

The SLIT–ROBO pathway: a regulator of cell function with implications for the reproductive system

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Abstract

The secreted SLIT glycoproteins and their Roundabout (ROBO) receptors were originally identified as important axon guidance molecules. They function as a repulsive cue with an evolutionarily conserved role in preventing axons from migrating to inappropriate locations during the assembly of the nervous system. In addition the SLIT–ROBO interaction is involved in the regulation of cell migration, cell death and angiogenesis and, as such, has a pivotal role during the development of other tissues such as the lung, kidney, liver and breast. The cellular functions that the SLIT/ROBO pathway controls during tissue morphogenesis are processes that are dysregulated during cancer development. Therefore inactivation of certain *SLITs* and *ROBOs* is associated with advanced tumour formation and progression in disparate tissues. Recent research has indicated that the SLIT/ROBO pathway could also have important functions in the reproductive system. The fetal ovary expresses most members of the *SLIT* and *ROBO* families. The *SLITs* and *ROBOs* also appear to be regulated by steroid hormones and regulate physiological cell functions in adult reproductive tissues such as the ovary and endometrium. Furthermore several *SLITs* and *ROBOs* are aberrantly expressed during the development of ovarian, endometrial, cervical and prostate cancer. This review will examine the roles this pathway could have in the development, physiology and pathology of the reproductive system and highlight areas for future research that could further dissect the influence of the SLIT/ROBO pathway in reproduction.

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Introduction

The Roundabout (*robo*) gene encodes a transmembrane receptor that was initially identified in *Drosophila* (Seeger *et al.* 1993). Six years later, the *Drosophila* SLIT protein was identified as the ligand for the ROBO receptor (Brose *et al.* 1999). There is a wealth of literature that supports an evolutionary conserved role for the SLIT–ROBO interaction in guiding axons during development of the nervous system (reviewed by Andrews *et al.* (2007)). A prime example is during the formation of the CN in the midline. The SLIT ligand, secreted by glial cells along the midline, binds to ROBO receptors expressed on crossing axons. This paracrine interaction acts as a repulsive cue to prevent the crossing axons from recrossing the midline. The SLIT–ROBO interaction has a similar function during the development of other processes in the nervous system including formation of the olfactory tract, optic chiasm, optic tract, forebrain and hindbrain (reviewed by Andrews *et al.* (2007)). Mounting evidence suggests that the SLIT–ROBO interaction also acts as a guidance cue during the development of a variety of organs (reviewed by Hinck (2004)). Furthermore, a loss of the SLIT–ROBO signal has been implicated in the aberrant growth and

migration of cells that occurs during cancer development (reviewed by Chédotal *et al.* (2005)). However, whether the SLIT–ROBO system has an important function in reproductive tissues is not well understood. This review will summarise the past and current literature to provide an overview of the cellular processes that the SLIT–ROBO system is thought to regulate. We will then discuss how, from these studies and present research, their potential roles in the reproductive system are starting to unravel.

Summary of the structure and signalling of SLITs and ROBOs

While invertebrates have a single SLIT protein; vertebrates have three homologous SLITs named SLIT1, SLIT2 and SLIT3. Since they lack any hydrophobic sequences that might indicate a transmembrane domain, they are predicted to be secreted proteins associated with the extracellular matrix. The protein sequence of all SLITs shows a high degree of conservation and has the same structure: an N-terminus signal peptide; four tandem leucine-rich repeat domains (LRR) termed D1–D4; six epidermal growth factor (EGF)-like

domains; a laminin G-like domain; a further one (invertebrates) or three (vertebrates) EGF-like domains; and a C-terminal cysteine knot domain (Brose *et al.* 1999; Fig. 1). It is possible that SLITs can be cleaved into N-terminal and C-terminal fragments as there is a putative proteolytic site between EGF5 and part of EGF6 in *Drosophila* SLIT, *Caenorhabditis elegans* SLIT, rat SLIT1, rat SLIT3 and human SLIT2. In the context of axon guidance, it seems that the N-terminal SLIT fragment retains full biological activity, as a ROBO ligand, while the C-terminal fragment is inactive (Nguyen-Ba-Charvet *et al.* 2001).

