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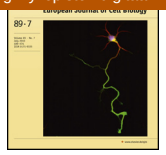

Mini-Review

# Netrin-1: A regulator of cancer cell motility?

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ABSTRACT

Netrins form a family of secreted and membrane-associated proteins, netrin-1 being the prototype and most investigated member of the family. The major physiological functions of netrin-1 lie in the regulation of axonal development as well as morphogenesis of different branched organs, by promoting the polarity of migratory/invasive front of the cell. On the other hand, netrin-1 acts as a factor preventing cell apoptosis. These events are mediated via a range of different receptors, including UNC5 and DCC-families. Cancer cells often employ developmental pathways to gain survival and motility advantage. Within recent years, there has been increasing number of observations of upregulation of netrin-1 expression in different forms of cancer, and the increased expression of netrin-1 has been linked to its functions as a survival and invasion promoting factor. We review here recent advances in the netrin-1 related developmental processes that may be of special interest in tumor biology, in addition to the known functions of netrin-1 in tumor biology with special focus on cancer cell migration.



## 1. Introduction

Netrins form a conserved family of extracellular matrix proteins. In vertebrates five secreted netrins, (netrins 1–5) and two membrane bound forms (netrin-G1 and netrin-G2) have been identified (Serafini et al., 1996, 1994; Yin et al., 2000; Nakashiba et al., 2002; Yamagishi et al., 2015). Netrin-2 is expressed in avians and zebrafish while netrin-3 is the counterpart in mammals. Uncoordinated-6 (UNC-6) and netrin-A and -B represent the *Caernohabtidis elegans* (*C. Elegans*) and *Drosophila Melanogaster* (*D. Melanogaster*) counterparts for the vertebrate netrins (Harris et al., 1996; Mitchell et al., 1996a). Structurally netrins resemble laminins and consist of three distinct parts (Fig. 1). The N-terminal domain of netrins 1–3 and netrin G1-2 is homologous to laminin gamma chain whereas in netrin-4 it resembles laminin beta chain. The central part of netrins contains three EGF repeats homologous to laminin domain VI (Serafini et al., 1994). In contrast, the C-terminal domain NTR domain is not related to the laminin domains. Instead, it has similarity with the C-termini of complement proteins, frizzled-related proteins, type I collagen C-proteinase enhancer proteins and tissue inhibitors of metalloproteinases (Banyai and Patthy, 1999). Additionally, the membrane bound netrin-Gs have glycosylphosphatidylinositol anchor in their C-terminal tail (Nakashiba et al., 2002). The newly found member of the family, netrin-5, lacks the N-terminal domain and one of the EGF repeats (Yamagishi et al., 2015).

Netrins were first investigated in *C. Elegans*. The loss of UNC-6 resulted in defects in axonal pathfinding (Brenner, 1974; Hedgecock et al., 1990). Subsequently, similar defects were discovered in vertebrates upon netrin-1 deletion (Serafini et al., 1996). Later, the complete loss of netrin-1 was found to be embryonically lethal due to failed axonal pathfinding (Bin et al., 2015). Netrin-1 is the prototype of the family and most widely studied. It plays a dual function in the axon guidance: it can act either as axon attracting or repelling cue (Hong et al., 1999). The role of other netrins in axonal guidance and other biological processes has been much less explored. Therefore, this review concentrates on the effects of netrin-1.

Classically netrin-1 effects have been believed to be mediated via two sets of receptors: the deleted in colorectal cancer (DCC) family that contains DCC and neogenin receptors (Keino-Masu et al., 1996; Engelkamp, 2002) and the uncoordinated5 (UNC5) family comprised of UNC5A-D (Leonardo et al., 1997). The axon attracting effects appear to be mediated via the DCC receptors in coordination with Down syndrome cell adhesion molecule (DSCAM) and repellent effects via binding to the UNC5 receptors

*Abbreviations:* *C. Elegans*, *Caernohabtidis Elegans*; DCC, deleted in colorectal cancer; *D. Melanogaster*, *Drosophila Melanogaster*; DSCAM, Down syndrome cell adhesion molecule PMEC primary midgut epithelial cell; UNC-6, uncoordinated-6; UNC5, uncoordinated5; YAP, yes associated protein.

