Slit2 suppresses endothelial cell proliferation and migration by inhibiting the VEGF-Notch signaling pathway

GUO-JIAN LI¹, YONG YANG¹, GUO-KAI YANG¹, JIA WAN¹, DAO-LEI CUI¹, ZHEN-HUAN MA¹, LING-JUAN DU¹ and GUI-MIN ZHANG²

¹Department of Vascular Surgery, The Fourth Affiliated Hospital, Kunming Medical University, Kunming, Yunnan 650021; ²Department of Cardiovascular Surgery, The First Affiliated Hospital, Kunming Medical University, Kunming, Yunnan 650032, P.R. China

Received November 24, 2015; Accepted November 10, 2016

DOI: 10.3892/mmr.2017.6240

Abstract. Slit homolog 2 (Slit2) is distributed in various tissues and participates in numerous cellular processes; however, the role of Slit2 in the regulation of angiogenesis remains controversial, since it has previously been reported to exert proangiogenic and antiangiogenic activities. The present study aimed to investigate the effects of Slit2 on vascular endothelial cell proliferation and migration in vitro, and to reveal the possible underlying signaling pathway. Aortic endothelial cells were isolated from Sprague Dawley rats and cultured. Cell proliferation assay, cell migration assay, immunocytochemistry and small interfering RNA transfection were subsequently performed. The results demonstrated that exogenous Slit2 administration markedly suppressed TNF-α-induced endothelial cell proliferation and migration in vitro. In addition, TNF-α application upregulated the protein expression levels of vascular endothelial growth factor (VEGF) and Notch in RAECs, whereas Slit2 administration downregulated VEGF and Notch expression in RAECs cultured in TNF-α conditioned medium. Further studies indicated that knockdown of VEGF suppressed the effects of TNF-α on the induction of RAEC proliferation and migration. VEGF knockdown-induced inhibition of RAEC proliferation and migration in TNF-α conditioned medium was also achieved without Slit2 administration. Furthermore, VEGF knockdown markedly decreased Notch1 and Notch2 expression. These results indicated that Slit2 suppresses TNF-α-induced vascular endothelial cell proliferation and migration in vitro by inhibiting the VEGF-Notch signaling pathway. Therefore, Slit2 may inhibit the proliferation and migration of endothelial cells during vascular development.

Introduction

Slit homolog 2 (Slit2) was initially identified in the development of the central nervous system (1,2). Further studies reported that Slit2 was also distributed in the kidney, liver, lung, spleen, embryo and bone marrow (3-5). Slit2 has also been detected in cardiomyocytes and endothelial cells from arterioles and venules (3). Previous studies have indicated that Slit2 and its receptor Roundabout (Robo) participate in various cellular processes, including cell proliferation, migration and adhesion (6-9). Studies regarding the Slit gene family have reported that secreted Slit2 proteins are able to guide neuronal migration (10,11). Due to embryonic Slit2 expression it has been hypothesized that Slit2 has potential roles in other systems, including the cardiovascular system. Secreted Slit2 interacts with Robo on the surface of vascular smooth muscle cells and monocytes, in order to inhibit migration of these cells toward diverse inflammatory chemoattractant cues in vitro and in vivo (12-14). Administration of Slit2 to atherosclerosis-prone low-density lipoprotein (LDL) receptor-deficient mice was able to inhibited monocyte recruitment to nascent atherosclerotic lesions, which supports a role for Slit2 in preventing early vascular inflammation (15). It is well known that endothelial dysfunction is a key step in the initiation of cardiovascular diseases, and endothelial cell proliferation, migration and tube formation are critical for neovascularization and angiogenesis. Angiogenesis has important roles in various physiological events, including embryonic development, tissue regeneration and wound healing, as well as in pathological processes, such as atherosclerotic plaque progression and tumor growth (16). Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is the best-characterized proangiogenic factor that acts as an upstream signal of Notch, is a key regulator of physiological angiogenesis and neovascularization (17-23). The Notch pathway is a highly conserved cell regulatory signaling system, which is associated with cell proliferation and migration. Therefore, the present study aimed to investigate the regulatory effects of Slit2 on endothelial cell proliferation.
and migration in vitro, and to reveal the potential role of VEGF-Notch signaling in this process.

