Published in final edited form as:

Curr Drug Targets. 2009 July ; 10(7): 602–610.

Netrin and DCC: Axon Guidance Regulators at the Intersection of Nervous System Development and Cancer

M. Duman-Scheel^{1,2,*}

¹Indiana University School of Medicine-South Bend at Notre Dame, Raclin-Carmichael Hall, 1234 Notre Dame Ave., South Bend, IN 46617, USA

²University of Notre Dame, Department of Biological Sciences, Walther Cancer Institute, and Eck Institute for Global Health, Notre Dame, IN 46556, USA

Abstract

In recent years, a number of axon guidance genes, including Netrin (Net) and Deleted in Colorectal Cancer (DCC), have been implicated in human cancers. Many of the hallmarks of human cancer, such as cell growth, invasion, evasion of apoptosis, and formation of a blood supply to the tumor, involve cellular processes that are critical during nervous system development. Here, the roles of Net-DCC in the regulation of these cellular processes in tumors and developing neurons are discussed. The advantages of using *Drosophila* to study the function of Net-DCC and other axon guidance molecules in these cellular processes, as well as the potential for cancer therapeutics targeting Net-DCC are highlighted.

Keywords

Netrin; DCC; axon guidance; cancer; apoptosis; metastasis; growth; angiogenesis

INTRODUCTION

During the process of nervous system formation in a developing embryo, axons must grow and make proper connections with targets within the body, a process that is often referred to as pathfinding. The leading edge of the axon, known as the growth cone, senses and responds to the neuron's external environment, allowing it to be guided to its final destination with precision. In recent years, developmental biologists have made great strides in understanding how axons find their targets [1]. Much of this research has focused on axons that function in the transfer of information between the two sides of the bilaterally symmetrical central nervous system (CNS) found in higher organisms. This transfer of information occurs through commissures formed by neurons that project axons across the midline to the opposite (contralateral) side of the CNS. In vertebrates, commissural axons, which originate dorsally, project toward the midline, extend contralaterally, and then project toward their final destinations [1]. Commissural axons behave similarly in many invertebrates, as well. For example, some of the earliest growth cones in the *Drosophila* embryo pioneer the axon commissures of the CNS (Fig. (1a)). Within each segment of the developing fruit fly, most interneuron growth cones generally cross the midline before they extend either rostrally or caudally in the longitudinal processes. Many motor neurons also cross the midline in the

anterior or posterior commissures prior to finding their peripheral targets. Thus, the guidance of axons toward the midline is conserved in both vertebrate and invertebrate species [2].

Specialized midline cells regulate the axon guidance of both crossing and noncrossing axons at the ventral midline of the developing vertebrate spinal cord and the *Drosophila* ventral nerve cord. These cells, the floor plate cells of higher vertebrates and the midline glia in *Drosophila*, secrete guidance molecules that regulate the growth of commissural axons [2]. In recent years, a number of midline axonal guidance molecules have been identified. The cloning of guidance genes in a variety of organisms has indicated that some of the primary molecules that control axon guidance are conserved from insects to higher mammals. For example, Netrin (Net) proteins are a family of evolutionarily conserved laminin-related diffusible proteins that regulate axon guidance in many organisms, from nematodes and insects to higher mammals (reviewed in [1], where protein structure is discussed in more detail). Vertebrate Net proteins were first identified in the chick, where they promote the outgrowth and guidance of spinal commissural axons toward the floor plate during embryogenesis [3]. Floor plate cells express *netrin-1* (*net-1*) RNA, and *netrin-2* (*net-2*) RNA is detected at lower levels in the ventral two-thirds of the spinal cord. Cells transfected with recombinant *net-1* or *net-2* secrete diffusible forms of the proteins which can guide commissural axons from a distance and promote their outgrowth [4]. Inactivation of *net-1* in vertebrates, such as the mouse, results in pathfinding defects that prevent most commissural axons from reaching the floor plate [3]. Thus, Net proteins play a significant role in commissural axon guidance during vertebrate development.

Upon their discovery in vertebrates, sequence comparisons indicated that chick *net-1* and *net-2* are related to *C. elegans unc-6*, a previously discovered gene required for circumferential migration of axons to the midline in nematodes [5,6]. *Netrin* genes were soon isolated in *Drosophila* [7,8]. *Drosophila* Netrin A (NetA) and Netrin B (NetB; Fig. (1b)) are also expressed at the midline and are required for proper guidance of posterior commissural axons. For example, deletion of *Net A* and *B* results in defective guidance of commissural axons in fruit flies [7,8]. Recent data suggest that *Drosophila* Nets act as short-range guidance cues that promote midline crossing in flies [9].

Receptors that bind Net proteins have been identified in both vertebrates and invertebrates. For example, *Drosophila* Frazzled (Fra, [10]), Deleted in Colorectal Cancer (DCC) in mice [11, 12], and Unc-40 in *C. elegans* [13] are homologous receptors for Net proteins [1]. Axon guidance functions of Net proteins are mediated by additional Net receptors, including the Unc5 receptor family [14]. The adenosine A2b receptor [15] and Integrin [16] proteins have also been reported to function as Net receptors. More recently, the Down's Syndrome Cell Adhesion Molecule (DSCAM) has been implicated as a Net receptor that can mediate turning responses to Net-1 [17,18]. Various combinations of these receptor proteins can elicit both attractive and repulsive responses [19].

Although a number of Net receptors mediate the impacts of Net on development and cancer, this review will focus on DCC [11,12] and its *Drosophila* homologue Frazzled (which is simply referred to as DCC in this review, [10]). These homologous receptors belong to a family of transmembrane proteins that possess immunoglobulin (IG) domains and fibronectin type III (FNIII) repeats (see [1] for a schematic and additional details on DCC protein structure). DCC is expressed on commissural axons and growth cones, where it functions as a cell-surface receptor for Net in axon attraction [1,20]. Mutation or inhibition of DCC receptors results in the failure of many commissural axons to reach the midline [5,10,12]. Redistribution of Net protein by DCC receptors creates positional information for other axons, even those lacking Net receptor expression [21].

