CMLS, Cell. Mol. Life Sci. 61 (2004) 1854–1866
1420-682X/04/151854-13
DOI 10.1007/s00018-004-3467-7

CMLS Cellular and Molecular Life Sciences

Review

Dependence receptors: between life and death

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Received 19 December 2003; received after revision 19 February 2004; accepted 26 February 2004

Abstract. The recently described family of dependence receptors is a new family of functionally related receptors. These proteins have little sequence similarity but display the common feature of inducing two completely opposite intracellular signals depending on ligand availability: in the presence of ligand, these receptors transduce a positive signal leading to survival, differentiation or migration, while in the absence of ligand, the receptors initiate or amplify a negative signal for apoptosis. Thus, cells that express these proteins manifest a state of dependence on their respective ligands. The mechanisms that trigger cell death induction in the absence of ligand are in large part unknown, but typically require cleavage by specific caspases. In this review we will present the proposed mechanisms for cell death induction by these receptors and their potential function in nervous system development and regulation of tumorigenesis.

Key words. Dependence receptor; apoptosis; development; tumor suppressor; caspase.

Introduction

Receptors are usually seen as inactive unless ligated by their ligands. However, recent studies have demonstrated that a number of receptors, involved in both nervous system development and cancer progression, exhibit different behavior. Studies of such receptors have revealed that in addition to their 'positive' effects on survival, differentiation and migration when bound to their ligands, these receptors transduce a 'negative' signal of cell death induction when unbound by their ligands. Thus, in the absence of ligand availability, these receptors induce programmed cell death, whereas in the presence of their trophic ligands, programmed cell death is inhibited (fig.1). Therefore, their expression leads to a state of cellular dependence on their respective ligands and that is why these receptors have been named 'dependence receptors'. To date, more than 10 receptors have been shown to display these two opposite activities: $p75^{NTR}$, the common neurotrophin receptor [1]; the netrin-1 receptors DCC [2], UNC5H1, UNC5H2 and UNC5H3 [3]; the androgen receptor (AR) [4]; RET, the receptor for GDNF (glial cell line-derived neurotrophic factor) [5]; integrins such as $\alpha_v\beta_3$ and $\alpha_5\beta_1$ [6, 7] and the receptor for Sonic hedgehog, Patched (Ptc) [8].

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$p75^{NTR}$: a concept is born

Levi-Montalcini and Hamburger demonstrated that developing neurons pass through a critical phase, during which approximately half of the neurons die [9]. Most developmental neuronal cell death is critically dependent on the availability of neurotrophic factors. Two distinct receptors have been identified to be receptors for nerve growth factor (NGF), the first neurotrophic factor dis-

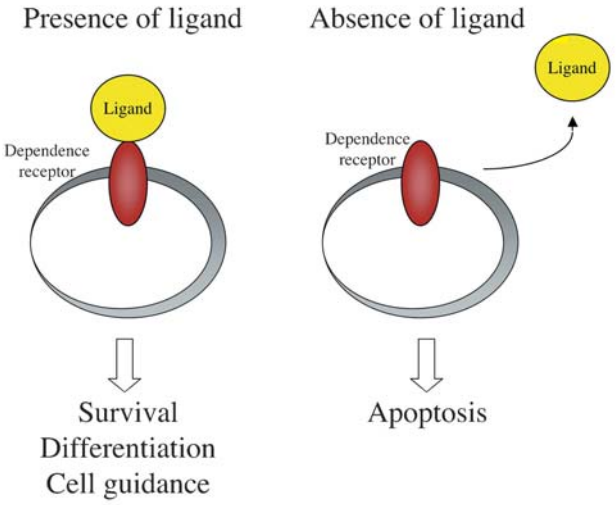

Figure 1. Dependence receptors as two-sided receptors. Current paradigm states that receptors function only in response to binding by a ligand. However, dependence receptors display two opposing signaling properties: in the presence of ligand, they induce a positive signal of differentiation, migration or survival; in the absence of ligand, they induce apoptosis. Thus, cells expressing these receptors are dependent on ligand availability for survival.