Materials and methods

Ethics. All procedures were conducted according to a protocol approved by the animal care and use committee of Kunming Medical University (Kunming, China). Animals were maintained and received care at the Laboratory Animal Care Center of Kunming Medical University.

Cell isolation and culture. Aortic endothelial cells were isolated from Sprague Dawley rats, which were purchased from Silaike Experimental Animal Corporation (Shanghai, China). The 20 Sprague Dawley rats (age, 8 weeks, body weight, 260-280 g) were housed in a standard animal room under a 12-h light/dark cycle, and were allowed ad libitum access to food and water. The temperature and humidity of the animal room were maintained at 25°C and 55%, respectively. Briefly, rats were anesthetized with 7.5% chloral hydrate (Aoxin Chemical Product Co., Ltd., Shanghai, China) and received an intraperitoneal injection of 1,250 units heparin (Yezhou BioTechnology, Shanghai, China). Rats were then sacrificed by rapid cervical dislocation and an incision was quickly made in the abdominal skin, in order to expose the aorta, which was perfused with PBS containing heparin and was then resected. The aortas were placed in Dulbecco’s modified Eagle’s medium (DMEM) and 2% collagenase II solution was injected and maintained inside the aorta for 45 min (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The aortas were then washed with DMEM supplemented with 20% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.) and endothelial cells were harvested by centrifugation at 800 x g for 10 min at 4°C. Subsequently, rat aortic endothelial cells (RAECs) were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin in an incubator containing 5% CO₂ at 37°C.

Immunocytochemistry. Briefly, cultured cells were fixed using 95% ethanol for 10 min. Antigen retrieval was performed using citrate buffer (pH 6.0) at 121°C for 2 min. After serial blocking with hydrogen peroxide and normal horse serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), the cells were incubated with a primary monoclonal antibody against Slit2 (1:500; cat. no. ab134166; Abcam, Cambridge, MA, USA) for 16 h at 4°C. The cells were then sequentially incubated with peroxidase-conjugated streptavidin (1:200, cat. no. 35105ES60; Shanghai Yeasen Biotechnology Co., Ltd., Shanghai, China) and were observed under a microscope (Leica AF6000; Leica Microsystems, Wetzlar, Germany).

Cell proliferation assay. RAECs were seeded in 96-well plates at a density of 1,500 cells/well. After being washed twice with serum-free medium, RAECs were incubated in endothelial basal medium, TNF-α conditioned medium (10 ng/ml) or Slit2 conditioned medium (100 ng/ml) (R&D System, Inc., Minneapolis, MN, USA) in a humidified incubator containing 5% CO₂ at 37°C for 48 h. Cell viability rate was assessed using the Cell Counting Kit-8 (CCK-8; ToYongBio, Shanghai, China). Briefly, 10 µl CCK-8 was added to each well and was incubated for 2 h at 37°C in a humidified incubator. Absorbance was measured at 450 nm.

Cell migration assay. RAEC migration was determined using a Transwell system (Corning, Inc., Corning, NY, USA). RAECs (cultured in TNF-α or Slit2 conditioned media) in 96-well plates were trypsinized and suspended with endothelial basal medium at a density of 5x10⁵ cells/ml. To the upper chamber of the Transwell system, 100 µl cell suspension was added. Endothelial basal medium, TNF-α conditioned medium or Slit2 conditioned medium was added to the lower chamber (R&D Systems, Inc.). Cells were incubated for 24 h at 37°C. Non-migrating cells on the top surface of the membrane were removed using cotton swabs. Cells that had migrated to the lower surface of the membrane were fixed with methanol and glacial acetic acid, and were stained with 20% Giemsa solution for 30 min at 37°C. The cells were washed twice with PBS. Stained cells were observed under an inverted microscope.