Several signaling molecules are targeted by Net-DCC signaling in the developing nervous system (reviewed by [14,19,22]). In general, activation of Net receptors promotes cytoskeletal rearrangements that regulate cell motility. Downstream of Net-DCC, crosstalk between adaptor proteins, kinases, phosphatases, and second messengers activate downstream responses (see [19] for a more detailed review of the signaling molecules downstream of Net-DCC, which are only summarized here). Net binding to DCC results in recruitment of ERK1 and ERK2 into a complex with DCC, leading to activation of MAPK signaling. Several other kinases function downstream of Net, including focal adhesion kinase (FAK), Src Tyr kinases, as well as other kinases (reviewed in [14]). Recent studies have shown that secondary messengers and ion channels function in the attraction and repulsion of axonal growth cones [14]. For example, turning behavior that is induced by Net requires Ca^{2+} influx through TRPC channels [23]. It has been proposed that at high ratios of cAMP to cGMP, Net-1 promotes attractive responses through DCC by triggering Ca^{2+} influx through L-type Ca^{2+} channels. Conversely, a low cAMP:cGMP ratio inhibits Ca^{2+} influx, resulting in repulsion [24]. It has been suggested that Rac and Rho GTPases, which are known to regulate Net-mediated actions [25] may serve as pivotal links between the pathways downstream of DCC and Ca^{2+} channels to ultimately regulate assembly/disassembly of Actin and direct cell motility [19]. Furthermore, Moore *et al.* [26] recently reported that Rho signaling reduces axonal chemoattraction to Net-1 by limiting the amount of DCC at the growth cone plasma membrane. They suggest that inhibition of RhoA by Net-1 activates a positive-feedback mechanism that facilitates chemoattraction to Net-1. Despite these advancements, identification of additional targets of Net signaling and understanding how known targets interact with each other to elicit proper guidance responses is a necessary and ongoing challenge to researchers in this field.

DCC, a Tumor Suppressor Gene: Cellular Processes Common to Oncogenesis and CNS Development

In addition to its role during nervous system development, DCC has also been linked to cancer. Loss of heterozygosity (LOH) of chromosome 18q, which includes the *DCC* gene, was identified in a large percentage of colorectal cancers [27]. Subsequently, LOH of 18q, typically associated with decreased DCC expression, was linked to many other types of cancers, ie. neuroblastoma, hematologic malignancies, and gastric, prostate, endometrial, ovarian, esophageal, breast, testicular, and glial cancers (reviewed in [20,28]). However, the status of *DCC* as a tumor suppressor gene has been questioned. As discussed by Mehlen and Furne [28], point mutations in the *DCC* coding sequence are rare, and other candidate tumor suppressor genes are located at 18q. Also, *DCC* mutant mice are not predisposed to tumors, and loss of *DCC* has not been shown to give tumor cells a selective advantage. These studies questioned the function of *DCC* as a tumor suppressor gene. Despite these concerns, a number of reports have documented roles of Net-DCC signaling in oncogenesis. Here, a number of these studies are reviewed, and the relevance of these cancer studies to nervous system development is discussed. Additionally, recent advances in our understanding of the roles of these genes during nervous system development, which may have implications for their functions in oncogenesis, are reviewed.

Many of the hallmarks of human cancer [29], such as cell survival, cell growth, formation of a blood supply to the tumor, and metastasis, involve cellular processes that are critical for nervous system development (Fig. (2)). For example, a number of molecules that function during CNS development, including Netrin, promote neuronal survival [30]. Also, neurons, like tumors, must increase their cellular mass. Take for example the giant axon of the squid, the largest known nerve cell in the animal kingdom, which can grow to a meter in length and 1 mm in diameter [31]. Given such immense growth requirements, it is not surprising that a number of axon guidance molecules, including Nets, not only can direct pathfinding, but can also promote cellular growth. Furthermore, both nervous system development and metastasis

involve invasive cell growth. Metastatic tumor cells migrate and invade surrounding tissues. Likewise, axons and dendrites migrate and invade surrounding tissues during neural development. Such invasive growth during metastasis and neural development is dependent upon the regulation of cell polarity, adhesion, and cytoskeletal organization. Understanding how modulating Net-DCC signaling regulates invasive cell growth will therefore impact our understanding of both oncogenesis and nervous system development. Finally, in recent years, the process of angiogenesis has been compared to axon guidance, and several axon guidance genes, including Net and DCC, have been implicated in both developmental and tumor angiogenesis [32,33]. Here, the function of Net-DCC signaling in each of these cellular processes is reviewed. The virtues of the *Drosophila* system for studying the function of Net-DCC and other axon guidance molecules in these cellular processes are also described.

Advantages of the *Drosophila* System for Studying Net-DCC Functions in Oncogenesis and CNS Development

Brumby and Richardson [34] recently reviewed the benefits of studying cancer in *Drosophila*, a number of which are shown in (Fig. (1)). Many of the hallmarks of human cancer, including self-sufficiency in growth and proliferative signals, insensitivity to anti-proliferative signals, evasion of apoptosis, and invasion/metastasis can be found in *Drosophila*. One great advantage is that the generation of genetic mosaics in *Drosophila* closely mimics the development of human cancers, which arise from cells that acquire successive mutations as cancers progress. In *Drosophila*, it is relatively simple to produce clones of somatic cells bearing one or more genetic modifications that are surrounded by adjacent wild-type cells. *Drosophila* researchers routinely generate somatic mutations in their studies using the FLP/FRT method of somatic recombination [35]. Furthermore, since multiple mutations typically cooperate to generate tumors, it is important to use animal models such as *Drosophila* in which multiple genetic manipulations can be performed simultaneously in somatic clones of cells. Such somatic mutation studies may more effectively simulate cancers in humans. Exploiting these advantages of the *Drosophila* system to understand the cellular roles of Net-DCC signaling is providing new insights into the functions of these signaling molecules during oncogenesis.

In addition to being an effective model organism for the study of cellular processes that are important for oncogenesis, *Drosophila* is an excellent model for analysis of CNS development (Fig. (1)). In short, the CNS of *Drosophila* is simple enough to analyze at both a genetic and cellular level, yet complicated enough to be both interesting and relevant to CNS development in organisms with more complex neural circuitries. Neural lineages are known, molecular markers for both neuroblasts and neurons have been identified, and axon trajectories are well defined. The embryonic nerve cord of *Drosophila* has been well characterized, and the roles of many genes that regulate axon growth and guidance have been investigated. The genetic analysis of nervous system development in *Drosophila* has served as a basis for understanding the genes which regulate this process in many organisms [36,37]. Given the advantages of the *Drosophila* system, it is not surprising that analysis of the roles of Net-DCC in this organism is providing insight into the function of these axon guidance molecules in oncology/CNS development. Many recent investigations have exploited the utility of the *Drosophila* system for understanding processes common to CNS development and cancer; these studies are discussed here, where they are presented in the context of vertebrate studies that have analyzed the function of Net-DCC.

Growth Regulation

Although tumor growth and neurite outgrowth have been linked to the Net/DCC signaling pathway (reviewed by [1,20,28]), relatively little is known about the mechanisms by which Net signaling induces cellular growth, defined here as the accumulation of mass. When analyzing the impact of axon guidance genes on cellular growth, it is important to discern

whether axonal defects associated with mutation of the genes of interest result from defects in axon guidance, defects in axon growth, or defects in both processes. Use of cell culture experiments in which axon outgrowth can be separated from axon guidance is one way to achieve such separation. For example, Forcet *et al.* [38] showed through culturing experiments that MAPK signaling promotes Net-induced growth in axons. When Net binds to DCC, ERK is recruited to the DCC receptor complex, and MAPK signaling is activated. They found that inhibition of ERK-1/2 in E13 rat dorsal spinal cord explants will block Net-mediated outgrowth. Neural cell culture experiments therefore indicate that MAPK signaling mediates the impacts of Net signaling on axon growth.