covered: $p75^{NTR}$ [10, 11], and TrkA [12]. TrkA was shown to be capable of mediating the known responses to NGF, such as neurite outgrowth and neuronal survival (for reviews, see [13, 14]). Subsequent studies demonstrated that $p75^{NTR}$ and TrkA collaborate to produce high-affinity sites for NGF binding [12] and that $p75^{NTR}$ expression may enhance the selectivity of neurotrophin binding for specific Trks (TrkA, B and C [15]). However, $p75^{NTR}$ was also shown to have Trk-independent effects. $p75^{NTR}$ is a member of a superfamily of receptors that includes the tumor necrosis factor receptors and Fas [16] (fig. 2). The relationship between these 'death receptors', which induce cell death upon binding of the pro-apoptotic factors TNF or FasL, and $p75^{NTR}$, which had been shown to bind a trophic factor that displays anti-apoptotic effects, led Bredesen and colleagues to investigate whether $p75^{NTR}$ works inversely to death receptors. It was then shown that $p75^{NTR}$ expression induced apoptosis when $p75^{NTR}$ is unoccupied by NGF, whereas binding of NGF blocks apoptosis [1, 17, 18]. The finding that $p75^{NTR}$ expression induces apoptosis in the absence of ligand but inhibits apoptosis following ligand binding suggested that $p75^{NTR}$ expression creates a state of cellular dependence on NGF. Follow-up studies provided further evidence for this notion [19–21]. However, it is important to point out that following the initial report of apoptosis induction by $p75^{NTR}$, $p75^{NTR}$ was also shown to mediate apoptosis in response to ligand binding rather than ligand withdrawal [22, 23]. This may be a Trk-dependent phenomenon, since to date it has very often been described in systems in which mismatched Trk members are expressed [24, 25].

Receptor name	Schematic representation	Ligand
$p75^{NTR}$	Chopper	NGF Nogo
DCC	(D1185) D1290	Netrin 1
UNC5H2	ZU-5 D412	Netrin 1
Integrins	MIDAS motif α β	EML (ex : laminin)
RET	Cadherin domain D707 D1017	GDNF
Ptc	D1392	Shh
AR	D146 DNA-BS PolyQ repeat Hormone-BS	Androgens

Immunoglobin-like domain
Thrombospondin type I-like domain
Fibronectin-like domain
Death domain
Cysteine-rich domain
Tyrosine kinase domain

Figure 2. Known dependence receptors. Schematic representation of $p75^{NTR}$, DCC, UNC5H, RET, Ptc, integrins and AR. For each protein, the various domains are indicated together with the positions of the caspase cleavage sites. EML, extracellular matrix ligand.

However, Miller and colleagues demonstrated that in sympathetic neurons, NGF induced $p75^{NTR}$-mediated cell death is somehow independent of Trk receptors [26]. Thus, alternatively, the decision between ligand-induced apoptosis and ligand-inhibited apoptosis mediated by $p75^{NTR}$ may depend on cell types and downstream mediators. However, it is fair to say that because the proposal of a receptor triggering apoptosis when unbound to its ligand ran counter to the dogma regarding receptor function, the view of $p75^{NTR}$ as being a more classic 'death receptor' somehow attracted more attention than the proposal for $p75^{NTR}$ as a dependence receptor. A search of other receptors that may show this ability to induce cell death in the absence of ligand was consequently performed and indeed led to the identification of other candidates, as described in the following section.

The dependence receptor family

The netrin-1 receptor, DCC (deleted in colorectal cancer) [27], was a good candidate. Netrin-1 was originally purified and identified as a diffusible molecule that can promote commissural axon outgrowth [28]. The classic view for netrin-1 action is that a gradient of this cue emanating from a ventral spinal cord structure, the floor plate, orients the growth of commissural axons as they extend circumferentially toward the ventral midline of the embryonic nervous system. The role of netrin-1 in mediating commissural axon development was fully supported by the defect of commissural neuron projections in netrin-1 null mice [29]. On a functional basis, netrin-1 not only induces axonal outgrowth but also directs the orientation of growth cones [30]. It also functions as a repellant for other axons [31], as well as playing a central role in neuronal migration (for a review, see [32]). The first proposed netrin-1 receptor was DCC, a type I transmembrane receptor of 175–190 kDa [33] whose gene is deleted through allelic loss in the majority of colorectal cancers [27, 34–36]. The sequences present in DCC's large extracellular domain of about 1100 amino acids bear strong similarity to those found in neural cell adhesion molecule (NCAM) protein family members, and include four immunoglobulin-like domains and six fibronectin type III-like motifs (fig. 2). The DCC cytoplasmic domain of 325 amino acids, however, shows little similarity to proteins with well-established functions (fig. 2).