Small interfering (si)RNA transfection. Silencer VEGFA siRNA (cat. no. AM16708) and non-specific negative control siRNA (cat. no. AM4641) were purchased from Invitrogen; Thermo Fisher Scientific, Inc. All siRNA transfections were performed using Lipofectamine™ 2000 Transfection Reagent, according to the manufacturer’s protocol (cat. no. LMRA001; Invitrogen; Thermo Fisher Scientific, Inc.). Briefly, RAECs were seeded in a 96-well plate at a density of 3x10⁴ cells/well in endothelial basal medium containing 2% charcoal stripped FBS. After 24 h at 37°C, the cells were transfected with 200 nM siRNA, using 0.25 µl Lipofectamine. A total of 16 h post-transfection, transfection reagents were removed, and the cells were treated TNF-α conditioned media (10 ng/ml) and Slit2 conditioned media (100 ng/ml) for 48 h at 37°C, as indicated in each experiment. VEGF knockdown was verified by western blot analysis. The number of viable cells and gene expression were determined at the end of the experiment.

Western blot analysis. Briefly, RAECs (cultured in TNF-α or Slit2 conditioned media, and/or transfected with VEGF or negative control siRNA) in 10 cm culture dish were harvested and total cellular proteins were extracted using lysis buffer (62.5 mmol/l Tris-HCl, pH 6.8; 100 mmol/l dithiothreitol; 2% SDS; 10% glycerol). The protein concentrations were then determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Equal amounts of protein (25 µg/well) were separated by 15% SDS-PAGE and were transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories, Inc.). The blots were blocked with TBS-1% Tween (TBST) containing 5% nonfat dry milk, and then incubated with VEGF (1:400; cat. no. ab53465), Notch1 (1:500; cat. no. ab52627), Notch2 (1:500; cat. no. ab8926) and GAPDH (1:400; cat. no. ab37168) primary antibodies (Abcam) in TBST containing 5% nonfat dry milk overnight at 4°C (Epitomics, Burlingame, CA, USA). Following secondary antibody (1:5,000; cat. no. A0208; Beyotime Institute of Biotechnology, Haimen, China) incubation for 2 h at room temperature, proteins were detected using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare Life Science, Little Chalfont, UK). Bands
were visualized using the ChemiDoc MP Imaging system and were semi-quantified with Quantity One v4.62 software (both Bio-Rad Laboratories, Inc.).

Reverse transcription (RT)-polymerase chain reaction (PCR). RAECs were incubated in endothelial basal medium, TNF-α conditioned medium (10 ng/ml) or Slit2 conditioned medium (100 ng/ml) in a humidified incubator containing 5% CO₂ at 37°C for 48 h. Total cellular RNA was extracted using the TRIZol™ Plus Purification kit (Thermo Fisher Scientific, Inc.), according to the manufacturer’s protocol. cDNA was synthesized at 50°C for 50 min and the reaction was terminated at 85°C for 5 min using SuperScript III First-Strand kit (Invitrogen; Thermo Fisher Scientific, Inc.). PCR was conducted in a total reaction volume of 25 µl, containing 18 µl PCR Master Mix, 5 µl cDNA template and 2 µl primers (TaqMan™ Gene Expression Assay; Thermo Fisher Scientific, Inc.). The PCR cycling conditions were as follows: Initial denaturation at 95°C for 5 min; 35 cycles at 94°C for 45 sec, 59°C for 45 sec and 72°C for 60 sec; and a final extension step at 72°C for 5 min. Subsequently, 5 µl amplification product was separated by 2% agarose gel electrophoresis to detect mRNA expression. Primers were designed, synthesized, purified and purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China) (Table I). GAPDH was used as an endogenous control. The results were analyzed with Quantity One v4.62 software (Bio-Rad Laboratories, Inc.).

VEGF determination. Levels of VEGF in the cell culture media were determined by electrochemiluminescence using an MSD® 96-Well Multi-Array Rat VEGF Assay kit (cat. no. L45RA-1; Meso Scale Diagnostics LLC, Rockville, MD, USA). The assay has no significant cross-reactivity (<0.6%) to basic fibroblast growth factor, placental growth factor or soluble VEGF receptor 1. The interassay and intra-assay coefficients of variation were <12%. The assay was conducted according to manufacturer’s protocol.