While cell culture methods have shed insight into the mechanisms by which Net signaling promotes growth, achieving such separation *in vivo* in a genetically tractable organism is desirable. One way to separate axon guidance vs. axon growth defects is to first analyze the impact of genes of interest on cellular growth in non-neural tissues. Preliminary studies in our laboratory have utilized the developing fly wing for this purpose. The developing wing has been used extensively to analyze cellular growth regulation in *Drosophila* [39,40]. The wing disc consists of a monolayer of epithelial cells surrounded by an external membrane. Neufeld *et al.* [39] induced ectopic gene expression using the Flp-out technique [41] in a single wing disc cell marked with GFP, allowed the cell to grow and divide over time, and then analyzed the size of the clone of cells formed. Examples of such clones are shown in Fig. (1c). The size of a clone in which the growth inhibitor Patched (Ptc) is expressed is smaller than that of a control clone, while the size of a clone in which the growth activator Cubitus interruptus (Ci, the *Drosophila* Gli homolog) is ectopically expressed is much larger (see [42] for detailed analysis of these clone sizes). Data from many clones can be collected such that statistical analyses can be performed. It is also possible to use this technique to measure cell proliferation rates *in vivo*, as the number of cells per clone can be counted. Individual cell sizes can be deduced by dividing the clone size by the number of cells per clone. An alternative measure of individual cell size includes analysis of cell size by measure of forward scatter (FSC). In these assays, discs in which clones have been generated as described above are briefly digested with trypsin and then analyzed by flow cytometry [39]. It is important to note that induction of cell proliferation does not necessarily lead to cellular growth, as it is possible to gain a larger number of smaller cells without visualizing increased clone size. Furthermore, it is possible to find larger clones that contain larger individual cells, but not an increased number of cells [39]. The latter is the case for three growth regulators that have been studied in wing disc growth assays: CycD-Cdk4 [43,44], Myc [45], and Ras [46].

We are currently analyzing the impact of Net-DCC on growth regulation using analyses comparable to those discussed above. These data suggest that ectopic Net-DCC signaling can induce growth in wing imaginal disc clone assays (VanZomerén- Dohm and Duman-Scheel, in preparation). The sizes of individual cells in which Net signaling has been activated are also increased with respect to wild type cells as detected by FSC analyses (VanZomerén-Dohm and Duman-Scheel, in preparation). Therefore, two different growth assays indicate that ectopic Net signaling induces growth during wing development. These studies have allowed us to establish the wing imaginal disc system as a useful way to study the impact of Net-DCC signaling on cellular growth and to separate the impacts of Net signaling on growth and guidance *in vivo* in a genetically tractable organism. We are now using this system to define and genetically test targets of Net signaling that may mediate its impacts on growth. The way that Net impacts cellular growth in the wing is reminiscent of clonal expression and flow cytometric analyses published for Myc [45], Cyc DCdk4 [44], and Ras [46]. Activation of these genes in wing disc clones leads to increased clone growth, as well as increased growth of individual cells. Thus, it is possible that these growth regulators mediate the impact of Net signaling on growth in the wing. Our preliminary results suggest that this may indeed be the case. We are using genetics assays to test the requirement of these and other candidate genes

which may function downstream of Net in the regulation of growth and are also pursuing more global approaches for the identification of unknown growth regulators that function downstream of Net-DCC signaling.

Invasive Cell Growth—Adherens junctions between epithelial cells allow for separation of apical and basolateral membranes which can vary in protein and lipid content. This molecular polarity is essential for cell function. For example, polarity is an important feature in neurons, which receive inputs through their dendrites and propagate action potentials to distant targets. During axon formation, a symmetrical cell generates an asymmetric domain, the leading edge of the growth cone (Fig. (2)). This loss of polarity is critical for invasive growth of the neuron [47,48]. Likewise, cell polarity is often altered in tumors. Advanced cancerous tumor cells lose their epithelial character and take on a more mesenchymal fate in a process known as the epithelial-mesenchymal transition (EMT), which is characterized by loss of apical-basal polarity and cell adhesion [49]. Such transitions accompany the progression toward malignancy (Fig. (2)). Although the majority of cancer fatalities are due to the ability of late stage tumors to metastasize, relatively little is known about the genetic alterations that cause noninvasive tumors to become metastatic [50-52]. Similarly, little is known about the mechanisms which drive asymmetric growth in neurons [48]. Recent analyses suggest that Net-DCC signaling is capable of inducing both neuronal asymmetry and metastasis.

Netrins have been linked to establishment of asymmetric neural growth. Recent studies implicate Net signaling in the establishment of asymmetrical Actin localization in developing neurons [53,54]. Binding of Net to DCC recruits an intracellular signaling complex consisting of Dcc, Nck1, Cdc42, Rac1, Pak1, and N-Wasp, which impacts the organization of Actin [53]. Furthermore, Net-1 signaling induces movement of Vg1 RNA-binding protein granules into the filopodia. Net activates asymmetric translation of eIF-4BP, a translation initiation regulator that induces a polarized increase in β -Actin, which is required for attractive neural responses.

Two recent studies in *C. elegans* also implicate Net signaling in establishment of asymmetrical growth [55]. Unc40, the *C. elegans* homolog of DCC, initiates, maintains, and asymmetrically orients neural growth. Net signaling breaks neural symmetry, and in the absence of Net signaling, the *C. elegans* HSN neuron remains symmetrical until late in development. Lipid modulators AGE-1/PI3K, and DAF-18/OPTEN drive the Actin-regulatory protein MIG-10/lamellipodin ventrally in the HSN, thereby promoting asymmetric growth. Nets therefore function not only to steer axon navigation, but also to regulate the polarized accumulation of presynaptic components through local exclusion [55]. Ziel *et al.* [56] analyzed the role of Net signaling in regulation of the anchor cell. Localized Net secretion directs DCC to the plasma membrane of the anchor cell, where it polarizes a specialized invasive membrane domain by enrichment of Actin regulators. Ultimately, these signals promote the formation of invasive protrusion from the anchor cell, which leads to disruption of the basement membrane. Ziel *et al.* [56] predict that these changes are relevant to the recently described ability of Net-1 to promote metastatic breast cancer [57].