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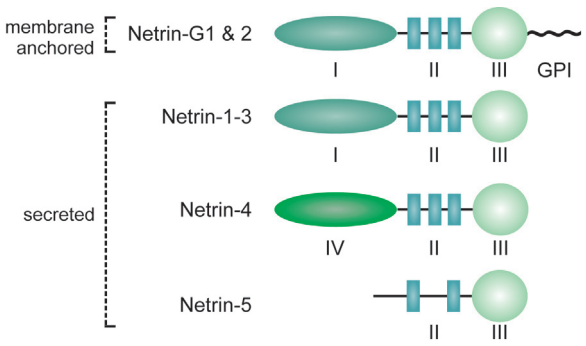


**Fig. 1.** Schematic illustration of the structure of netrins. Different netrin domains are marked as follow I: N-terminal domain related to laminin γ-chain, II: 3 EGF repeats III: NTR domain IV: N-terminal domain related to laminin β-chain ~: GPI-anchor.

(Keino-Masu et al., 1996; Leonardo et al., 1997; Ly et al., 2008; Liu et al., 2009). However, evidence supporting and against the role of DSCAM as netrin-1 receptor has been presented (Palmesino et al., 2012; Huang et al., 2015).

In addition to axon guidance, netrin-1 functions have been connected to several other cellular guidance events. Via binding to integrins alpha6 beta4 and alpha3 beta1 netrin-1 participates in the branching morphogenesis of mammary gland, pancreas and lungs (Yebra et al., 2003; Srinivasan et al., 2003; Dalvin et al., 2003; Liu et al., 2004). Netrin-1 also influences the embryonic angiogenesis. However, it has been for long controversial whether it is pro- or antiangiogenic factor (Lu et al., 2004; Wilson et al., 2006; Larrivee et al., 2007; Castets et al., 2009).

During tumor evolution various developmental signaling pathways are upregulated. Netrin-1 signaling has been shown to be upregulated in several cancer types (Table 1). The involvement of netrin-1 in many developmental processes regulating cell motility and polarity raises questions of its possible functions in cancer biology. We review here the known functions of netrin-1 in tumor cell biology as well as the possible links between the roles in developmental and cancer biology.

## 2. Roles of Netrin-1 in cancer

### 2.1. Netrin-1 as survival promoting factor

Netrin-1 has been shown to act as a survival factor in many forms of cancer. Based on these observations Mehlen and colleagues have presented dependence receptor model where the apoptotic functions of netrin-1 receptors are prevented by interaction with netrin-1 (Mehlen et al., 1998; Llambi et al., 2001; Mehlen and Bredesen, 2004). Both DCC and UNC5 receptors have a so called death domain in their cytoplasmic tail. When the receptor is not engaged with netrin-1 ligand the death domain is exposed and allows the binding of caspase-9. This leads to caspase-3 activation and subsequent apoptosis. However, the caspase activation cascade is prevented by the binding of netrin-1 to DCC/UNC5 receptor, leading to resistance to apoptosis. Indeed, netrin-1 upregulation has been suggested to be a way to gain survival advantage for several cancers including colorectal cancer, non-small cell lung cancer, neuroblastoma, glioblastoma and metastatic breast cancer (Delloye-Bourgeois et al., 2009a,b; Fitamant et al., 2008; Mazelin et al., 2004). However, no increase in apoptosis within spinal cord was observed in netrin-1 knockout mouse model (Bin et al., 2015) suggesting that the survival promoting functions of netrin-1 are not universal.

As cancer cells get survival advantage by downregulating apoptotic signaling of netrin receptors, the simplest way to achieve this would be to downregulate death receptor expression. This occurs in several cases (Fearon et al., 1990; Krimpenfort et al., 2012). However, the increased netrin-1 expression in various cancers suggest that those cancer types receive additional benefit from netrin-1 expression.