Statistical analysis. Data are presented as the mean ± standard deviation. One-way analysis of variance was used to compare the differences among more than three groups. Bonferroni post-hoc test was subsequently used to analyze the differences between two groups. Statistical analysis was performed using SPSS 19.0 statistical software (SPSS IBM, Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

TNF-α stimulates endothelial cell proliferation and migration in vitro. RAECs were treated with various doses of TNF-α for 48 h. As shown in Fig. 1, treatment with TNF-α, at doses ranging between 5 and 15 ng/ml for 48 h, resulted in a dose- and time-dependent induction of RAEC proliferation. Compared with the control group, the number of viable cells was increased by 30, 35 and 42% following treatment with 5, 10 and 15 ng/ml TNF-α for 24 h, respectively (all P<0.05). After 48 h of treatment with 5, 10 and 15 ng/ml TNF-α, the number of viable cells was increased by 46, 60 and 70%, respectively (all P<0.05). A Transwell migration assay was performed to determine the migratory ability of RAECs. As presented in Fig. 2, the number of migrated cells increased in the TNF-α conditioned medium group compared with the endothelial cell medium group (P<0.05).

TNF-α stimulation upregulates VEGF and Notch expression in RAECs. TNF-α administration increased VEGF, Notch1 and Notch2 expression in RAECs. In order to determine the effects of TNF-α on gene expression in RAECs, RT-semi-quantitative PCR was used to determine the mRNA expression levels of VEGF, Notch1 and Notch2. To determine the effects of TNF-α on protein expression in RAECs, western blotting was used to determine VEGF, Notch1 and Notch2 protein expression levels. A total of 48 h after treatment with 10 ng/ml TNF-α, the protein and mRNA expression levels of VEGF, Notch1 and Notch were significantly increased (Fig. 3, all P<0.05). Slit2 expression was not altered following treatment with TNF-α (data not shown).

Slit2 inhibits endothelial TNF-α-induced cell proliferation and migration. To determine whether Slit2 affects TNF-α-induced cell proliferation and migration in RAECs, RAECs were treated with various doses of Slit2 for 1 or 2 days. Treatment with Slit2, at doses ranging between 25 and 100 ng/ml for 24 or 48 h, resulted in a dose- and time-dependent decrease in TNF-α-induced cell proliferation and migration (Fig. 4; all P<0.05). These results indicated that Slit2 may inhibit RAEC proliferation and migration in a concentration-dependent manner.

Slit2 attenuates TNF-α-induced VEGF and Notch overexpression in RAECs. The present study initially indicated that TNF-α increased VEGF, Notch1 and Notch2 expression in RAECs. To determine the role of Slit2 in VEGF, Notch1 and Notch2 expression, various doses of Slit2, between 25 and 100 ng/ml, were added to RAECs for 48 h. A dose-dependent reduction in VEGF, Notch1 and Notch2 expression was detected following Slit2 administration (Fig. 5). The greatest reduction in VEGF, Notch1 and Notch2 expression was observed in the 100 ng/ml Slit2 group, compared with the other two doses (Fig. 5, all P<0.05). These results suggested that Slit2 inhibited the TNF-α-induced increase in VEGF and Notch expression.

### Table I. Polymerase chain reaction primers.

| Gene   | Primer sequence (5’-3’)                  |
|--------|-----------------------------------------|
| VEGF   | F:GAGGGCAGAATCATCACGAA                  |
|        | R:GGCTCAGGCCGATTAAGCA                   |
| Notch1 | F:AGCTACTCTCGCTGTGACAA                  |
|        | R:ACATTAGGTGCGCGACGAGGA                 |
| Notch2 | F:AAAAATGGGGCACAAGCCGAGAC              |
|        | R:TTGATCCAGAGGGCGACACAA                |
| GAPDH  | F:AGCCACATCGCTCACACA                   |
|        | R:TGGACTCCAGCAGTACT                    |

F, forward; R, reverse; VEGF, vascular endothelial growth factor.
Slit2 decreases cell proliferation and migration via the VEGF-Notch pathway. VEGF siRNA transfection was performed to investigate the role of VEGF in TNF-α-induced RAEC proliferation and migration. The results confirmed that VEGF siRNA transfection silenced VEGF expression, and reduced Notch1 and Notch2 expression (Fig. 6A and B). The present study previously indicated that TNF-α increased cell proliferation and migration, and upregulated VEGF expression. Conversely, VEGF knockdown prevented TNF-α-induced cell proliferation and migration (Fig. 6C and D). Therefore, these data indicated that TNF-α increased cell proliferation and migration via upregulating VEGF expression. In addition, Slit2 inhibited TNF-α-induced RAEC proliferation and migration, and reduced TNF-α-induced VEGF and Notch overexpression. These results suggested that Slit2 suppressed TNF-α-induced RAEC proliferation and migration via the VEGF-Notch pathway.