Although *C. elegans* can function with a single ROBO receptor, three ROBO proteins were identified in *Drosophila* and they were named as follows: ROBO1; ROBO2; and ROBO3. However, four ROBO receptors have been characterised in vertebrates: ROBO1/DUTT1; ROBO2; ROBO3/Rig-1; and ROBO4/Magic Roundabout. ROBO1, ROBO2 and ROBO3 share a common extracellular domain structure that is reminiscent of cell

adhesion molecules. This region contains five immunoglobulin-like (Ig) domains followed by three fibronectin type 3 (FN3) repeats (Fig. 1). The D2 LRR domain of the SLITs and IG1 and IG2 of the ROBOs are evolutionary conserved and are crucial for the binding interaction in vertebrates and invertebrates. However, ROBO4 is unusual in that it contains only two Ig and FN3 domains (reviewed by Hohenester (2008)), and the major SLIT-binding residues within IG1 are not conserved. It has therefore been suggested that ROBO4 may not be a *bona fide* ROBO receptor (Seth *et al.* 2005). However, recent research suggests that SLIT2 does indeed bind to the ROBO4 receptor (Jones *et al.* 2008).

In order to direct changes in cell motility, the binding of SLIT to the ROBO receptor leads to reorganisation of the actin cytoskeleton. This interaction is enhanced, in an evolutionary conserved way, by heparin sulphate proteoglycans (Ronca *et al.* 2001). The binding of the SLITs modifies the cytoplasmic domains of the ROBO receptors. These cytoplasmic domains are highly divergent through