Because of gene deletion [37] and probably because of promoter methylation [38], DCC expression is lost or reduced in colorectal cancer. This has led to the suggestion that DCC expression somehow represents a constraint for tumor development and that consequently DCC is a putative tumor suppressor. This notion is supported by the findings that (i) decreased *DCC* expression has also been demonstrated in various other cancers (for reviews [37, 39]), (ii) loss of *DCC* expression has been associated with poor prognosis [40, 41] and (iii) restoration of *DCC* expression can suppress tumorigenic growth properties in vitro and in nude mice [42, 43]. However, the rarity of point mutations identified in tumors in *DCC* coding sequences, the presence of other known and candidate tumor suppressor genes on chromosome 18q [44, 45] and the lack of a tumor predisposition phenotype in mice heterozygous for *DCC*-inactivating mutation [46] have raised questions about DCC's candidacy as a tumor suppressor. As discussed elsewhere, however [37], this evidence is insufficient to exclude a proposed tumor suppressor function for DCC.

Such a dual role for a receptor – positive role during development and putative tumor suppressor activity – appears to be a common theme for dependence receptors (see below). It was then shown that DCC expression in various cell lines lacking endogenous DCC expression led to cell death induction [2, 47]. Moreover, addition of the ligand of DCC, netrin-1, was shown to be sufficient to inhibit apoptosis [2, 48, 49].

In addition to DCC, netrin-1 also binds another family of receptors – the UNC5 family receptors, which were also good candidates for dependence receptors. Based on genetic screens, the *Caenorhabditis elegans* netrin-1 ortholog – UNC6 – was predicted to interact with UNC40 (the ortholog of DCC) and with UNC5 [50, 51]. Four homologs of UNC5 have been discovered in mammals, named UNC5H1, H2, H3 and H4 in rodents and UNC5A, 5B, 5C and 5D in humans [32, 52]. These proteins encode type 1 transmembrane receptors of roughly 110–120 KDa, each composed of two extracellular immunoglobulin(Ig)-like domains, two thrombospondin type I domains and an intracellular sequence with a ZU5 domain and a death domain (fig. 2) [52]. While DCC/UNC40 is involved in the chemoattractive response of growth cone to netrin-1, UNC5H/UNC5 are related more to the chemorepulsive effect of netrin-1. In mammals, Hong and colleagues proposed that netrin-1-mediated axon attraction occurs in axons expressing only DCC, whereas axon repulsion occurs when both DCC and UNC5H are expressed, with the pair cooperating by interacting in their intracellular domains [53]. However, this report was performed in a system in which UNC5H2 was truncated to remove its death domain, since expressing the full-length protein appears to induce cell death [53]. The presence of this death domain and the fact that UNC5Hs were described as netrin-1 receptors led us to ask whether UNC5Hs were also netrin-1 dependence receptors [3]. Similarly to DCC, expression of the different UNC5H receptors induces apoptosis in various cell lines, and the presence of netrin-1 inhibits this effect [3, 54]. Thus, the UNC5H receptors also belong to the dependence receptor family.

The third identified dependence receptor is RET (rearranged during transfection), a classical tyrosine kinase receptor. As such, it is a type I transmembrane protein that displays an extracellular ligand-binding domain, a transmembrane domain and an intracytoplasmic tyrosine kinase domain [55] (fig. 2). In addition, RET displays a cadherin-like domain extracellularly, suggesting the possibility that it may function in cell-cell interaction [56]. RET forms part of the receptor complex for GDNF and its related trophic factors neurturin, artemin and persephin [57]. These four trophic factors are related to the transforming growth factor-β (TGF-β) family. The receptor complex includes RET and a glycosylphosphatidylinositol (GPI)-anchored protein that is required for RET dimerization, the latter of which may be GFRα-1, 2, 3 or 4 [57, 58]. RET and GFRα-1 mediate a GDNF signal that plays a role in the development of the enteric nervous system and the kidney; this was clearly demonstrated by the striking similarity of the phenotype of GDNF, RET and

GFRα-1 null mice [59–61]. Ligand binding to the RET complex leads to RET autophosphorylation followed by interaction with effectors that include phospholipase Cγ, Shc, Enigma, Grb2, Grb7/Grb10, Src kinase and Ras-GAP [57].

Mutations in the RET protooncogene have been associated with both neoplasia and neural development. Mutations of RET are associated with multiple endocrine neoplasia type 2 (MEN-2), a neoplastic syndrome that includes the development of specific endocrine tumors – tumors of the parathyroid, adrenal gland (pheochromocytoma) and thyroid (medullary carcinoma of the thyroid) [62]. However, other mutations of RET have been shown to be associated with Hirschsprung syndrome, a relatively common (1 in 5000 live births) genetic defect in which neural crest-derived parasympathetic neurons of the hindgut are congenitally absent [63, 64]. Such a dual manifestation of RET – mediating aspects of both neoplasia and neural development – led to the question whether RET may function as a dependence receptor. It was then observed that the expression of RET in the absence of GDNF induced apoptosis, whereas GDNF blocked this effect, a piece of data that suggests that RET is a dependence receptor [5].