Discussion

The results of the present study confirmed that TNF-α induced RAEC proliferation and migration, and demonstrated that VEGF-Notch signaling mediated TNF-α-induced RAEC proliferation and migration. Conversely, administration of Slit2 inhibited TNF-α-induced endothelial cell proliferation and migration, and the inhibitory effects of Slit2 on endothelial cell proliferation and migration were dependent on the VEGF-Notch signaling pathway.

At present, it has yet to be fully elucidated how vessels choose specific paths to induce angiogenesis. However, the regimented and conserved pattern of the vascular network suggests that specific genetic programs are responsible for its formation. It is well known that vascular endothelial proliferation and migration are required for vascular tube formation, neovascularization and angiogenesis. Slit2 is regarded as a chemorepellent that controls migration of growth cones during central nervous system development (24). Slit2, which is secreted by midline glia, prevents axons from crossing the midline, whereas growth cones that express low levels of Robo1 are allowed to cross (24). Slit2 has previously been reported to not only act as a chemorepellent, but also as a chemoattractant (25). Schmid et al demonstrated that Slit2 could exert functions as a chemokine, in order to promote breast cancer cell migration (26). In retrospective clinical studies, it has been reported that the expression of Slit2 in patients with breast cancer and pancreatic ductal adenocarcinoma was associated with incidence and the extent of lymph node metastasis (27,28).
Qin et al demonstrated that Slit2 was involved in brain metastasis of breast cancer, and low expression of Slit2 was associated with poor prognosis and high morbidity of breast carcinoma (24). The role of Slit proteins in the regulation of angiogenesis remains controversial. Slit2 can either promote or inhibit angiogenesis, depending on the molecular context (12,29-36). The present study provided evidence to suggest that Slit2 may inhibit vascular endothelial cell migration in vitro in a dose-dependent manner, which is consistent with the findings of previous studies (12,33-35,37). Youngblood et al reported that inhibiting Slit activity rescued VEGF-induced angiogenesis in vitro and in vivo, as well as VEGF-dependent tumor angiogenesis in EPH receptor A2 (EphA2)-deficient endothelial cells and animals (38). Furthermore, suppressing Slit activity or Slit2 expression in EphA2-deficient endothelial cells has been revealed to...
restore VEGF-induced activation of Src and Rac, which are required for VEGF-mediated angiogenesis (38).

The present study indicated that VEGF is a major mediator of the inhibitory effects of Slit2 on TNF-α-induced endothelial cell proliferation and migration. The VEGF family is a subfamily of growth factors, which is required for promoting endothelial cell proliferation, initiating angiogenic sprouting and creating vascular structures (39). VEGFs include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-F and placental growth factor; VEGF-A is the most important...
factor in mediating endothelial cell proliferation (39). VEGF receptor 2 is the main receptor that mediates the actions of VEGF-A in endothelial cells, such as endothelial cell proliferation and migration, sprouting activity and the formation of tubule-like structures (40). VEGF regulates endothelial cell activation, proliferation, migration and morphogenesis; however, it does not act in isolation. The present study demonstrated that TNF-α-induced VEGF-A expression was crucial for vascular endothelial cell proliferation and migration. Conversely, Slit2 administration attenuated VEGF-A expression, and endothelial cell proliferation and migration. Furthermore, the knockdown of VEGF-A expression, using a specific VEGF-A siRNA, completely suppressed the proliferation and migration of endothelial cells. In addition, VEGF-A knockdown decreased Notch1 and Notch2 expression in RAECs, which is consistent with the findings of a previous study that revealed that VEGF acts upstream of the Notch pathway to determine arterial and venous endothelial cell fate (19,41). It has previously been demonstrated that the Notch pathway is involved in the regulation of endothelial cell proliferation, migration and vascular development, since the single gene deletion of Notch1 results in severe defects in early arterial development (42-44).