Fitamant *et al.* [57] recently implicated Net-1 in metastatic breast cancer. In comparison to non-metastatic breast tumors, a large proportion of metastatic breast cancers have elevated levels of Net-1. Reduction of Net-1 expression or application of decoy soluble receptor ectodomains inhibits metastasis in a mouse model of lung colonization of a mammary cancer cell line, as well as in a model of lung metastasis in xenografted human breast tumors. Fitamant *et al.* [57] concluded that autocrine expression of Net-1 induces metastasis by promoting tumor cell survival and suggested that anticancer therapeutic strategies might target Net-1. In addition to promoting cell viability, Net-1 is also likely promoting remodeling of the cytoskeleton/changes in cell adhesion that directly promote a metastatic phenotype. As indicated by

Rodrigues *et al.* [58], who described metastatic phenotypes associated with Net-1 activation in human colorectal cancer cells, many of the signaling pathways that function downstream of Net, such as Rac, Cdc42, MapK, Src, and FAK, have been implicated in cancer metastasis. These proteins may therefore function downstream of Net signaling to promote invasive phenotypes.

As described above, analysis of Net signaling in *C. elegans* is providing insight into the mechanisms by which Net promotes invasive migration. *Drosophila* is also emerging as an excellent invertebrate model for studying metastasis (Fig. (1)). *Drosophila* models of tumor progression display many aspects of metastatic behaviors observed in human malignant cancers, including basement membrane degradation, loss of E-cadherin expression, migration, invasion, and metastasis to other organ sites. Analysis of *Drosophila* metastatic tumor models has provided insight into the cellular mechanisms driving tumor metastasis [59,60]. For example, recent work in *Drosophila* has uncovered a group of genes referred to as neoplastic tumor suppressors which are key regulators of epithelial polarity. These genes include *lethal giant larvae (lgl)*, *discs large (dlg)*, and *scribble (scrib)*, all of which have mammalian homologues. These three tumor suppressors function in a common pathway to regulate cell polarity and proliferation (reviewed in [61-63]). Recent studies demonstrate that neoplastic tumor suppressor mutations cooperate with other oncogenes, such as Ras, to promote tumor growth and invasion [34,52,59].

A Ras-induced tumor progression model in *Drosophila* has provided insight into the mechanisms that drive tumor metastasis. Activated Ras induces non-invasive tumors. However, mutations in genes that regulate apical-basal cell polarity, including the neoplastic tumor suppressors, cooperate with activated Ras to promote excess tumor growth and metastatic behavior. Cells in which neoplastic tumor suppressor genes are mutated normally die through activation of proapoptotic JNK signaling. However, in the presence of activated Ras, the proapoptotic role of JNK is switched to a progrowth effect. This excess growth in combination with loss of cell polarity promotes metastasis [34,52,59]. JNK signaling has been shown to induce Fos-dependent transcriptional activation of matrix metalloproteinase 1 (Mmp1), a zinc-dependent endopeptidase that cleaves components of the basement membrane/extracellular matrix [64]. The basement membrane is a specialized form of extracellular matrix found on the basal side of polarized epithelia and is composed of protein networks containing collagen and other glycoproteins. Basement membrane remodeling occurs during tumor invasion. In flies, JNK-induced Mmp dependent degradation of the basement membrane is an early event during the invasion process of metastasizing cells [65].

Recent work in our laboratory suggests that *Drosophila* is an excellent system in which to study the impact of Net-DCC signaling on metastasis. Our somatic clonal assays suggest that modulations in Net-DCC signaling can induce metastatic phenotypes in *Drosophila* larvae (VanZomeran-Dohm and Duman-Scheel, in preparation). Our preliminary work suggests that genes which can induce metastatic phenotypes in flies, such as JNK, may be responsible for inducing these phenotypes; use of *Drosophila* will allow us to genetically test this possibility and to identify other factors that function downstream of Net signaling to promote metastasis. Approaches to identify these targets will include the use of global microarrays, which were made available through the *Drosophila* genome projects. Furthermore, genetic screens could be used to identify suppressors of these metastatic phenotypes, which could lead to the identification of additional therapeutic agents that could target metastatic cancers linked to Net-DCC.

Net-DCC and Cell Survival

Recent studies have proposed that DCC functions as a tumor suppressor by promoting apoptosis. DCC can be cleaved by caspases, major proteases of the apoptotic pathway. *In*

vitro, DCC can be cleaved by caspase-3 at position 1290, which is believed to expose/release the proapoptotic addition dependence domain, ADD. In support of this model, mutation of the cleavage site inhibits the proapoptotic activity of DCC. According to the dependence receptor model, DCC induces cell death only in the absence of Net ligand. In the presence of Net ligand, DCC-induced apoptosis is suppressed. When Net-1 is withdrawn, Caspase-9 is recruited into a complex with Caspase-3 and DCC. This leads to caspase activation, cleavage of the death domain of DCC, and apoptosis. DCC-interacting protein (DIP13) can interact with the death domain and promote apoptosis. Therefore, cells expressing DCC become dependent upon the presence of Net for their survival. It has been proposed that this system is a mechanism by which DCC functions as a tumor suppressor, as pre-tumorous cells which grow in regions of ligand unavailability, are eliminated [57,66-68].

An excellent example of the dependence receptor phenomenon can be found in the developing intestine. During gut development, Net-1 promotes maintenance, integrity, migration, and renewal of the intestinal epithelium by promoting cell survival in proliferating intestinal crypt progenitors exposed to high Net levels. As cells differentiate, they migrate to the surface of the intestinal luminal epithelium, where lack of Net results in DCC-induced apoptosis. Ectopic expression of Net-1 in the mouse gastrointestinal tract results in spontaneous formation of hyperplastic and neoplastic lesions. Overexpression of Net-1 in an *APC* mutant background promotes intestinal tumor development, presumably by blocking DCC-induced apoptosis [68,69]. Recent studies in metastatic breast cancer cells [57] provide further support for the dependence receptor model. A high percentage of metastatic breast cancers have elevated Net-1 levels, which give these cells a selective advantage. When Net-1 levels are experimentally decreased or decoy soluble receptor domains are added, cell death is induced in metastatic tumor cell lines [57]. Thus, experiments in both the gastrointestinal tract and in metastatic breast cancer cells indicate that one function of Net in tumors is to inhibit cell death.

The impact of Net signaling on cell survival has mainly been studied in the context of cancer. However, as discussed by Furne *et al.* [30], *Net-1* deficient mice have increased cell death in the developing brainstem [67], suggesting that Net may promote cell survival in the nervous system. Furne *et al.* [30] analyzed the impact of Net-1 on cell survival in the mouse and chick nervous systems. They showed that Net-1 can promote survival of mouse or chick commissural neurons grown in culture. Their study demonstrated that Net-1 does not promote survival like classic neurotrophic factors such as the neurotrophins, but that Net-1 promotes neural survival by inhibiting the pro-apoptotic functions of DCC. They concluded that Nets regulate both axon guidance and neural survival in the developing nervous system. This study is an excellent example of the concept that Net-DCC signaling regulates comparable cellular processes in both neurons and tumors.