A recent report proposed that the integrins, classic receptors that mediate cellular interactions with extracellular matrix ligands such as laminins, collagens and fibronectins, may also function as dependence receptors [65]. Indeed, integrins are heterodimeric ($\alpha\beta$) type I transmembrane receptors (fig. 2), and provide a connection between the matrix and the cytoskeleton. Integrins have traditionally been considered to be pro-survival receptors, based on the concept of 'anchorage dependence' [66]. Integrin-mediated adhesion supports the formation of cytoskeletal and contractile elements, promotes cellular resistance to exogenous apoptotic stimuli and facilitates signaling by trophic factor receptors. Most cells require integrin-mediated adhesion to respond to trophic factors. This has led to the proposal that integrin control of cell adhesion and geometry, enabling responsiveness to survival factors, may be the critical role played by integrins in maintaining cell viability. However, expression of certain β3 or β1 integrins can also induce apoptosis if immobilized substrates are not available as ligands. These 'non-liganded integrins', which are either unligated or occupied by a soluble antagonist, not only disrupt survival signaling but also actively induce apoptosis, hence supporting the view of integrins as dependence receptors [6].

The most recently characterized dependence receptor is Patched (Ptc), a receptor of Sonic hedgehog (Shh). Sonic hedgehog is a secreted and diffusible glycoprotein expressed by the notochord and floor plate during development. Shh exists as a ventro-dorsal gradient of concentration in the ventral neural tube. The classical view suggests that this graduated expression determines the induction and specification of ventral neurons in the vertebrate neural tube [67]. The module of Shh signal integration in the target cells is composed of at least two receptors, Smoothened (Smo) [68, 69] and Ptc [70, 71]. Smo, a 7-transmembrane receptor, is thought to be the intracellular transducer of the positive signal of differentiation via the downstream activation of transcription factors such as Gli-1/3 [68], but it does not interact with Shh. In contrast, Ptc is a 12-transmembrane receptor, a glycoprotein of about 200 kDa that, unlike Smo, physically interacts with Shh (fig. 2). The prevailing model is that Shh binding to Ptc inhibits the repressing effect of Ptc on Smo activity, thus allowing the transducing activity of Smo [68, 69, 72]. However, the view of Shh only as a morphogen was recently challenged by LeDouarin and colleagues, who observed that the experimental withdrawal of Shh in chick embryos by partial destruction of the notochord leads to massive cell death in the developing neural tube. These results suggested that Shh is also a survival factor [73, 74]. Moreover, Ptc was described as a tumor suppressor, because Ptc is frequently mutated in association with nevoid basal cell carcinoma syndrome and basal cell carcinoma and medulloblastoma [75]. These findings led us to evaluate Ptc as a potential dependence receptor. It was found that both in vitro and in ovo, expression of the 12-transmembrane receptor Ptc in settings of Shh absence leads to apoptosis induction, while Shh addition blocks this cell death induction [8].

Finally, dependence receptors are not only transmembrane receptors. Indeed, Ellerby and colleagues proposed the androgen receptor can also share this behavior. The androgen receptor is a nuclear/cytosolic steroid receptor that includes an aminoterminal-region polyglutamine stretch, a DNA binding domain, and a carboxyterminal-region ligand-binding domain (fig. 2). Binding of androgens such as testosterone by the androgen receptor leads to nuclear translocation and transcriptional activity. Gene regulation by the androgen receptor affects widespread processes such as male gonadal development, cell survival and muscular development, among many others [76].

Mutations in the androgen receptor may be associated both with prostate cancer and neurodegeneration [77]. Neurodegeneration-associated mutants give rise to Kennedy's disease, also referred to as spinobulbar muscular atrophy (SBMA) [78]. This syndrome is associated with the degeneration of motor neurons in the brainstem and spinal cord, resulting in weakness and muscular atrophy. The associated mutations in the androgen receptor are expansions of the polyglutamine tract. Disease-associated polyglutamine tracts are typically longer than 30 glutamines, whereas those with fewer than 30 glutamines in the polyglutamine tract of the androgen receptor do not develop Kennedy's disease [79]. Interestingly, Ellerby and colleagues showed that the androgen receptor displays a profile similar to that of other dependence receptors: ex-

pression of the androgen receptor induces apoptosis in the absence of ligand, whereas the addition of ligand inhibits the receptor-induced cell death [4].