In conclusion, these findings indicated that Slit2, by acting as a suppressor of VEGF-Notch signaling, may inhibit TNF-α-induced endothelial cell proliferation and migration. Although the precise steps regarding how Slit2 governs vascular endothelial proliferation and migration in vivo are not well understood, the present study provided a novel insight into the regulatory mechanism underlying vascular endothelial cell proliferation and migration. These findings may contribute to a novel therapeutic target for the control of vascular endothelial cell proliferation and migration.

Acknowledgements

The authors would like to thank Professor Ming Yang for helping with the language of the present study, and Professor Pengfei Hu for suggestions regarding the cell migration assay. This study was supported by the National Science Foundation of China (31360227) and fund 2014NS047.

References

1. Brose K, Bland KS, Wang KH, Arndt D, Henzel W, Goodman CS, Tessier-Lavigne M and Kidd T: Slit proteins bind robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell 96: 795-806, 1999.
2. Yuan W, Zhou L, Chen JH, Wu JY, Yao Y and Ornitz DM: The mouse slit family: Secreted ligands for ROBO expressed in populations. Cell cycle 11: 675-682, 2012.
3. Wu JY, Feng L, Park HT, Haviroglo N, Wen L, Tang H, Bacon KB, Jiang ZH, Zhang XC and Yao Y: The neuronal repellent slit inhibits leukocyte chemotaxis induced by chemotactic factors. Nature 410: 948-952, 2001.
4. Ma WJ, Zhou Y, Li D, Dong D, Tian XJ, Wen JX and Zhang J: Reduced expression of slit2 in renal cell carcinoma. Med Oncology 31: 768, 2014.
5. Smith-Berdan S, Schepers K, Ly A, Passegué E and Forsberg EC: Dynamic expression of the robo ligand slit2 in bone marrow cell populations. Cell cycle 11: 675-682, 2012.
6. Piper M, Georgas K, Yamada T and Little M: Expression of the vertebrate slit gene family and their putative receptors, the robo genes, in the developing murine kidney. Mech Dev 94: 213-217, 2000.
7. Nones K, Waddell N, Song S, Patch AM, Miller D, Johns A, Wu J, Kassahn KS, Wood D, Bailey P, et al.: Genome-wide DNA methylation patterns in pancreatic ductal adenocarcinoma reveal epigenetic deregulation of SLIT/ROBO, ITGA2 and MET signaling. Int J Cancer 135: 1110-1118, 2014.
8. Álvarez C, Tapia T, Cornejo V, Fernandez W, Muñoz A, Camus M, Alvarez M, Devoto L and Carvallo P: Silencing of tumor suppressor genes RASSF1A, SLIT2 and WI/F1 by promoter hypermethylation in hereditary breast cancer. Mol Cancer Res 52: 475-487, 2013.
9. Qiu H, Zhu J, Yu J, Pu H and Dong R: SLIT2 is epigenetically silenced in ovarian cancers and suppresses growth when activated. Asian Pac J Cancer Prev 12: 791-795, 2011.
10. Wu W, Kong K, Chen J, Jiang Z, Dupuis S, Wu JY and Yao Y: Directional guidance by neuronal notch in the olfactory system by the protein slit. Nature 400: 331-336, 1999.
11. Hu H: Chemorepulsion of neuronal migration by slit2 in the developing mammalian forebrain. Neuron 23: 703-711, 1999.
12. Liu D, Hou J, Hu X, Wang X, Xiao Y, Mou Y and De Leon H: Neuronal chemorepellent slit2 inhibits vascular smooth muscle cell migration by suppressing small GTPase Rac1 activation. Circ Res 98: 480-489, 2006.
13. Prasad A, Qamri Z, Wu J and Ganju RK: Slit-2/robo-1 modulates the cxc12/ccx4-induced chemotaxis of t cells. J Leukoc Biol 82: 474-486, 2007.
14. Tole S, Mukovozov IM, Huang YW, Magalhaes MA, Yan M, Crow MR, Liu JY, Sun CX, Durocher Y, Gologlu M and Robinson LA: The axonal repellent, slit2, inhibits directional migration of circulating neutrophils. J Leukoc Biol 86: 1403-1415, 2009.