Although the dependence receptor model and the concept that apoptosis is driven by cleavage of the ADD domain in DCC are well-accepted, the recent discovery of the impacts of Net signaling on TrpC channels raises the question of whether Net signaling impacts cell survival through additional mechanisms. Net signaling is known to increase Ca^{2+} levels in neurons. Binding of Net to DCC activates phospholipase C leading to both release of Ca^{2+} from intracellular stores and Ca^{2+} influx through TrpC channels at the plasma membrane, which regulates axon turning during development [23]. In addition to regulating growth cone turning, BDNF-induced stimulation of Ca^{2+} influx has a prosurvival impact on neurons. This impact on survival is dependent upon activation of CREB [70]. Although it has not yet been tested, it is possible that activation of Trp channels by Net signaling might have a pro-survival impact on cancer cells. TrpC levels are known to be elevated in some cancers and are believed to contribute to the process of tumorigenesis [71], making this an interesting possibility.

Our laboratory has begun to analyze the impact of modulating Net-DCC signaling in somatic clones of cells during larval development. Preliminary data suggest that *DCC* mutant clones in the eye and wing disc fail to survive (Fig. (1f)). These results (VanZomerén-Dohm and Duman-Scheel, in preparation) suggest that *DCC* is required for cell viability in some developing *Drosophila* tissues. One interesting aspect of this phenotype is that it is amenable for the design of genetic suppressor screens which might provide more insight into the mechanisms by which Net-DCC promotes cell survival. Another interesting aspect of studying Net-DCC signaling in flies is that these assays are being performed in somatic clones of cells which more closely mimic human cancers. Furthermore, in this system, it is possible to combine mutations in *DCC* with mutations in other oncogenes. The ability to combine mutations in *Drosophila* has led to important advancements in recent years. For example, while somatic clones of cells bearing mutations in some genes, such as various neoplastic tumor suppressor mutations, fail to survive, combining such mutations with second site mutations in other genes can sometimes promote their survival and unleash their oncogenic potential [34, 52, 59].

Netrins and Angiogenesis—The recent discovery that Net proteins may act as regulators of tumor angiogenesis [33] has elucidated another potential avenue for cancer therapies that target Nets. The success of therapeutic angiogenesis relies on the development of strategies for establishment of a mature and functional vascular network. Establishment of this network requires i) the induction of blood vessel growth and ii) the guidance of these vessels into a properly organized network [32]. Although the molecular mechanisms underlying vasculogenesis and angiogenesis have been heavily researched in recent years, the molecular events underlying the invasive growth of blood vessels along specific paths are poorly understood. Progress in understanding how neural networks are formed during development has provided insight into the molecular pathways that drive invasive growth during cardiovascular development [32,33]. Structurally, both neurons and blood vessels exist in hierarchically ordered networks that exhibit reproducible branching patterns. The guidance of neural axons to their target destinations is dependent upon the growth cone, located at the distal tip of the axon, which senses and responds to the surrounding environment. Similarly, in the vascular system the leading edge of capillaries have tip cells (Fig. (2)), motile cells that regulate extension of the capillary sprout [33]. Given these similarities, it is not surprising that in recent years several major signaling families, including Neuropilins, Semaphorins, Ephrins, Slits, and Nets, have been implicated in the regulation of guidance events during both axonal and vascular morphogenesis [32,33].

Most studies have implicated *Unc5* as the primary receptor for Nets in the regulation of vascular morphogenesis. *Unc5B* is expressed in arteries, capillaries, and endothelial tip cells during blood vessel development in a number of vertebrate species, and analysis of the *Unc5b* knock-out mouse indicates that Net-1 inhibition of blood vessel branching is mediated by the *Unc5b* receptor [72,73]. While other studies have implicated Nets as positive regulators of angiogenesis, it is not entirely clear which Net receptor mediates these impacts. It is possible that different levels of Net may have diverse impacts on angiogenesis. It has also been proposed that the type of Net receptor differs depending on the type of angiogenic process [74]. In support of this concept, Hoang *et al.* [75] recently demonstrated that intracerebroventricular administration of Net-4 in the ischemic brain increases blood vessel density and endothelial proliferation. Net-4 expression is upregulated in the ischemic core one day post-cerebral ischemia. While *Unc5A* and *B* are not upregulated in this context, increased *DCC* levels can be detected on neuronal processes in the peri-infarct cortex. Future experiments can aim to better elucidate the roles of Net and its various receptors during angiogenesis *in vivo*.

Since a number of axon guidance molecules function in developmental angiogenesis, it is not surprising that several axon guidance genes have been implicated in tumor angiogenesis [74]. To date, studies that examine the roles of Nets in tumor angiogenesis have focused on the

potential role of Unc5 in this process. It has been proposed that Net-1 functions in the initial vascularization of tumors by repelling capillary sprouts that express Unc5b [74]. As a result of their experiments in endothelial cells, Lejmi *et al.* [76] proposed that Net-4 acts as an anti-angiogenic factor through binding to neogenin and recruiting Unc5B. Based on developmental and ischemic angiogenesis studies, it is possible that a variety of Net receptors may function in a number of different tumor types in various tissues. Further studies in additional models and multiple tumor types may uncover roles for additional Net receptors, such as DCC, in tumor angiogenesis.

CONCLUSIONS

In recent years, a number of axon guidance genes, including Netrin and DCC, have been implicated in human cancers. Many of the hallmarks of human cancer, such as cell growth, invasion, evasion of apoptosis, and formation of a blood supply to the tumor [29] involve cellular processes that are also critical during nervous system development. Net and DCC have emerged as key regulators of all of these processes (Fig. (2)). These studies indicate that the impacts of Net signaling are context dependent. For example, Net can induce either attractive or repulsive responses depending on the cAMP:cGMP ratios in neurons [24]. Furthermore, although this review focuses on the DCC receptor, other Net receptors exist, and the context-dependent nature of Net signaling is likely dependent on the presence/absence of other Net receptors. Understanding the context dependent nature of Net signaling is a key research challenge and requisite step toward the development of therapeutics that will specifically target this pathway in human cancers.

Given the context-dependent nature of Net signaling, it is critical to study it in genetically tractable organisms in which cell-cell interactions can be investigated *in vivo*. *Drosophila* is therefore an excellent system in which to study Net-DCC signaling (Fig. (1)). As discussed above, the ability to generate somatic clones of cells bearing one or more mutations in imaginal disc tissues is providing new insight into the mechanisms by which Net-DCC signaling regulates cellular processes that are critical during both nervous system development and cancer. Furthermore, analysis of Net-DCC function in *Drosophila* allows for the use of genetic screens as well as whole-genome microarrays to identify modulators and mediators of Net signaling. Since many of the cellular targets known to function downstream of Net signaling in mammalian systems are conserved in *Drosophila* imaginal disc cells (M. Duman-Scheel, unpublished), it is likely that modulators and mediators of Net signaling identified in flies will have conserved roles in humans.