Caspase amplification by the dependence receptors

Faced with such a variety of receptors with different sequences and structures, some may argue that these receptors do not constitute a family but rather a palette of distinct receptors with a similar effect on cell fate. However, aside from the fact that they induce apoptosis in the absence of their ligand, they seem to share general functional similarities. The first similarity is the important role of caspases for the induction of cell death by these receptors. All these receptors failed to induce apoptosis in the presence of general caspase inhibitors such as zVAD.fmk or the baculovirus protein p35 [5, 8, 48]. More specifically, most of them appear to be caspase substrates. In vitro, DCC is cleaved roughly at the middle of its intracellular domain (D1290) [2]. Similarly, AR, RET, UNC5H1-3 and Ptc are all cleaved by caspases within their intracellular domains (see fig. 2) [3–5, 8]. For each protein, abrogation of the caspase cleavage by mutation of the caspase site markedly inhibits cell death induction. Thus, these receptors need to be cleaved to induce apoptosis. Interestingly, the caspase site seems conserved among mammalian species but not in *C. elegans* or *Drosophila*. For example, UNC5H has a DXXD(S) site conserved in all four mammalian proteins but not in *C. elegans,* i.e, PLKPQ. Similarly, Ptc displays a conserved Asp residue in human, mouse and even chick but not in *Drosophila*. Although it remains unproven, these findings may suggest that the appearance as a caspase substrate – and therefore the mediation of the dependent state – was a relatively late event in the evolution of these proteins. This may make sense given the greater plasticity of the mammalian and avian nervous systems, in comparison to those of the relatively hard-wired simple invertebrates. It is, however, unclear whether $p75^{NTR}$ and integrins require cleavage by caspases for activation. $p75^{NTR}$ is indeed processed proteolytically by an unknown protease [80, 81]. In addition, $p75^{NTR}$ can be cleaved in vitro by caspases [S. Rabizadeh et al., unpublished data], but it is still unclear whether such cleavage is required for the pro-apoptotic effect of $p75^{NTR}$.

Another common feature of these receptors is the presence of a domain required for the pro-apoptotic activity of these unligated receptors. These domains, called addiction/dependence domains (ADDs) [25], are required and often sufficient for cell death induction. Such a specific domain has been mapped to the intracellular domain of DCC, just upstream of the caspase cleavage site (D1290), from amino acid 1243–1264 [2]. The deletion of this domain is sufficient to eradicate the pro-apoptotic activity of DCC.

In the case of $p75^{NTR}$ and UNC5H, two regions have been shown to be important for the pro-apoptotic activity of these receptors. The first region, located near the carboxy-terminus, is the death domain. This domain bears structural resemblance to the death domains of Fas, tumor necrosis factor receptor I (TNFR I), and other death receptors. However, both $p75^{NTR}$ and UNC5H death domains are distinct from the type I death domains of Fas and TNFR I [25, 82]. Within the six-helical death domain of $p75^{NTR}$, a 29-amino acid region that lies in the fourth and fifth helices has been shown to be required for cell death induction [83]. Deletion of these ADDs has been shown to block the pro-apoptotic potential of these receptors [3, 83]. A second ADD region has been mapped for $p75^{NTR}$ and UNC5H more recently. Interestingly, this region appears very similar in both proteins and is located in the juxtamembrane region of $p75^{NTR}$ – i.e. chopper domain [84] – and in the first third of the UNC5H intracellular domain – i.e. the ZU domain [85]. These similar regions appear to mediate cell death through an interaction with NRAGE, a protein expressed in the nervous system during early development [85, 86].

The polyglutamine region was defined as the ADD of AR [4]. Forced expression of AR with an expanded polyglutamine domain led to more cell death as compared with the wild-type androgen receptor, while AR with the polyglutamine repeat deleted is only poorly pro-apoptotic [4]. It is noteworthy, however, that deletion of the polyglutamine tract did not completely suppress the pro-apoptotic activity of the AR, arguing that the pro-apoptotic dependence domain is probably not only restricted to the polyglutamine repeat [4].

Only fragmented information regarding the ADDs of RET, Ptc and integrins is known. The RET ADD is located between the two caspase sites (D707 and D1017), because expression of this region is sufficient to trigger cell death [5]. Similarly, the ADD of Ptc lies in the seventh intracellular domain, upstream of the caspase cleavage site. Indeed, expression of this seventh intracellular domain deleted of the all C-terminal portion lying after the caspase cleavage site, induces cell death [8]. In the case of integrins, a juxtamembrane domain with the sequence KLLITIHDRKEF appears to be required for cell death induction [6].