### 2.2. Regulation of cancer cell invasion by Netrin-1

Netrin-1 promotes the motility of many epithelial cancer cell types. In a majority of colorectal cancer tumors DCC and UNC5 receptors are downregulated, whereas most of the colorectal tumors express netrin-1 (Paradisi et al., 2008). In addition, netrin-1 expression is upregulated in colon carcinoma and adenoma cell lines, whereas the expression of DCC or UNC5 receptors are downregulated (Rodrigues et al., 2007). Netrin-1 upregulation promotes the invasiveness and growth of the cancer cells lacking DCC both *in vitro* and *in vivo* (Rodrigues et al., 2007). The invasion promoting effects of netrin-1 are mediated by RhoA, Rac1, Cdc42 and PI3K signaling pathways. On the contrary, DCC acts as a tumor suppressor gene by reducing cancer cell invasion and metastasis *in vivo* (Rodrigues et al., 2007). DCC expressing tumor xenografts grow equally to control tumors but invade significantly less to lymph nodes or lungs of the mice (Rodrigues et al., 2007). Furthermore, DCC expression increases the percentage of apoptotic cells under hypoxic conditions. Interestingly, netrin-1 upregulation cannot rescue DCC induced apoptosis supporting the pro-invasive rather than survival promoting function of netrin-1 in this model (Rodrigues et al., 2007).

In breast cancer patient biopsies netrin-1 is strongly expressed in lymph node or other distant organ metastases while tumors localized to breast area show only modest expression (Fitamant et al., 2008). The correlation of netrin-1 expression and lymph node metastasis is even stronger than the association between lymph node metastasis and known metastasis markers CXCR4 or VEGFR (Fitamant et al., 2008). Furthermore, in a panel of 48 human breast cancer cell-lines netrin-1 expression is the most abundant in cell lines derived from aggressive tumors. Similarly, netrin-1 expression is lower in 67NR non-metastasizing mouse breast cancer cell line compared to metastatic 4T1 cell line. However, the expression patterns of classical netrin receptors do not differ between these cell lines. Inhibition of the autocrine netrin-1 expression either via netrin-1 silencing or by preventing netrin-1 binding to the classical receptors in the 4T1 cells lowers the cell survival and their capability to metastasize. Authors suggested that netrin expression confers selective advantage for cell survival outside the primary tumor by blocking the apoptotic functions of the DCC or UNC5 receptors. (Fitamant et al., 2008). However, the cellular mechanisms how netrin-1 increases the cell motility remained unclear.

Furthermore, netrin-1 expression increases pancreatic ductal adenocarcinoma (PDAC) invasiveness and apoptotic resistance and serves as adhesive surface for the cells (Dumartin et al., 2010). Netrin-1 was also observed to mediate the neural invasion of PDAC in a mouse model (Wang et al., 2015). Netrin-1 expression was regulated via mucin4 induced HER2 activation and subsequent NFkb signaling activation. Similarly, inflammation induced NFkb signaling acted as an upstream regulator of netrin-1 expression in colorectal cancer progression (Paradisi et al., 2008, 2009). In addition, netrin-1 serves as prognostic factor in pancreatic cancer (Link et al., 2007). On the contrary, a recent study suggests netrin-1 to be a tumor suppressor in low-grade pancreatic adenocarcinoma via UNC5b mediated integrin beta4 downregulation (An et al., 2016). However, netrin-1 upregulation was observed in high grade pancreatic adenocarcinomas (An et al., 2016). Moreover, netrin-1 increases the proliferation and migration of hepatocellular carcinoma cells. Netrin-1 treatment upregulates yes associated protein (YAP) expression, an oncogene that belongs to the Hippo signaling pathway (Qi et al., 2015). Netrin-1 increases YAP dephos-

**Table 1**
Summary of the netrin-1 expression levels, suggested functions and novel pathways in different cancers. The expression of netrin-1 in various cancer tissues is indicated as follows: + expression has no change in comparison to normal tissue, ++ modest increase, +++ strong upregulation and − indicates decreased expression level. N/R stand for not researched and N/A for not applicable. * Netrin-1 expression has been determined from cell-line.