15. Mukovozov I, Huang YW, Zhang Q, Liu SY, Gao A, Sokolovsky Y, Patel S, Hydak SJ, Kutryk MJ, Cybulsky MS and Robinson LA: The neurorepellent slit2 inhibits postadhesion stabilization of monocytes tethered to vascular endothelial cells. J Immunol 195: 3334-3344, 2015.
16. Carmeliet P and Jan RK: Molecular mechanisms and clinical applications of angiogenesis. Nature 473: 298-307, 2011.
17. Tie J and Desai J: Antiangiogenic therapies targeting the vascular endothelia growth factor signaling system. Crit Rev Oncog 17: 51-67, 2012.
18. Waldner MJ and Neurath MF: Targeting the VEGF signaling pathway in cancer therapy. Expert Opin Ther Targets 16: 5-13, 2012.
19. Lawson ND, Vogel AM and Weinstein BM: Sonic hedgehog and vascular endothelial growth factor act upstream of the notch pathway during arterial endothelial differentiation. Dev Cell 3: 127-136, 2002.
20. Zheng Y, Hao Z, Ding Y, Wang Q, Li S, Xiao G, Luo H, Shi Q and Tong S: Expression of delta-like 4 (drosophila) and vascular endothelial growth factor a in colon cancer and association with tumour angiogenesis. J Int Med Res 43: 535-543, 2015.
21. Yamaishi Y, Hayashi Y, Suda M, Tateno K, Okada S, Moriya J, Yokoyama M, Nojima A, Yamashita M, Kobayashi Y, et al.: Notch signaling regulates the lifespan of vascular endothelial cells via a p16-dependent pathway. PLoS One 9: e100359, 2014.
22. Chintala H, Krupiska I, Yan L, Lau L, Grant M and Chaqour B: The matricellular protein ccn1 controls retinal angiogenesis by targeting VEGF, src homology 2 domain phosphatase-1 and notch signaling. Development 142: 2364-2374, 2015.
23. Zhang P, Yan X, Chen Y, Yang Z and Han H: Notch signaling in blood vessels: From morphogenesis to homeostasis. Sci China Life Sci 57: 774-780, 2014.
24. Qin F, Zhang H, Ma L, Liu X, Dai K, Li W, Gu F, Fu L and Ma Y: Low expression of slit2 and robo1 is associated with poor prognosis and brain-specific metastasis of breast cancer patients. Sci Rep 5: 14430, 2015.
25. Kramer SG, Kidd T, Simpson JH and Goodman CS: Switching repulsion to attraction: Changing responses to slit during transition in mesoderm migration. Science 292: 737-740, 2001.
26. Schmid BC, Reznicek GA, Fabjani G, Yoneda T, Leodolter S and Zillinger R: The neuronal guidance cue slit2 induces targeted migration and may play a role in brain metastasis of breast cancer cells. Breast Cancer Res Treat 106: 333-342, 2007.
27. Chang PH, Hwang-Sversules WW, Chang YC, Chen CC, Hsiao M, Jeng YM, Chang KJ, Lee EY, Shew JY and Lee WH: Activation of Notch1 signaling of breast cancer cells by slit2 from stromal fibroblast restrains tumorigenesis via blocking p33/akt/b-catenin pathway. Cancer Res 72: 4652-4661, 2012.
28. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529-1540, 2014.

29. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

30. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

31. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

32. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

33. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

34. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

35. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

36. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

37. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

38. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

39. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

40. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

41. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

42. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.

43. Göhrig A, Detjen KM, Hilfenhaus G, Körner JL, Welzel M, Arsenic R, Schmuck R, Bahra M, Wu JY, Wiedenmann B and Fischer C: Axon guidance factor SLIT2 inhibits neural invasion and metastasis in pancreatic cancer. Cancer Res 74: 1529 -1540, 2014.