemorepellent Slit2 inhibits vascular smooth muscle cell migration by suppressing small GTPase Rac1 activation. Circ Res 2006;98:480–489.
[29] Kaur S, Samant GV, Pramanik K, et al. Silencing of directional migration in roundabout4 knockdown endothelial cells. BMC Cell Biol 2008;9:61.
[30] Seth P, Lin Y, Hanai J, et al. Magic roundabout, a tumor endothelial marker: expression and signaling. Biochem Biophys Res Commun 2005;332:533–541.
[31] Wang B, Xiao Y, Ding BB, et al. Induction of tumor angiogenesis by Slit–Robo signaling and inhibition of cancer growth by blocking Robo activity. Cancer Cell 2003;4:19–29.
[32] Singh RK, Indra D, Mitra S, et al. Deletions in chromosome 4 differentially associated with the development of cervical cancer: evidence of slit2 as a candidate tumor suppressor gene. Hum Genet 2007;122:71–81.
[33] Sharma G, Mirza S, Prasad CP, et al. Promoter hypermethylation of p16INK4A, p14ARF, CyclinD2 and Slit2 in serum and tumor DNA from breast cancer patients. Life Sci 2007;80:1873–1881.
[34] Gorn M, Anige M, Burkholder I, et al. Serum levels of Magic Roundabout protein in patients with advanced non-small cell lung cancer (NSCLC). Lung Cancer 2005;49:71–76.
[35] Dai CF, Jiang YZ, Li Y, et al. Expression and roles of Slit/Robo in human ovarian cancer. Histochem Cell Biol 2011;135: 475–485.
[36] Dallol A, Da Silva NF, Viacava P, et al. SLIT2, a human homologue of the Drosophila Slit2 gene, has tumor suppressor activity and is frequently inactivated in lung and breast cancers. Cancer Res 2002;62:5874–5880.
[37] Dickinson RE, Fegan KS, Ren X, et al. Glucocorticoid regulation of SLIT/ROBO tumour suppressor genes in the ovarian surface epithelium and ovarian cancer cells. PLoS ONE 2011;6:e27792.
[38] Faulkner SW, Friedlander ML. Molecular genetic analysis of malignant ovarian germ cell tumors. Gynecol Oncol 2000;77:283–288.
[39] Xian J, Aitchison A, Bobrow L, et al. Targeted disruption of the 3p12 gene, Dutt1/Robo1, predisposes mice to lung adenocarcinomas and lymphomas with methylation of the gene promoter. Cancer Res 2004;64:6432–6437.
[40] Latil A, Chene L, Cochant-Priollet B, et al. Quantification of expression of netrins, slits and their receptors in human prostate tumors. Int J Cancer 2003;103:306–315.
[41] Prasad A, Qamri Z, Wu J, Ganju RK. Slit-2/Robo-1 modulates the CXCL12/CXCR4-induced chemotaxis of T cells. J Leukoc Biol 2007;82:465–476.
[42] Schmid BC, Rezniczek GA, Fabjani G, et al. The neuronal guidance cue Slit2 induces targeted migration and may play a role in brain metastasis of breast cancer cells. Breast Cancer Res Treat 2007;106:333–342.
[43] Ma S, Liu X, Geng JG, Guo SW. Increased SLIT immunoreactivity as a biomarker for recurrence in endometrial carcinoma. Am J Obstet Gynecol 2010;202:68.
[44] Kim HK, Zhang H, Li H, et al. Slit2 inhibits growth and metastasis of fibrosarcoma and squamous cell carcinoma. Neoplasia 2008;10:1411–1420.
[45] Smith-Berdan S, Nguyen A, Hassanein D, et al. Robo4 cooperates with CXCR4 to specify hematopoietic stem cell localization to bone marrow niches. Cell Stem Cell 2011;8:72–83.
[46] Dunwell TL, Dickinson RE, Stankovic T, et al. Frequent epigenetic inactivation of the SLIT2 gene in chronic and acute lymphocytic leukemia. Epigenetics 2009;4:265–269.
[47] Wellbrock J, Vettorazzi E, Vohwinkel G, et al. Expression of roundabout receptor 4 (Robo-4) confers a negative prognosis on patients with acute myeloid leukemia. ASH Annu Meet Abstr 2012;120:1412.