| Cancer type | | Colorectal cancer | Breast cancer | Pancreatic cancer | Lung cancer | Hepatic carcinoma | Glioblastoma | Medulloblastoma | Neuroblastoma |
|---|---|---|---|---|---|---|---|---|---|
| Netrin-1 expression in tumor tissue | low grade/non-metastatic tumors | N/R | + | − | +++ | +++ | +* | N/R | N/R |
| | high grade/metastatic tumors | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | brain metastases | + | ++ | N/R | +++ | N/R | N/A | N/A | N/A |
| Netrin-1 effects | protection from apoptosis | x | x | x | x | N/R | x | x | x |
| | motility promoting effect | x | x | x | x | x | x | x | x |
| | suggested as biomarker | N/R | N/R | x | N/R | N/R | x | x | x |
| Suggested alternative signaling pathways | | NFkB, PKA, Rho GTPases/ROK, and PI3K- pathways | | Her2 induced NFkB signaling | Hippo-pathway | NFkB signaling and subsequent IL8 and IL6 release, Hippo-pathway | Notch-Jagged signaling pathway, Hippo-pathway, cytoskeleton remodelling via RhoA | | |
| References | | Rodrigues et al., (2007), Paradisi et al. (2008), Paradisi et al. (2009); Harter et al. (2014) | Fitamant et al. (2008), Harter et al. (2014) | Dumartin et al. (2010), Wang et al. (2015) | Delloye-Bourgeois et al. (2009a), Harter et al. (2014), Qi et al. (2015) | Yan et al. (2014), Qi et al. (2015), Han et al. (2015) | Ramesh et al. (2011), Jarjour et al. (2011), Shimizu et al. (2013), Ylivinkka et al. (2013), Qi et al. (2015) | Akino et al. (2014) | Delloye-Bourgeois et al. (2009b) |

phorylation which stabilizes it and leads to its prolonged nuclear accumulation. These netrin-1 effects were mediated by UNC5b and DCC. Previously it has been observed that under hypoxic conditions netrin-1 also induces epithelial to mesenchymal transition of the hepatocellular cancer cells (Yan et al., 2014). In addition, netrin-1 upregulation leads to production of many inflammatory molecules which are thought to aid cancer invasiveness (Yan et al., 2014; Han et al., 2015).

Netrin-1 promotes also the invasiveness of tumors of the nervous system. It is expressed in glioblastoma, the most malignant human brain cancer, and in glioblastoma derived cell-lines that show infiltrative growth *in vivo* but not in less malignant cells (Ylivinkka et al., 2013; Jarjour et al., 2011). On the contrary, DCC expression is downregulated in netrin-1 expressing glioblastoma cells but upregulated in less invasive, netrin-negative U87MG glioblastoma cells (Jarjour et al., 2011). Netrin-1 upregulation or usage as chemoattractant promotes the invasiveness of glioblastoma cells even further (Ylivinkka et al., 2013; Shimizu et al., 2013).

A screen for interacting proteins revealed that netrin-1 associates with important general cellular signaling pathways such as integrin signaling, proteolysis and endocytosis in glioblastoma cells (Ylivinkka et al., 2013). In addition, netrin-1 bound to the complex of Notch2 and Jagged1, and activated the Notch signal pathway and subsequent cell invasiveness (Ylivinkka et al., 2013). Another study proposed that the increase in invasiveness is regulated via RhoA mediated actin cytoskeleton modulation (Shimizu et al., 2013). Furthermore, similarly to hepatocytic carcinoma cells netrin-1 is suggested to promote the proliferation and migration of glioblastoma cells via stabilizing the YAP-kinase (Qi et al., 2015). Moreover, netrin-1 expression increased the invasiveness of medulloblastoma cells via neogenin and UN5b receptors (Akino et al., 2014), pointing out the complexity in netrin-1 induced signal pathways.

Furthermore, netrin-1 has been suggested to function as a potential biomarker for both glioblastoma and medulloblastoma (Akino et al., 2014; Ramesh et al., 2011). Increased levels of netrin-1 were detected in the blood of glioblastoma patients and in the urine of medulloblastoma patients. In the medulloblastoma patients surgical resection of the tumor decreased the levels of urine netrin-1 significantly (Akino et al., 2014). Interestingly, in subcutaneous glioblastoma and medulloblastoma mouse xenograft models netrin-1 upregulation also increased the vascularity of the tumors (Shimizu et al., 2013; Akino et al., 2014).