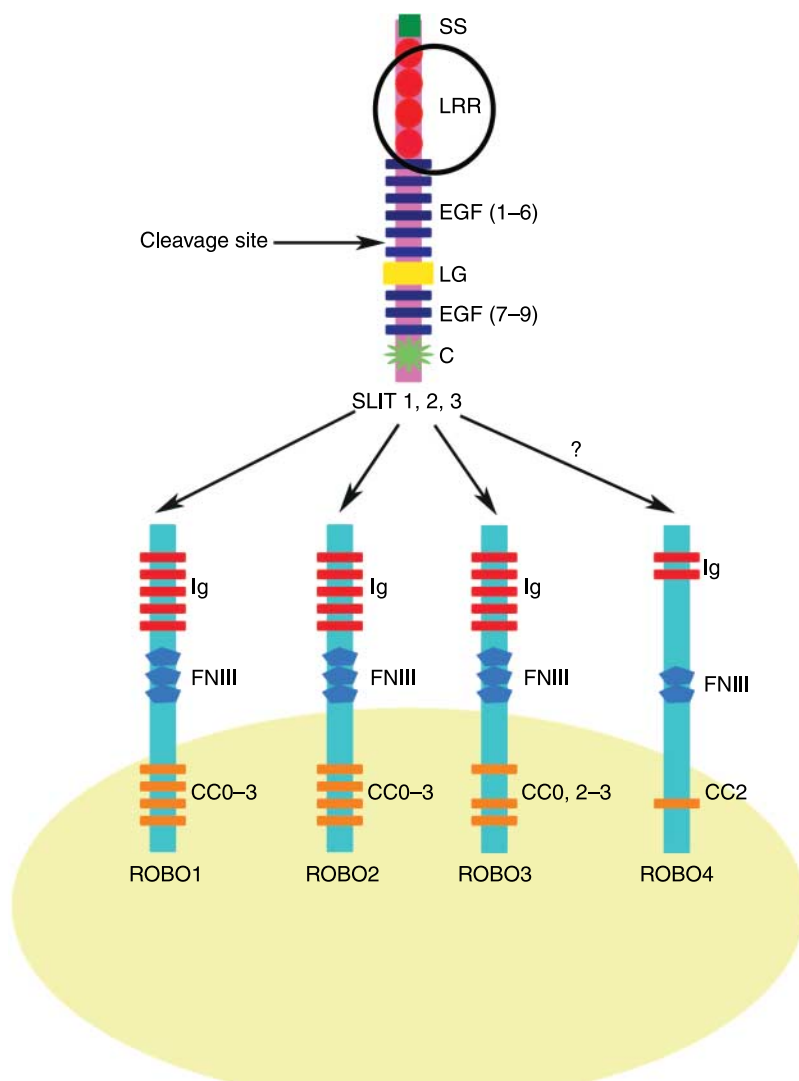


Figure 1 The domain structure of the vertebrate SLIT and ROBO proteins. All three vertebrate SLIT proteins have the same structure. From the N-terminus: a putative signal peptide (SS); four tandem leucine-rich repeat domains (LRR); six epidermal growth factor (EGF)-like domains; a laminin G (LG)-like domain; a further three EGF-like domains and a C-terminal cysteine knot (C) domain. A putative proteolytic cleavage site has been mapped to EGF5 and part of EGF6. Four ROBO receptors have been identified in vertebrates. ROBO1, ROBO2 and ROBO3 share a common extracellular domain structure of five immunoglobulin-like (Ig) domains and three fibronectin type 3 (FNIII) repeats. The intracellular domains of ROBO1 and ROBO2 share the same conserved cytoplasmic motifs (CC0, CC1, CC2 and CC3). The CC1 motif is absent in ROBO3. ROBO4, which has the lowest homology with other ROBO family members, contains only two Ig and FNIII domains along with one CC motif, CC2. The second LRR domain of SLIT along with IG1 and IG2 of ROBO is essential for the ligand-receptor interaction. The LRR domain is circled to highlight its importance in the SLIT-ROBO interaction. SLIT has the same affinity for ROBO1-3 receptors. However, whether ROBO4 is a receptor for SLIT is debatable.

different species, but there are four conserved motifs that occur in various combinations in different ROBOs (Fig. 1). Actin polymerisation is regulated by several adaptor proteins that can bind to the cytoplasmic motifs of the ROBO receptors. This has been elegantly reviewed elsewhere (Hohenester 2008), and potential ROBO adaptor proteins, in the context of the nervous system, include abelson tyrosine kinase, enabled, SLIT-ROBO Rho GTPase-activating protein (srGAP), son of sevenless and Dreadlocks (Bashaw *et al.* 2000, Wong *et al.* 2001, Yang & Bashaw 2006). A SLIT-dependent inactivation of the Rho GTPase CDC42 directly alters the actin cytoskeleton, and an associated increased activity of the Rho GTPase RAC1 augments the adhesive strength of cadherin-mediated intracellular contacts.

SLIT-ROBO signalling can also promote cell adhesion by stimulating the interaction between E-cadherin and β -catenin at the plasma membrane. Consequently, clumps of cells are less prone to cell-cell dissociation and scattering (Prasad *et al.* 2004, 2008, Stella *et al.* 2009). As well as migration and adhesion, SLIT action can regulate other cellular processes involved in cell growth. It can inhibit hepatocyte growth factor, stromal cell-derived factor-1 (SDF1) and β -catenin activity (Prasad *et al.* 2004, 2008, Stella *et al.* 2009), and this leads to inhibition of other signal transduction pathways including phosphatidylinositol 3-kinase and p44/42 MAP kinase activity. Therefore, in addition to a well-described function in controlling cytoskeletal rearrangements affecting cell migration, the SLIT-ROBO pathway can also regulate other cellular processes including cell proliferation and survival.

The SLIT-ROBO interaction and organogenesis

With the possible exception of *Slit1* and *Robo3*, expression of the *Slits* and *Robos* is regulated in a spatial and temporal manner during development in a wide variety of non-neuronal tissues (reviewed by Hinck (2004)). Gonadal development, for example, involves tightly regulated cell migration, proliferation and cell death. We have demonstrated that the SLIT-ROBO system is expressed during sheep fetal ovary development (Dickinson *et al.* 2010). Expression of *Robo2* and *Robo4* was maximal during days 60–70 of gestation, corresponding to the early stages of follicle formation. In the developing primordial follicle, ROBO1 was localised to pre-granulosa cells, while ROBO2 and SLIT2 were localised to the oocytes. Although ROBO4 is thought to be expressed primarily in the vasculature (Seth *et al.* 2005), we could also localise this protein in the germ cells suggesting that it may have a role outside of the vascular system. However, the role of the SLIT-ROBO interaction in the fetal ovary and its relationship with germ and somatic cell migration have not been clarified yet. Interestingly, the increased SLIT-ROBO expression in the fetal ovary occurs at the same time as a reduction in

the number of proliferating oocytes (Dickinson *et al.* 2010), and SLITs have an anti-proliferative role in the nervous system (Andrews *et al.* 2008). Whether the SLIT-ROBO system regulates the development of other reproductive tissues is not known at present. However, the SLIT-ROBO system is involved in the formation of the hypothalamic paraventricular nucleus and supraoptic nucleus. These nuclei contain neuroendocrine cells that modulate pituitary secretion, suggesting that the SLIT-ROBO interaction may have a role in the formation of the hypothalamic-pituitary axis (Xu & Fan 2008).