It is clear that Net and DCC function in diverse cellular processes. Net-DCC signaling has also been linked to a variety of different human cancers [20,28]. The development of methods to target this pathway in human cancers is therefore of great interest. Recent studies in metastatic breast cancer cells [57] indicate that targeting Net signaling has a high therapeutic potential. A high percentage of metastatic breast cancers have elevated Net-1 levels, which give these cells a selective advantage. When Net-1 levels are experimentally decreased or decoy soluble receptor domains are added, cell death is induced in metastatic tumor cell lines [57]. Based upon these results, Fitamant *et al.* [57] proposed that Net is a promising target for alternative therapeutic strategies. They concluded that disrupting the interaction between Net and DCC could treat two-thirds of metastatic breast cancers. It is likely that comparable strategies could be applied to combat a multitude of other human cancers that have been linked to Net signaling [20,28].

ACKNOWLEDGEMENTS

Thanks to Adrienne VanZomeran-Dohm and Nicole Siddall for comments on this manuscript. Adrienne VanZomeran-Dohm contributed substantially to our own experiments described herein and illustrated in Fig. (1). Anthony Clemons photographed the adult flies shown in Fig. (1). Li Weng and Wei Du photographed the Ci/Ptc clones shown in Fig. (1). Netrin projects in the Scheel laboratory have been supported by NIH NINDS Grant # R15 NS 048904-01 and an Indiana University School of Medicine Research Enhancement Grant to MDS.

REFERENCES

1. Kaprielian Z, Runko E, Imondi R. Axon guidance at the midline choice point. *Dev Dyn* 2001;221:154–181. [PubMed: 11376484]
2. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. *Science* 1996;274:1123–1133. [PubMed: 8895455]
3. Serafini T, Colamarino SA, Leonardo ED, Wang H, Beddington R, Skarnes W, et al. Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* 1996;87:1001–1014. [PubMed: 8978605]
4. Kennedy TE, Serafini T, de la Torre JR, Tessier-Lavigne M. Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* 1994;78:425–435. [PubMed: 8062385]
5. Hedgecock EM, Culotti JG, Hall DH. The unc-5, unc-6, and unc-40 genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. *Neuron* 1990;4:61–85. [PubMed: 2310575]
6. Ishii N, Wadsworth WG, Stern BD, Culotti JG, Hedgecock EM. UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. *Neuron* 1992;9:873–881. [PubMed: 1329863]
7. Mitchell KJ, Doyle JL, Serafini T, Kennedy TE, Tessier-Lavigne M, Goodman CS, et al. Genetic analysis of Netrin genes in *Drosophila*: Netrins guide CNS commissural axons and peripheral motor axons. *Neuron* 1996;17:203–215. [PubMed: 8780645]
8. Harris R, Sabatelli LM, Seeger MA. Guidance cues at the *Drosophila* CNS midline: identification and characterization of two *Drosophila* Netrin/UNC-6 homologs. *Neuron* 1996;17:217–228. [PubMed: 8780646]
9. Brankatschk M, Dickson BJ. Netrins guide *Drosophila* commissural axons at short range. *Nat Neurosci* 2006;9:188–194. [PubMed: 16429137]
10. Kolodziej PA, Timpe LC, Mitchell KJ, Fried SR, Goodman CS, Jan LY, et al. frazzled encodes a *Drosophila* member of the DCC immunoglobulin subfamily and is required for CNS and motor axon guidance. *Cell* 1996;87:197–204. [PubMed: 8861904]
11. Keino-Masu K, Masu M, Hinck L, Leonardo ED, Chan SS, Culotti JG, et al. Deleted in Colorectal Cancer (DCC) encodes a netrin receptor. *Cell* 1996;87:175–185. [PubMed: 8861902]
12. Fazeli A, Dickinson SL, Hermiston ML, Tighe, RV, Steen RG, Small CG, et al. Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene. *Nature* 1997;386:796–804. [PubMed: 9126737]
13. Chan SS, Zheng H, Su MW, Wilk R, Killeen MT, Hedgecock EM, et al. UNC-40, a *C. elegans* homolog of DCC (Deleted in Colorectal Cancer), is required in motile cells responding to UNC-6 netrin cues. *Cell* 1996;87:187–195. [PubMed: 8861903]
14. Cirulli V, Yebra M. Netrins: beyond the brain. *Nat Rev Mol Cell Biol* 2007;8:296–306. [PubMed: 17356579]
15. Corset V, Nguyen-Ba-Charvet KT, Forcet C, Moyse E, Chedotal A, Mehlen P. Netrin-1-mediated axon outgrowth and cAMP production requires interaction with adenosine A2b receptor. *Nature* 2000;407:747–750. [PubMed: 11048721]
16. Yebra M, Montgomery AM, Diaferia GR, Kaido T, Silletti S, Perez B, et al. Recognition of the neural chemoattractant Netrin-1 by integrins $\alpha 6 \beta 4$ and $\alpha 3 \beta 1$ regulates epithelial cell adhesion and migration. *Dev Cell* 2003;5:695–707. [PubMed: 14602071]
17. Andrews GL, Tanglao S, Farmer WT, Morin S, Brotman S, Berberoglu MA, et al. Dscam guides embryonic axons by Netrin-dependent and -independent functions. *Development* 2008;135:3839–3848. [PubMed: 18948420]