In an extracranial neural tumor, neuroblastoma, netrin-1 is associated with poor patient prognosis (Delloye-Bourgeois et al., 2009b). Netrin-1 is strongly expressed in aggressive, stage 4 neuroblastoma. On the contrary, expression of the DCC receptor is low and UNC5A-B modest in these tumors. Netrin-1 expression was connected with the increased apoptosis resistance of the neuroblastoma cells (Delloye-Bourgeois et al., 2009b). Interestingly, netrin-1 inhibition also led to a decrease in the migratory capacity of the neuroblastoma cells. Furthermore, netrin-1 negative primary neuroblastoma tumors xenografted into mice showed netrin-1 positive metastatic lesions in lungs or heart. Moreover, a recent study suggests that netrin-1 is an independent prognostic factor in brain metastases of various cancers including non-small cell lung cancer, breast cancer and melanoma (Harter et al., 2014).

These observations raise a question: why netrin-1 is upregulated during the metastatic cascade? Does netrin give advantage in this process? It has been suggested that netrin-1 expression allows the tumor cells to survive better outside the primary tumor (Delloye-Bourgeois et al., 2009b). However, netrin-1 seems to be strongly linked to cell invasion and metastatic growth. In contrast to the mechanisms netrin-1 plays in the prevention of apoptosis, less is known about molecular mechanisms in netrin-1 driven cancer cell invasion. On the other hand, the mechanisms of cell motility that netrin-1 regulates during developmental processes have been more widely characterized and may shed light also on the events leading to cancer cell migration.

## 3. Netrin-1 in development: regulator of cell migration, polarity and adhesion

### 3.1. Directing the turning of the axon

The functions of netrin-1 have been most widely characterized in axonal guidance events during embryogenesis. The growth and turning of the axon is a diligent and carefully regulated process which eventually leads to the growth of the axon towards its correct target. The axons sense their environment via a specialized cell structure called growth cone. The growth cone is very motile and responsive to many extracellular guidance signals including diffusive netrin gradient (Lowery and Van Vactor, 2009; Geraldo and Gordon-Weeks, 2009; Kolodkin and Tessier-Lavigne, 2011). It contains filopodial and lamellipodial protrusions which undergo fast changes during the axon guidance. These structural changes are dependent on the microtubule and actin dynamics within the growth cone (Geraldo and Gordon-Weeks, 2009). To turn the growth cone must be able to grow from the other side while retract from another. This is mediated via continuous formation and breaking down of lamellipodia and filopodia. Netrin-1 has been suggested to be a factor leading to uneven filopodia extension on the growth cone. It plays several different roles in this process. Netrin-1 binding to the growth cone leads to the coupling of DCC, DSCAM and TUBB3, the major constituent of microtubules (Huang et al., 2015; Qu et al., 2013). The binding leads to the increased number and growth of plus-ended microtubules. The netrin-1 induced association of DCC and DSCAM occurs only with stable, phosphorylated microtubules on lamellipodia and axon branchpoints. In addition, netrin-1 increases local protein translation in the growth cone (Tcherkezian et al., 2010). Because the axon extensions are very far away from the cell body and nucleus it is essential that the proteins needed for the growth of the axon can be produced locally. In this process the microtubules are needed for polypeptide chain elongation and for the delivery of ribonucleoparticles whereas the F-actin is needed for the initiation of the protein synthesis (Piper et al., 2015). These observations suggest that netrin-1 acts as a polarizing agent leading to DCC and DSCAM clustering. These receptors then function as signaling platforms which can regulate both the local protein synthesis and microtubule growth to orient the axon steering.