Mouse models with deletions in certain SLITs and ROBOs have also implicated this pathway in morphogenesis of other tissues throughout the body. *Robo1* homozygous mutant mice frequently died at birth from respiratory failure. These mice had lung defects that included abnormal bronchioles, possibly caused by aberrant branching (Xian *et al.* 2001). *Slit2* and *Robo2* homozygous mutant mice had very similar phenotypes and died shortly after birth from kidney abnormalities (Grieshammer *et al.* 2004). These mouse models suggested that SLIT2-ROBO2 signalling suppresses supernumerary bud formation possibly by directly regulating transcription or translation of genes encoding crucial morphogenetic cues such as glial cell-derived neurotrophic factor. Recently, *Slit3* homozygous mutant mice have been generated which also have kidney defects (Liu *et al.* 2003). These *Slit3* mutant mice also had an enlarged right ventricle in their heart (Liu *et al.* 2003). The SLIT-ROBO interaction seems to play an important role in the development of the heart. In early heart tube genesis in *Drosophila*, SLIT, ROBO1 and ROBO2 guide migrating cardioblasts and pericardial cells in the dorsal midline. Furthermore, they regulate adhesion and alignment between groups of migrating cardioblasts. SLIT-ROBO signalling seems to hinder E-cadherin activity, and this is crucial to the formation of the lumen in the *Drosophila* heart (Santiago-Martínez *et al.* 2006). Likewise, SLIT2 is thought to act as an adhesive cue during ductal morphogenesis in the mammary gland (Strickland *et al.* 2006). Furthermore, these studies suggest that SLIT-ROBO signalling involves both the autocrine and paracrine interactions that we observed during sheep fetal ovary development (Dickinson *et al.* 2010). Overall, during organogenesis, the SLIT-ROBO pathway regulates numerous processes including cell proliferation, migration and adhesion that seem to be important in the development of disparate tissues including those of the reproductive system.

The SLIT-ROBO interaction in reproductive and hormone-dependent cancers

Pathways with crucial roles in tissue growth and development are often dysregulated during tumourigenesis. The SLIT-ROBO interaction is no exception, and there is accumulating evidence to indicate that it has a

fundamental function during cancer development (reviewed by Chédotal *et al.* (2005)). The majority of published research has indicated that *ROBO1/DUTT1*, *ROBO2*, *SLIT1*, *SLIT2* and *SLIT3* are candidate tumour suppressor genes that are inactivated through deletions and hypermethylation of their promoter regions in a variety of epithelial tumour types, including cervical cancer (Narayan *et al.* 2006). In addition, deletion of the *SLIT2* locus was associated with poor survival in cervical cancer patients (Singh *et al.* 2007). Conversely, however, a recent study has implied that tumour *SLIT2* and *ROBO1* expression may be higher in patients with recurrent endometrial cancer in contrast to those without recurrence (Ma *et al.* 2010).

While there is no evidence in the literature that the *SLITs* and *ROBOs* are inactivated through promoter region hypermethylation in ovarian cancer, the *SLIT3* locus was deleted in ovarian germ line tumours (Faulkner & Friedlander 2000). Around 90% of ovarian tumours are thought to originate in the ovarian surface epithelium (OSE; Leung & Choi 2007) and we have demonstrated that ovarian cancer epithelial samples have reduced expression of *SLIT2*, *SLIT3*, *ROBO1*, *ROBO2* and *ROBO4* compared to the normal human OSE (Dickinson *et al.* 2009). There is no evidence in the current literature that the *SLIT-ROBO* pathway has a role in testicular cancer. However, decreased expression of *ROBO1* was detected in prostate tumours (Latil *et al.* 2003).

The pivotal role of *SLIT-ROBO* in inhibiting aberrant migration during development has implicated this pathway in cancer progression, and its inactivation is associated with increased metastasis. Re-expression of *SLIT2* significantly inhibited the invasion and migration of endometrial carcinoma and ovarian carcinoma lines (Stella *et al.* 2009). Other published functional studies have focused on the role of the *SLIT-ROBO* pathway in breast cancer development. Injection of exogenous *SLIT2*-expressing cells into nude mice reduced breast carcinoma size by 65% (Prasad *et al.* 2008). In contrast to these reports, one study has implied that *SLIT2* may act as a chemoattractant and induce brain metastasis of breast cancer cells (Schmid *et al.* 2007). It therefore seems that the role of the *SLIT-ROBO* interaction in cancer is fundamentally important but increasingly complex.