18. Ly A, Nikolaev A, Suresh G, Zheng Y, Tessier-Lavigne M, Stein E. DSCAM is a netrin receptor that collaborates with DCC in mediating turning responses to netrin-1. *Cell* 2008;133:1241–1254. [PubMed: 18585357]
19. Round J, Stein E. Netrin signaling leading to directed growth cone steering. *Curr Opin Neurobiol* 2007;17:15–21. [PubMed: 17254765]
20. Chedotal A, Kerjan G, Moreau-Fauvarque C. The brain within the tumor: new roles for axon guidance molecules in cancers. *Cell Death Differ* 2005;12:1044–1056. [PubMed: 16015381]
21. Hiramoto M, Hiromi Y, Giniger E, Hotta Y. The Drosophila Netrin receptor Frazzled guides axons by controlling Netrin distribution. *Nature* 2000;406:886–889. [PubMed: 10972289]
22. Barallobre MJ, Pascual M, Del Rio JA, Soriano E, Barallobre MJ, Pascual M, Del Rio JA, Soriano E. The Netrin family of guidance factors: emphasis on Netrin-1 signalling. *Brain Res Rev* 2005;49:22–47. [PubMed: 15960985]
23. Wang GX, Poo MM. Requirement of TRPC channels in netrin-1-induced chemotropic turning of nerve growth cones. *Nature* 2005;434:898–904. [PubMed: 15758951]
24. Nishiyama M, Hoshino A, Tsai L, Henley JR, Goshima Y, Tessier-Lavigne M, et al. Cyclic AMP/GMP-dependent modulation of Ca²⁺ channels sets the polarity of nerve growth-cone turning. *Nature* 2003;423:990–995. [PubMed: 12827203]
25. Causeret F, Hidalgo-Sanchez M, Fort P, Backer S, Popoff MR, Gauthier-Rouviere C, et al. Distinct roles of Rac1/Cdc42 and Rho/Rock for axon outgrowth and nucleokinesis of precerebellar neurons toward netrin 1. *Development* 2004;131:2841–2852. [PubMed: 15151987]
26. Moore SW, Correia JP, Lai Wing Sun K, Pool M, Fournier AE, Kennedy TE. Rho inhibition recruits DCC to the neuronal plasma membrane and enhances axon chemoattraction to netrin 1. *Development* 2008;135:2855–2864. [PubMed: 18653556]
27. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759–767. [PubMed: 2188735]
28. Mehlen P, Furne C. Netrin-1: when a neuronal guidance cue turns out to be a regulator of tumorigenesis. *Cell Mol Life Sci* 2005;62:2599–2616. [PubMed: 16158190]
29. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
30. Furne C, Rama N, Corset V, Chedotal A, Mehlen P. Netrin-1 is a survival factor during commissural neuron navigation. *Proc Natl Acad Sci U S A* 2008;105:14465–14470. [PubMed: 18796601]
31. Hodgkin AL, Katz B. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J Physiol* 1949;108:37–77.
32. Autiero M, De Smet F, Claes F, Carmeliet P. Role of neural guidance signals in blood vessel navigation. *Cardiovasc Res* 2005;65:629–638. [PubMed: 15664389]
33. Klagsbrun M, Eichmann A. A role for axon guidance receptors and ligands in blood vessel development and tumor angiogenesis. *Cytokine Growth Factor Rev* 2005;16:535–548. [PubMed: 15979925]
34. Brumby AM, Richardson HE. Using *Drosophila melanogaster* to map human cancer pathways. *Nat Rev Cancer* 2005;5:626–639. [PubMed: 16034367]
35. Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* 1993;117:1223–1237. [PubMed: 8404527]
36. Doe CQ, Skeath JB. Neurogenesis in the insect central nervous system. *Curr Opin Neurobiol* 1996;6:18–24. [PubMed: 8794042]
37. Pearson BJ, Doe CQ. Neurogenesis in the insect central nervous system. *Annu Rev Cell Dev Biol* 2004;20:619–647. [PubMed: 15473854]
38. Forcet C, Stein E, Pays L, Corset V, Llambi F, Tessier-Lavigne M, et al. Netrin-1-mediated axon outgrowth requires deleted in colorectal cancer-dependent MAPK activation. *Nature* 2002;417:443–447. [PubMed: 11986622]
39. Neufeld TP, de la Cruz AF, Johnston LA, Edgar BA. Coordination of growth and cell division in the *Drosophila* wing. *Cell* 1998;93:1183–1193. [PubMed: 9657151]
40. Su TT, O'Farrell PH. Size control: cell proliferation does not equal growth. *Curr Biol* 1998;8:R687–689. [PubMed: 9768354]

41. Struhl G, Basler K. Organizing activity of wingless protein in *Drosophila*. *Cell* 1993;72:527–540. [PubMed: 8440019]
42. Duman-Scheel M, Weng L, Xin S, Du W. Hedgehog regulates cell growth and proliferation by inducing Cyclin D and Cyclin E. *Nature* 2002;417:299–304. [PubMed: 12015606]
43. Meyer CA, Jacobs HW, Datar SA, Du W, Edgar BA, Lehner CF. *Drosophila* cdk4 is required for normal growth and is dispensable for cell cycle progression. *EMBO J* 2000;19:4533–4542. [PubMed: 10970847]
44. Datar SA, Jacobs HW, de La Cruz AF, Lehner CF, Edgar BA. The *Drosophila* cyclin D-cdk4 complex promotes cellular growth. *EMBO J* 2000;19:4543–4554. [PubMed: 10970848]
45. Johnston LA, Edgar BA. Wingless and Notch regulate cell-cycle arrest in the developing *Drosophila* wing. *Nature* 1998;394:82–84. [PubMed: 9665132]
46. Prober DA, Edgar BA. Interactions between Ras1, dMyc, and dPI3K signaling in the developing *Drosophila* wing. *Genes Dev* 2002;16:2286–2299. [PubMed: 12208851]
47. Weiner OD. Regulation of cell polarity during eukaryotic chemotaxis: the chemotactic compass. *Curr Opin Cell Biol* 2002;14:196–202. [PubMed: 11891119]
48. Adler CE, Fetter RD, Bargmann CI. UNC-6/Netrin induces neuronal asymmetry and defines the site of axon formation. *Nat Neurosci* 2006;9:511–518. [PubMed: 16520734]
49. Fish EM, Molitoris BA. Extracellular acidosis minimizes actin cytoskeletal alterations during ATP depletion. *N Engl J Med* 1994;330:1580–1588. [PubMed: 8177249]
50. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2002;2:563–572. [PubMed: 12154349]
51. Pagliarini RA, Xu T. A genetic screen in *Drosophila* for metastatic behavior. *Science* 2003;302:1227–1231. [PubMed: 14551319]
52. Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumor growth and invasion through JNK activation in *Drosophila*. *Curr Biol* 2006;16:1139–1146. [PubMed: 16753569]
53. Shekarabi M, Moore SW, Tritsch NX, Morris SJ, Bouchard JF, Kennedy TE. Deleted in colorectal cancer binding netrin-1 mediates cell substrate adhesion and recruits Cdc42, Rac1, Pak1, and N-WASP into an intracellular signaling complex that promotes growth cone expansion. *J Neurosci* 2005;25:3132–3141. [PubMed: 15788770]
54. Leung KM, van Horck FP, Lin AC, Allison R, Standart N, Holt CE. Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat Neurosci* 2006;9:1247–1256. [PubMed: 16980963]
55. Poon VY, Klassen MP, Shen K. UNC-6/netrin and its receptor UNC-5 locally exclude presynaptic components from dendrites. *Nature* 2008;455:669–673. [PubMed: 18776887]
56. Ziel JW, Hagedorn EJ, Audhya A, Sherwood DR. UNC-6 (netrin) orients the invasive membrane of the anchor cell in *C. elegans*. *Nat Cell Biol* 2009;11:183–189. [PubMed: 19098902]
57. Fitamant J, Guenebeaud C, Coissieux MM, Guix C, Treilleux I, Scoazec JY, et al. Netrin-1 expression confers a selective advantage for tumor cell survival in metastatic breast cancer. *Proc Natl Acad Sci U S A* 2008;105:4850–4855. [PubMed: 18353983]
58. Rodrigues S, De Wever O, Bruyneel E, Rooney RJ, Gespach C. Opposing roles of netrin-1 and the dependence receptor DCC in cancer cell invasion, tumor growth and metastasis. *Oncogene* 2007;26:5615–5625. [PubMed: 17334389]
59. Pagliarini RA, Quinones AT, Xu T. Analyzing the function of tumor suppressor genes using a *Drosophila* model. *Methods Mol Biol* 2003;223:349–382. [PubMed: 12777740]
60. Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumor growth and invasion through JNK activation in *Drosophila*. *Curr Biol* 2006;16:1139–1146. [PubMed: 16753569]
61. Wodarz A, Nathke I. Cell polarity in development and cancer. *Nat Cell Biol* 2007;9:1016–1024. [PubMed: 17762893]
62. Bilder D. Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. *Genes Dev* 2004;18:1909–1925. [PubMed: 15314019]
63. Humbert P, Russell S, Richardson H. Dlg, Scribble and Lgl in cell polarity, cell proliferation and cancer. *Bioessays* 2003;25:542–553. [PubMed: 12766944]