A final common feature of these dependence receptors is that ligand binding inhibits their pro-apoptotic activity. Such an inhibitory effect can occur either through inhibition of cleavage of these receptors – i.e. because this cleavage has been shown to be a prerequisite for cell death induction – or through inhibition of a downstream effect – e.g. the pro-apoptotic activity of the ADD domain. The hypothesis that the cleavage should be blocked upon ligand binding is tempting, but because the resultant fragments may have relatively short half-lives and therefore be difficult to detect in vivo, clear demonstration that

ligand blocks receptor processing by caspases has been provided to date only for the androgen receptor and UNC5H2 [4, 54]. Indirect evidence suggests that it is also probably true for Ptc, since a truncated mutant of Ptc lacking the domain lying carboxyterminal to the caspase cleavage site induced apoptosis irrespective of ligand binding [8]. This finding was interpreted to suggest that Shh binding to Ptc is likely to control Ptc cleavage or a mechanistic step upstream of cleavage. A similar mechanism is also probably utilized by netrin-DCC, although only indirect evidence supports this notion [C. Thibert et al., unpublished data]. However, this mechanism is probably not used in the case of GDNF binding to RET, since the cleavage of RET was shown to occur to a similar extent in the absence or in the presence of GDNF [5]. Thus, at least in the case of RET, it is speculated that the ligand blocks cell death downstream of RET cleavage. A tempting view would then be that in this case, the positive signaling mediated upon GDNF binding may induce a cell survival pathway that counteracts RET-induced cell death. This speculation may be extended to the other receptors, and thus ligand binding may not only block critical receptor cleavage but may also initiate, via classical signal transduction, signals blocking caspase activation. Interestingly, with the exception of integrins and Ptc, all of these receptors display homo-multimerization properties in the presence of their respective ligands [57, 87]. It is therefore tempting to hypothesize that multimerization is required not only for positive signaling as shown for RET and DCC [57, 88], but also for inhibiting cell death induction. This notion has been supported by chemical multimerization studies: $p75^{NTR}$ was found to exert its pro-apoptotic effects as a monomer, with multimerization completely suppressing this effect [83, 88]. Similar results were obtained for DCC [C. Thibert et al., unpublished data] and for UNC5H (F. Llambi et al., in prep.). In any case, the events that surround the switch between the bound-uncleaved multimeric receptors and the unbound-cleaved monomeric pro-apoptotic receptors require further definition.

Based on these common features, we propose a hypothetical model for cell death initiation by these receptors: in the presence of ligand, bound receptors transduce positive signals for differentiation ($p75^{NTR}$, AR, RET, Ptc via Smo), migration (DCC, UNC5H, $p75^{NTR}$, RET, Ptc via Smo) or survival (RET, integrins). In the absence of ligand, these receptors are recognized and cleaved by active caspases (or possibly other active proteases), leading to either (i) the release from the membrane receptor of a pro-apoptotic fragment or (ii) the exposure of a pro-apoptotic domain that, prior to cleavage, was masked by the presence of the C-terminal domain of these receptors (fig. 3). The former seems to occur for RET and UNC5H, resulting in release of the pro-apoptotic fragments comprising residues 708–717 (for RET) and 413–946 (for UNC5H). The latter mechanism is utilized by Ptc, and probably for DCC [2]. This model raises two additional questions: (i) How does the domain released or exposed trigger apoptosis and (ii) how are the receptors initially cleaved in settings in which caspases are usually thought to be inactive?

The pro-apoptotic fragments that are produced – i.e. either released or exposed – lead to the processing of caspase zymogens to active caspases either directly or indirectly. Indeed, in the case of $p75^{NTR}$, this activation appears indirect because the $p75^{NTR}$ ADD located within the death domain is capable of triggering cytochrome c release by its insertion into the mitochondrial membrane [83]. By contrast, DCC appears to directly trigger caspase activation. Indeed, DCC seems to interact directly with caspase-3 and indirectly with caspase-9. Moreover, using a cell-free system, it was shown that the DCC intracellular domain, once cleaved (amino acid 1121–1290), is able to trigger slight but significant caspase-3 activation. Hence, a putative model for DCC would be that in the absence of netrin-1, DCC, once cleaved, interacts through an adaptor protein with caspase-9, allowing caspase-3 activation [48]. Similarly, unliganded integrins form clusters on the surface of dying cells and colocalize with FAM-VAD, a covalent reporter of caspase activity. Biotin-VAD pull-downs revealed active caspase-3 and caspase-8 in these cells, and caspase-8 could be coprecipitated with integrins after antibody-mediated integrin clustering [6]. Thus, it is possible that caspases interact with dependence receptors and consequently are directly activated. However, interactions between DCC and caspase-9, on the one hand, and integrin and caspase-8, on the other hand, are probably indirect [6, 48]. Moreover, caspase-3 activation by DCC does not occur in vitro unless a cell lysate is added, suggesting that additional molecules are required [48]. One of them may be DIP13 α (DCC-interacting protein-13 α), a protein recently identified by Chen and colleagues as interacting directly with the DCC ADD [49]. Moreover, it was shown that DIP13 α is important for DCC-induced cell death. However, whether DIP13 α recruits caspase-9 remains to be determined. Finally, in some case such as the ZU domain of UNC5H or the chopper region of $p75^{NTR}$, the activation of caspase does not appear to require the formation of a caspase-activating complex, but rather requires interaction with a protein such as NRAGE [85, 86]. Thus, it is still unclear how dependence receptors trigger caspase amplification, and further studies are required to know whether dependence receptors use the same mechanism.