Another role of the *SLIT-ROBO* pathway in tumourigenesis, like in organogenesis, may be to affect cell proliferation and death. We have shown that *SLIT-ROBO* activity could induce programmed cell death through caspase-3 activation in ovarian tumour cell lines (Dickinson *et al.* 2009). Loss of *SLIT2*, *SLIT3* or *ROBO1* in murine mammary gland outgrowths leads to the formation of hyperplastic disorganised lesions, an increase in the number of proliferating cells and stimulation of the *SDF1-CXCR4* axis within the epithelium (Marlow *et al.* 2008). Research using tumours derived from other tissues has supported these findings. In *SLIT2*-transfected fibrosarcomas and squamous cell

carcinomas, there was a higher number of apoptotic cells and reduced proliferation (Kim *et al.* 2008). Furthermore, expression of anti-apoptotic *Bcl-xL* and the proliferation-associated cell cycle proteins *CDK6* and *cyclin D1* was reduced in tumours from nude mice injected with *SLIT2*-expressing cells (Kim *et al.* 2008), and the proliferation rate was also lower in *SLIT2*-expressing hepatocellular carcinoma cells (Jin *et al.* 2009).

The mechanisms by which the *SLIT-ROBO* pathway inhibits proliferation and promotes apoptosis have not been determined. However, there is a plausible theory about how *SLIT-ROBO* signalling may stimulate programmed cell death. *ROBO* can physically interact with another axon guidance receptor called deleted in colorectal cancer (*DCC*) through their cytoplasmic domains (Stein & Tessier-Lavigne 2001). This may cause *DCC* to disassociate from its ligand *netrin-1*. *SLIT2* can also bind and sequester *netrin-1* and prevent its interaction with *DCC*. *DCC* can transmit pro-survival signals in the presence of *netrin-1*, but induces apoptosis, by activating caspases-3 and -9, when its ligand is absent. *ROBO* binding to *DCC* could induce apoptosis since that association inhibits *netrin-1* signalling, and the binding of *SLIT* to *netrin-1* could also block the transmission of pro-survival signals (reviewed by Chédotal *et al.* (2005)). Previous research demonstrated that *DCC* and *netrin-1* are expressed in the ovary, and expression of *DCC* was lost during ovarian tumourigenesis (Saegusa *et al.* 2000). Overall, these findings indicate that the *SLIT-ROBO* pathway mainly suppresses tumour formation and progression by regulating processes including invasion, migration, proliferation, adhesion and apoptosis.

The *SLIT-ROBO* interaction in adult vasculature

As well as its roles in fetal development and the regulation of tumourigenesis in adult cells, the *SLIT-ROBO* pathway is also thought to have an important role in angiogenesis and the function of adult vascular system. Reproductive tissues including the ovary, endometrium and placenta are highly vascular, with dynamic and tightly regulated angiogenesis and vascular remodelling. It is therefore possible that the *SLIT-ROBO* interaction may regulate processes such as endothelial cell migration in these tissues. Certainly, the *SLITs* and *ROBOs* are widely expressed in the human corpus luteum (CL; Dickinson *et al.* 2008) and can be localised to the endometrium (Shen *et al.* 2009; Fig. 2). In addition, *SLIT2*, *ROBO1* and *ROBO4* are expressed in endothelial enriched cultures isolated from the human luteinising follicle (Dickinson *et al.* 2008), and *SLIT3* promotes ovine fetoplacental artery endothelial cell migration and tube formation (Liao *et al.* 2009). Most published research investigating the role of the *SLIT-ROBO* pathway in the vasculature, however, has