### 3.2. Regulation of the invertebrate development

It is well known that the loss of both epithelial hierarchy and apico-basal polarization expose cells to malignant growth. Tumor cells of epithelial cancers undergo epithelial to mesenchymal transformation where the tumor cells lose their epithelial hierarchy and gain the motile properties of the mesenchymal cell type (reviewed in Hanahan and Weinberg, 2011). One key consequence of this process is the ability of the cancer cells to break the basement membrane and leave the primary tumor. This phenomenon is similar to the cell migration events during organ development.

The migration of specialized gonadal cell, anchor cell, of *C. Elegans* is dependent on the guidance of UNC-6 and UNC-40, the *C. Elegans* counterparts for netrin-1 and DCC, respectively (Sherwood and Sternberg, 2003; Ziel et al., 2009; Hagedorn et al., 2013). Prior to anchor cell invasion through basement membrane, the integrin receptor in the anchor cell polarizes the actin cytoskeleton to the cell-basement membrane interface. Integrin activity promotes the formation of invadopodia-small membrane associated F-actin structures that breach the basement membrane (Hagedorn

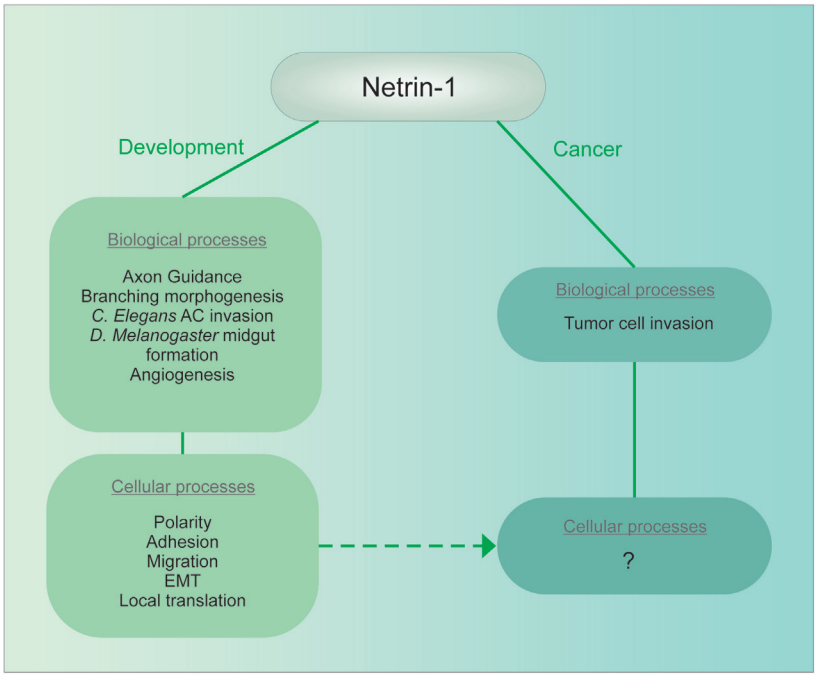


**Fig. 2.** Motility related functions of netrin-1 in development and cancer. The developmental processes are presented on left side and cancer in right side. The upper boxes represent the biological processes and the lower boxes the cellular processes.

et al., 2013). Integrin also helps the trafficking of the netrin receptor UNC-40 to the cell membrane, which concentrates at the breach site before invadopodia-mediated basement membrane penetration (Hagedorn et al., 2013, 2009). UNC-40 clustering at the breach site may occur in response to UNC-6, which accumulates in the basement membrane (Ziel et al., 2009). At the breach site, UNC-40 recruits actin regulators and focus F-actin formation on this site to build a large invasive protrusion that crosses the basement membrane and enters the neighboring tissue (Hagedorn et al., 2013). If the location of the UNC-6 gradient changes, the clustering of UNC-40 and F-actin and the formation of the invasive protrusion changes accordingly (Wang et al., 2014).

Netrin-1 is involved in the development of the midgut of *D. Melanogaster*. In this process two masses of mesenchymal primary midgut epithelial cells (PMEC) first reside in the opposite ends of the embryo and start to migrate towards each other (Tepass and Hartenstein, 1994). The migration occurs along the visceral mesoderm cells that are on the outer membranes of the embryo. During the course of the migration the PMECs undergo mesenchymal to epithelial transition and form epithelium. This epithelium formation depends on the contact with visceral mesoderm on the basal side of the PMECs (Tepass and Hartenstein, 1994).