Because dependence receptors appear to act as functional caspase amplifiers, an important question regards the initiation of this process: if caspase activation by these receptors requires caspase cleavage of the same receptors, how is the process initiated? How can a receptor that requires caspase cleavage to produce a pro-apoptotic

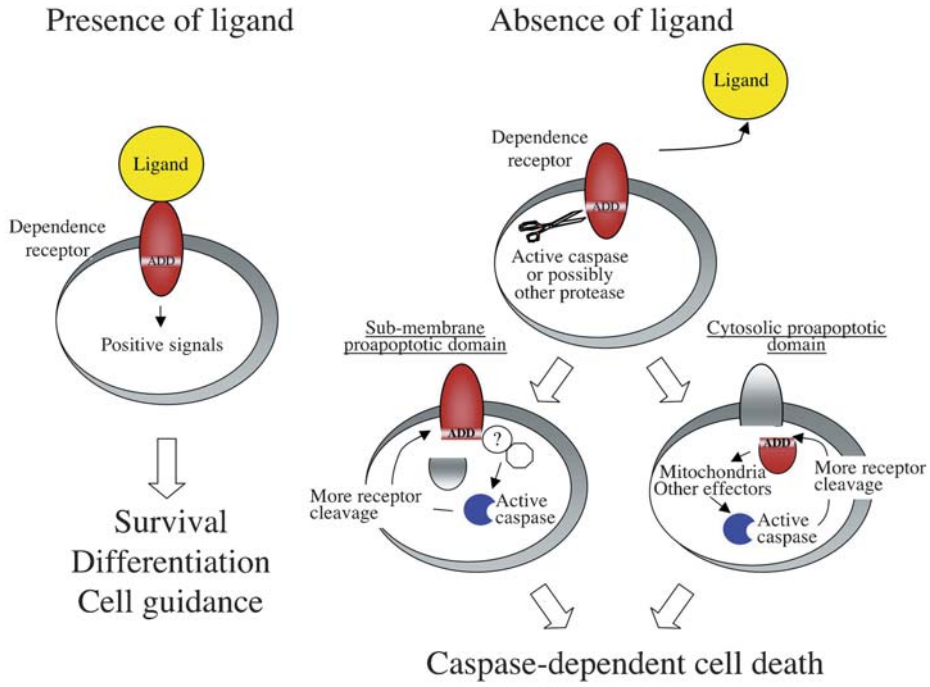

Figure 3. Putative mechanisms for apoptosis induction by dependence receptors. Dependence receptors, when bound by their ligand, mediate classical signal transduction that affects either differentiation or migration but may also inhibit the pro-apoptotic activity of these receptors; however, when unbound (or bound by an antagonist ligand), a pro-apoptotic signal occurs via negative signal transduction. In at least some cases, this is mediated by conformational change in the receptor, proteolytic cleavage and resulting pro-apoptotic dependence peptides. Such cytotoxic peptides (either at the membrane or cytoplasmic) may potentially act through mitochondria, through formation of a caspase-activating complex or through other as yet undescribed intermediates to induce cell death. ADD, addiction/dependence domain.