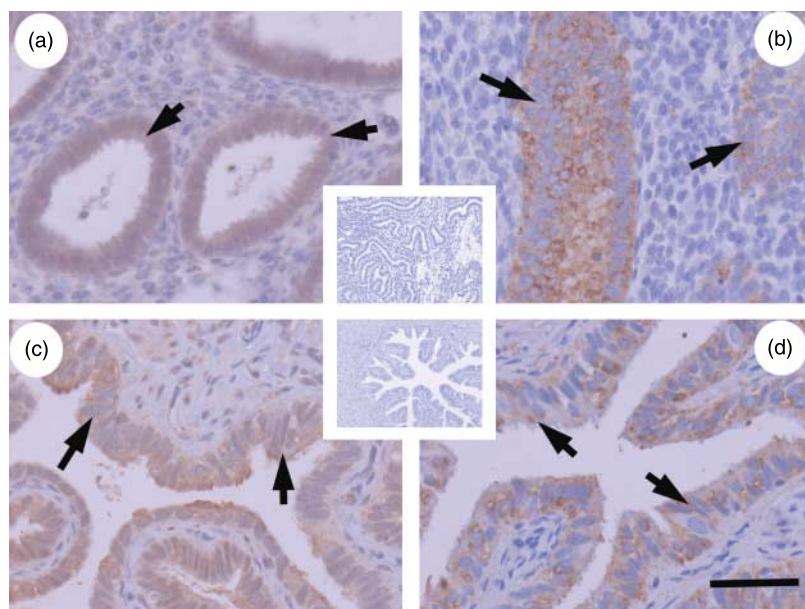


Figure 2 Localisation of SLIT2 and ROBO1 in the endometrium and fallopian tube. Immunohistochemistry for SLIT2 and ROBO1 (brown) using protocols and pre-absorption controls using the techniques reported in Dickinson *et al.* (2008, 2010). (a) SLIT2 in human endometrium with inset showing negative control and (b) ROBO1 in human endometrium. The epithelial glandular staining is highlighted by the arrows. (c) SLIT2 in human fallopian tube with inset showing negative control and (d) ROBO1 in human fallopian tube. The luminal epithelial staining is highlighted by the arrows. Scale bar represents 50 μ m.

used cells derived from non-reproductive tissues that could provide further clues for the potential function of this system in a reproductive context.

However, the exact role of the SLIT-ROBO pathway in the regulation of angiogenesis and vascular function remains controversial. Activation of ROBO4 by SLIT2 inhibited vascular endothelial growth factor-induced migration, tube formation and permeability of mouse lung endothelial cells (Jones *et al.* 2008). Furthermore, in their mouse models of retinal and choroidal vascular disease, the SLIT2-ROBO4 interaction inhibited angiogenesis and vascular leak (Jones *et al.* 2008). SLIT2 also inhibited human aortic smooth muscle cell, human umbilical cord vascular endothelial cell (HUVEC) and human microvascular endothelial cell migration (Seth *et al.* 2005, Liu *et al.* 2006, Kaur *et al.* 2008). The exact mechanism by which SLIT2 exerts its effects is unclear at present. A recent study has suggested that in the absence of SLIT, ROBO1 and ROBO4 form heterodimers which keep them in an inactive state that results in increased HUVEC migration (Kaur *et al.* 2008). However, when SLIT2 was present, it could bind to either ROBO1 or ROBO4 and inhibit HUVEC migration. In contradiction to these reports, one study was unable to convincingly demonstrate SLIT-ROBO4 binding in endothelial cells (Seth *et al.* 2005), and a further study suggested that a SLIT2-ROBO1 interaction may actually promote HUVEC migration (Wang *et al.* 2003) and facilitate angiogenesis (Stollman *et al.* 2009). However, changes in intracellular concentrations of cyclic nucleotides and calcium can switch axon guidance cues from being repulsive to attractive or vice versa (reviewed by Chédotal *et al.* (2005)). Therefore, overall, it seems that

the SLIT-ROBO pathway is involved in the regulation of endothelial cell migration, but the molecular effects are complex and they require further investigation particularly in reproductive tissues.

Regulation of the SLIT-ROBO interaction in adult reproductive tissues

As well as pivotal duties during organogenesis, tumourigenesis and angiogenesis, it is likely that the SLIT-ROBO interaction plays a role in regulating physiological adult cell function in reproductive tissues. We have shown that *SLIT2*, *SLIT3*, *ROBO1*, *ROBO2* and *ROBO4* are expressed in the CL of the human ovary. Furthermore, expression of *SLIT2*, *SLIT3* and *ROBO2* was maximal in the late luteal phase, at the time of increasing cell death, when the CL is starting to regress (Dickinson *et al.* 2008). Since *SLIT2*, *SLIT3*, *ROBO1* and *ROBO2* were expressed in the steroidogenic luteinised granulosa cells and luteal fibroblast-like cells of the CL, the SLIT-ROBO pathway could regulate cell function in this tissue through an autocrine and/or paracrine mechanism.