The visceral mesoderm cells secrete netrins-A and B while the PMECs express netrin receptor frazzled (*D. Melanogaster* counterpart for DCC) (Mitchell et al., 1996b). The depletion of both netrins leads to significant delay in the migration of the PMECs (Pert et al., 2015). However, depletion of either netrin-A or netrin-B alone had no significant effect on the migration of the PMECs. The effect could be rescued by re-expressing either netrin suggesting parallel and compensatory functions for them. In addition, the shape of the migrating PMECs and the clustering of F-actin to the contact points with the visceral mesoderm was altered upon depletion of netrins. Similar effects were observed with frazzled depletion. Interestingly, the delay in the PMECs migration was even greater than in the netrin-null embryos suggesting that frazzled may have some netrin independent functions. Furthermore, netrins and frazzled were co-localized and endocytosed together on the basal side of the PMECs (Pert et al., 2015). Clustering and subsequent endocytosis of frazzled to the basal side of the PMECs failed upon netrin deletion. In addition, mesenchymal to epithelial transition mediator proteins filamin-1, F-Actin and E-cadherin also failed to polarize to the basal side of PMECs. As a result the formation of the epithelium was prevented. These findings suggest that netrin-frazzled signaling regulates the motility and mesenchymal to epithelial transition of the PMECs. Similarly to the development of *C. Elegans*, netrins appear to be critical in regulating the polarization of the cells.

### 3.3. Role in branching morphogenesis of epithelial structures

Netrins have been suggested to play a role in several developmental processes of various epithelial structures in vertebrates as well. It regulates the branching morphogenesis of several organs. Here we review the development processes of lung, mammary gland and pancreas.

During lung development netrin-1 and -4 are expressed by the epithelial cells on the stalk area of the developing lung bud (Dalvin et al., 2003; Liu et al., 2004). After secretion netrin accumulates at the basement membrane or at the epithelial cells located behind the tip cells (Liu et al., 2004). UNC5B receptor has opposite expression pattern than netrins and is expressed by the tip cells of the budding lung. In addition, the tip cells show increased phosphorylation of ERK-proteins. Interestingly, addition of exogenous netrin to 3D-lung bud cultures abolishes the budding or even reverse its direction towards the lumen of the lung bud (Liu et al., 2004). It also lowers the overall levels of phosphorylated ERK. This suggests that netrins at the stalk area prevent excess lung budding by reducing ERK phosphorylation. Authors examined possible receptors involved and suggest UNC5B to mediate this cascade but do not exclusively rule out integrins or other netrin receptors.

During mammary gland development netrin-1 is expressed at the prelumenal cells of the terminal end buds (Srinivasan et al., 2003). Neogenin on the other hand is expressed by the cap cells neighboring the prelumenal cells. During the mammary gland development the branches elongate via the growth of the cap cells on the terminal end buds. It is important that the terminal end buds keep their correct organization where the cap cells are in

contact with the luminal cells behind them (Silberstein, 2001). Deletion of both netrin-1 and neogenin in mammary gland led the cap cells to move uncoordinatedly and create gaps between the luminal cells and the cap cells although their expression of E- and P-cadherin remained normal (Srinivasan et al., 2003). Therefore netrin-neogenin interaction is important stabilizer for the epithelial cell adhesions. Furthermore, netrin-1/neogenin mediated effects seem to be acting synergistically with another axon guidance protein (SLIT2/ROBO1) signaling (Strickland, 2006).

In the developing human pancreas netrin-1 is expressed in various populations of epithelial cells in the ductal structures, developing islet cells and around the undifferentiated acinar structures and co-localizes with alpha6beta4 and alpha3beta1 integrin receptors (Yebra et al., 2003). Netrin-1 binds to integrins via its C-terminal domain and serves as adhesive surface to pancreatic epithelial cells and induces their migratory phenotype *in vitro*. Netrin-1 is as prominent migratory surface as laminin-1 or other abundant extracellular matrix components. However, the *in vivo* concentrations of netrin-1 may not be as high as laminin, for example. The migration inducing effect of netrin-1 was only observed in haptotactic manner and not chemotactically as during axon guidance. Interestingly, majority of the cells migrating along netrin-1 are undifferentiated pancreatic progenitor cells. The two integrin receptors co-operate in binding to netrin-1 and possibly activate c-MET receptor and subsequent epithelial cell migration pathways.