64. Uhlirova M, Bohmann D. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in *Drosophila*. *EMBO J* 2006;25:5294–5304. [PubMed: 17082773]
65. Srivastava A, Pastor-Pareja JC, Igaki T, Pagliarini R, Xu T. Basement membrane remodeling is essential for *Drosophila* disc eversion and tumor invasion. *Proc Natl Acad Sci U S A* 2007;104:2721–2726. [PubMed: 17301221]
66. Mehlen P, Rabizadeh S, Snipas SJ, Assa-Munt N, Salvesen GS, Bredeisen DE. The DCC gene product induces apoptosis by a mechanism requiring receptor proteolysis. *Nature* 1998;395:801–804. [PubMed: 9796814]
67. Llambi F, Causeret F, Bloch-Gallego E, Mehlen P. Netrin-1 acts as a survival factor via its receptors UNC5H and DCC. *EMBO J* 2001;20:2715–2722. [PubMed: 11387206]
68. Mehlen P, Fearon ER. Role of the dependence receptor DCC in colorectal cancer pathogenesis. *J Clin Oncol* 2004;22:3420–3428. [PubMed: 15310786]
69. Bernet A, Fitamant J. Netrin-1 and its receptors in tumour growth promotion. *Expert Opin Ther Targets* 2008;12:995–1007. [PubMed: 18620521]
70. Jia Y, Zhou J, Tai Y, Wang Y. TRPC channels promote cerebellar granule neuron survival. *Nat Neurosci* 2007;10:559–567. [PubMed: 17396124]
71. Prevarskaya N, Zhang L, Barritt G. TRP channels in cancer. *Biochim Biophys Acta* 2007;1772:937–946. [PubMed: 17616360]
72. Larrivee B, Freitas C, Trombe M, Lv X, Delafarge B, Yuan L, et al. Activation of the UNC5B receptor by Netrin-1 inhibits sprouting angiogenesis. *Genes Dev* 2007;21:2433–2447. [PubMed: 17908930]
73. Lu X, Le Noble F, Yuan L, Jiang Q, De Lafarge B, Sugiyama D, et al. The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. *Nature* 2004;432:179–186. [PubMed: 15510105]
74. Freitas C, Larrivee B, Eichmann A. Netrins and UNC5 receptors in angiogenesis. *Angiogenesis* 2008;11:23–29. [PubMed: 18266062]
75. Hoang S, Liauw J, Choi M, Guzman RG, Steinberg GK. Netrin-4 enhances angiogenesis and neurologic outcome after cerebral ischemia. *J Cereb Blood Flow Metab* 2009;29:385–397. [PubMed: 18985053]
76. Lejmi E, Leconte L, Pedron-Mazoyer S, Ropert S, Raoul W, Lavalette S, et al. Netrin-4 inhibits angiogenesis via binding to neogenin and recruitment of Unc5B. *Proc Natl Acad Sci U S A* 2008;105:12491–12496. [PubMed: 18719102]

Fig. (1). Analysis of Net-DCC function in CNS Development and Cancer in *Drosophila melanogaster*
 Analysis of Net-DCC function in *Drosophila* is providing insight into the roles of these axon guidance regulators during development and oncogenesis. The functions of many axon guidance molecules have been examined in the *Drosophila* nerve cord, where axon trajectories (marked by BP102 staining in **a**) have been defined, and many neural and midline cell markers (*Net B* expression at midline is shown in **b**) have been identified. Furthermore, analysis of the function of Net-DCC and other oncogenes during larval development is providing insight into the roles of these and other oncogenes in cancer (see text). The generation of FLP-mediated somatic clones during larval development is a useful way to study cell growth (wild-type clone is shown in **c1**; growth inhibitor *Ptc* clone is shown in **c2**; growth activator *Ci/Gli* clone is shown in **c3**). Furthermore, metastatic phenotypes are being investigated in *Drosophila*, where metastasizing cells (green cells in **d1** are magnified in **d2**, red = Dlg staining of eye in both panels) can be seen exiting the third instar eye disc during development. Such metastatic phenotypes in the eye can also be visualized in the adult (**e**), in which red-pigmented metastasizing cells have left the eye field and entered the head region. The *Drosophila* eye is also a useful system for investigating cell death. Most *DCC* mutant somatic clones generated in the eye fail to survive; a rare *DCC* mutant clone (white-colored clone marked by arrow) is shown in **f**, where a number of dark-red twin spots lacking a *DCC* mutant twin are observed. Such scorable phenotypes in the adult eye may aid in the design of genetic screens aiming to identify modulators and mediators of Net-DCC signaling.

Fig. (2). Netrin-DCC signaling in cancer, nervous system development, and angiogenesis
Many of the hallmarks of human cancer involve cellular processes that are critical during nervous system development. The ability of Net and DCC to regulate cellular growth, cell survival, and changes in polarity at the growth cone in developing neurons (shown in the schematic here) promotes proper nervous system formation. However, the ability of Net-DCC signaling to regulate these cellular processes can also contribute to formation of metastatic tumors (above). Given that Net is known to regulate angiogenesis, a process which has recently been compared to axonogenesis [32,33], it is likely that Net signaling has comparable impacts on endothelial tip cells, which must also invade surrounding tissues. Additional details are provided in the text.