During maternal recognition of pregnancy, human chorionic gonadotrophin (hCG), from the trophoblast of the implanting blastocyst, acts through the luteinising hormone receptor to 'rescue' the CL from luteolysis and maintain its structural and functional integrity (Duncan 2000). Treating CL *in vivo* and primary cultures of luteinised granulosa cells *in vitro* with hCG, to mimic early pregnancy, caused a reduction in *SLIT2*, *SLIT3* and *ROBO2* expression (Dickinson *et al.* 2008). In addition, we have found that hCG stimulates luteal *HSD11B1*

expression (Myers *et al.* 2007), and this results in increased local glucocorticoid production. We believe that locally generated cortisol exerts an additional luteotrophic action on luteal cells (Duncan *et al.* 2009). Interestingly, cortisol negatively regulated *SLIT2* and *SLIT3* expression in primary cultures of luteal fibroblast-like cells and luteinised granulosa cells.

The SLIT-ROBO pathway also hindered the migration of luteal fibroblast-like cells from the adult human CL and the luteinising follicle (Dickinson *et al.* 2008). Furthermore, blocking SLIT-ROBO activity decreased apoptosis, through a caspase-3-mediated mechanism, in human luteinised granulosa cells and luteal-fibroblast like cells from the luteinising follicle (Dickinson *et al.* 2008). In the immune system, SLIT2 can regulate leukocyte chemotaxis (Wu *et al.* 2001). Since luteolysis of the CL involves an influx of macrophages (Duncan 2000), further studies should investigate whether the SLIT-ROBO pathway can act on these cells. Overall, these results indicate the SLIT-ROBO pathway may promote luteolysis and its expression is hormonally regulated in the adult CL.

The SLIT-ROBO pathway is also expressed in endometrium and fallopian tube epithelium (Fig. 2),

although it is not yet known whether its expression is regulated in these steroid-responsive tissues. Recent research has suggested that expression of SLIT2 and ROBO1 is higher in the ectopic endometrium of ovarian endometriomas when compared to eutopic normal endometrium (Shen *et al.* 2009). SLIT2 expression correlated with microvascular density in endometrioma tissue, and SLIT2 and ROBO1 immunostaining was also elevated in recurrent in contrast to non-recurrent endometriomas (Shen *et al.* 2009). It is attractive to speculate that the SLIT-ROBO interaction may have a role in endometrial function and implantation, but as yet, it is not known if their expression is regulated during implantation or if the SLIT-ROBO pathway is involved in trophoblast function.

The SLITs and ROBOs seem to be involved in the functional regulation of the human ovarian surface after ovulation. Our recent studies have suggested that the human OSE expresses *SLIT2*, *SLIT3*, *ROBO1*, *ROBO2* and *ROBO4*. After ovulation, when the associated inflammation and follicular rupture have damaged the surface epithelium, local production of cortisol increases. Cortisol acts as an anti-inflammatory agent to promote tissue repair (Hillier & Tetsuka 1998).