The localization of netrin-1 during branching morphogenesis seems to be different compared to the localization in axonal guidance and *C. Elegans* anchor cell invasion. In branching organs netrin-1 is localized to invading structures themselves, whereas in neuronal guidance the source of netrin-1 is distant from the target cells, and the target cells are either attracted to or retracted from netrin-1. Mechanisms related to axonal cell polarization are relatively well known, whereas mechanisms related to branching morphogenesis are poorly known. However, these studies suggest that during branching morphogenesis netrin-1 regulates cell–cell adhesions and maintains the correct morphology of the organs. Similarly to axon guidance, the receptor complexes on the affected cells dictate whether the effect of netrin-1 is pro- or anti-migratory.

Furthermore, the source of netrins seem to be different in these two modes of migration. In axonal guidance netrin-1 acts in paracrine manner. In branching morphogenesis at least the localization is in invading structures, and netrin-1 seems to act there as autocrine manner. Cancer cells also have autocrine netrin-1 expression (Fitamant et al., 2008), suggesting that tumors may get invasive advantage from netrin-1 in autocrine manner. However, poorly known mechanisms warrant thorough investigations on the involvement of netrin-1 and the various receptors of it on these processes, especially in cancer.

## 4. Concluding remarks

Within recent years there has been increasing numbers of observations of elevated netrin-1 expression in various cancers (Table 1). In many studies netrin-1 has been suggested to be a survival promoting factor for cancer cells and to be acting via its classical receptors, DCC and UNC5 families (Delloye-Bourgeois et al., 2009a,b; Fitamant et al., 2008; Mazelin et al., 2004). However, in tumors the functions and expression of netrin-1 and the classical receptors are not always overlapping. For example in glioblastoma and breast cancer, there are cell lines which differ in their netrin-1 expression pattern but not in their expression patterns of the classical netrin receptors (Fitamant et al., 2008; Ylivinkka et al., 2013; Shimizu et al., 2013). Despite the lack of netrin-1 expression the netrin-1 negative cells do not exhibit increased apoptosis or defects in their survival. In fact, the major difference between the netrin-1 positive and negative cells is their motility and capability to metastasize. In both tumor types the netrin-1 negative cells are not metastatic unlike the netrin-1 positive cells. Therefore, netrin-1 may have functions independent of the classical receptors and may interact with other signaling pathways in cancer cells. Indeed, based on recent studies netrin-1 has been linked to new receptors and signaling pathways including the Notch signaling pathway, the Hippo pathway and the NFkB signaling pathway as well as to many other interacting proteins including proteases and endocytosis related proteins (Wang et al., 2015; Paradisi et al., 2009; Qi et al., 2015; Ylivinkka et al., 2013). These findings support the role of netrin-1 as multifunctional protein which is involved in many cellular events yet to be identified. Furthermore, the molecular mechanisms of netrin-1 induced cancer cell invasion remain largely unknown and the involvement of DCC and UNC5 receptors in netrin-1 induced cancer cell motility cannot be ruled out. During developmental processes netrin-1 regulates the rearrangement of the cytoskeleton, cell polarity, adhesion, migration through basement membranes, local translation and epithelial to mesenchymal transition. The key biological and cellular processes that netrin-1 regulates during development and their possible connection to cancer are summarized in Fig. 2. In addition, since the Hippo pathway and YAP kinase are linked to the regulation of contact inhibition of cells it will be of interest to explore whether netrin-1 regulates this in cancer cells (Zhao et al., 2007). All of these cellular events are also of great importance in tumorigenesis and in the metastatic spreading of the cancer cells. Therefore it is of interest to explore if netrin-1 regulates cancer cell motility via these processes.

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