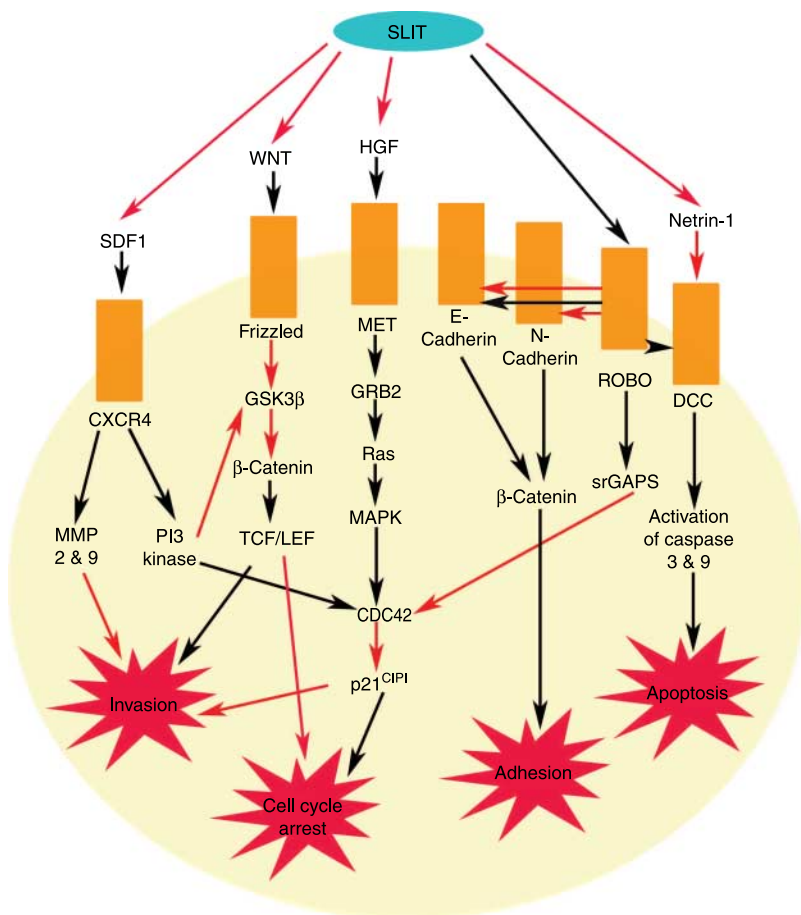


Figure 3 The SLIT-ROBO interaction and their regulation of cell function. SLIT can inhibit invasion and promote a cell cycle arrest by blocking WNT, HGF and SDF1 signalling. The SLIT-ROBO can also prevent invasion and stimulate a cell cycle arrest directly by negatively regulating CDC42 activity. SLIT binding to ROBO also relieves inhibition of DCC by netrin-1. This allows the activation of pro-apoptotic pathways through caspases-3 and -9. SLIT can also bind and sequester netrin-1, preventing its interaction with DCC and inhibitory role in apoptosis. Depending on the particular cellular environment, the SLIT-ROBO interaction can also promote and inhibit adhesion. The SLIT-ROBO interaction promotes adhesion in breast tumour cells and during mammary gland development, possibly by enhancing the association between E-cadherin and β-catenin at cell borders. However, during the formation of the heart lumen, SLIT-ROBO signalling antagonises E-cadherin/β-catenin-mediated cell-cell adhesion. During neural development, SLIT binding promotes an interaction between ROBO and N-cadherin. Subsequently, β-catenin becomes disassociated from the complex, and there is a reduction in cadherin-mediated cell-cell adhesion. Black arrows represent promoting an activity, while red arrows depict inhibiting an action.

Moreover, cortisol treatment caused a significant reduction in the expression of all these genes (Dickinson *et al.* 2009). Since the SLITs and ROBOs promote apoptosis and inhibit cell proliferation, cortisol may inhibit the expression of these genes to allow cell division and migration to repair the surface epithelium after ovulation.

Since there are no published reports at present, it will be interesting to investigate whether SLIT-ROBO expression is regulated by additional factors in the ovary and steroid hormones in other reproductive tissues. Interestingly, the srGAP has been identified as a potentially tamoxifen-sensitive gene in breast cancer cells (Zarubin *et al.* 2005). Furthermore, *Slit1* expression is reduced by oestrogen and selective oestrogen receptor modulators in the bone of ovariectomised rats (Helvering *et al.* 2005). The steroid regulation of the SLIT-ROBO pathway and its manipulation is therefore of major interest and further research is needed. Taken together, however, the current literature suggests that there are parallels between the multiple processes that the SLIT-ROBO pathway regulates in organogenesis, tumourigenesis and normal adult physiology (Fig. 3).

Concluding remarks

Along with a well-established function as an axon guidance-repulsive cue, the SLIT-ROBO pathway has key roles during organ development, tumourigenesis and normal physiology. Importantly, recent studies have suggested new functions for the SLIT-ROBO system beyond their well-described role in regulating cell migration. The SLIT-ROBO interaction seems to regulate proliferation, apoptosis, adhesion and angiogenesis in normal and tumour cells. Furthermore, in the adult ovary, expression of SLITs and ROBOs was physiologically regulated by steroid hormones and gonadotrophins during the normal reproductive cycle. Recent research has also indicated that the SLIT-ROBO pathway is expressed in the uterus. It is therefore likely that there are disparate roles for the physiological and pathological functions of the SLIT-ROBO interaction in reproductive tissues, and wider study is indicated. Future research should also investigate the possible function of the SLIT-ROBO interaction in the fetal and adult male reproductive system which is an area that has been particularly neglected so far. Furthermore, the generation of transgenic mice that have mutant copies of SLIT-ROBO in male and female reproductive organs would provide further clues on the role of this pathway in these tissues. Identifying hormonal and other factors that may influence SLIT-ROBO expression in adult and fetal reproductive tissues is also an important area for further analysis, and such research could have important implications for fertility and for the treatment of hormone-dependent cancers.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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