molecule participate in apoptosis induction, rather than simply serving a passive role in the process? One possibility is that the process may be initiated by a non-caspase protease, then propagated via caspase cleavage. Only a few cleavage events by a non-caspase protease would then be sufficient to initiate the cell death pathway by activating caspase locally enough to generate a caspase amplification loop via these receptors. An alternative view could also be that the now old dogma suggesting that caspases are completely inactive in non-apoptotic cells and are only activated massively upon pro-apoptotic stimuli is just wrong. Indeed, recent findings have shown that caspase zymogens display some protease activity [89–91]. Along the same line, it is noteworthy that cells express endogenous inhibitors of active caspases such as IAPs (inhibitor of apoptosis proteins), suggesting that cells are equipped with inhibitors that function at the point of the active caspases [91]. Similarly, local caspase activation without cell death induction is beginning to be documented [92, 93]. Thus, it seems that cell death induction could be the result of caspase activation amplification rather than caspase initiation per se. That would argue for the importance of cellular control of caspase activation/inhibition in cell fate determination: low/local activation as a positive input for the cell, high/distributed activation for cell death induction. Thus, it is possible that the balance between low/local caspase activation and high/distributed caspase activation is modulated by endogenous caspase inhibitors such as IAPs, and by endogenous caspase amplifiers such as the dependence receptors, as well as other parameters (fig. 3).

Role of pro-apoptotic activity of these receptors during nervous system development

It is fair to say that while the positive side of the different receptors presented above has been clearly demonstrated to be important in vivo, it is still unclear whether the negative side of these receptors has any relevance in vivo. However, it is interesting to note that all these receptors are implicated during nervous system development, and evidence is accumulating that cell death regulation by pairs of dependence receptors and their ligands may be crucial for shaping nervous system development.

As an example, the dependence receptors DCC and UNC5H were first described as netrin-1 receptors, and as such, involved in axon outgrowth and turning and in neuronal migration [29, 46, 52, 94]. However, both DCC and UNC5H receptors have been shown to induce apoptosis in the absence of netrin-1 [2, 3]. Hence, netrin-1 may be considered not only as a chemotropic molecule that guides axons and neurons but also as a survival factor that may determine neuron fate – i.e. survival or death – during

migratory pathfinding. Along these lines pontine cells and olivary neurons in netrin-1 knockout mutant mice are not simply incorrectly targeted but are actually absent [95, 96], probably because they undergo premature developmental neuronal death [3]. Indeed, in these netrin-1 knockout mutant mice, Llambi and colleagues demonstrated increased cell death in olivary neuron precursors that are known to express DCC and UNC5H [3]. These data support the view that netrin-1 acts as a survival factor as well as a guidance factor. As would be predicted from the dependence receptor profiles described above, DCC knockout mutant mice display a much less pronounced increase of olivary neuronal cell death than netrin-1 knockout mice [E. Bloch-Gallego, personal communication], supporting the view that the olivary neuronal death is the result of not only a loss of the positive signaling of the pair DCC/netrin-1 but also of apoptosis triggering by unbound DCC. Hence, the presence of netrin-1 along the pathway of migration may be important not only to attract or repel axons or neurons, but may be key to support survival of these neurons as well. Similarly, Jiang and colleagues recently reported that neural crest cells that migrate and colonize the developing bowel and pancreas are also dependent on DCC/netrin-1 not only for migration but also for survival [97].

Similarly, RET is expressed in the migratory neural crest cells that colonize the enteric nervous system, and RET plays a critical role in this migration, since the loss of function of RET or its ligand GDNF by gene inactivation in mice led to aganglionosis [59, 60]. However, Hirschsprung disease, which is a congenital malformation of the gut, cannot, in every case be explained simply by mutations that affect only the positive side of RET signaling [98]. Therefore, it has been hypothesized that Hirschsprung disease is not due simply to aberrant or reduced migration of neural crest cells but also to an increase in their developmental neuronal death [5]. Supporting this hypothesis, Bordeaux and colleagues observed that five Hirschsprung-associated mutations convert RET's cell death-inducing profile from that of a typical dependence receptor to that of a constitutive pro-apoptotic molecule, i.e. cell death is induced irrespective of ligand presence [5]. This finding suggests that adequate neural crest cell migration is dictated by both positive signaling that is mediated by the binding of GDNF (and other RET ligands) to RET, and by inhibition of negative signaling – here, cell death induction by RET – that regulates cell migration outside the region of adequate ligand availability.

Ptc and its ligand, Shh, are crucial for determining cell fate during development, and in particular during early development of the neural tube [67]. The currently accepted scheme is that a ventro-dorsal gradient of Shh is produced by the floor plate and the notochord, allowing the determination and differentiation of all of the ventral neurons of the neural tube. LeDouarin and colleagues have demonstrated that Shh not only determines the type of neurons but also determines their fate, since withdrawal of Shh leads to massive cell death in the neural tube and, at later stages, to strongly reduced or completely absent neural tube [73, 74]. Thibert and colleagues, using a mutant of Ptc that blocks endogenous Ptc-induced cell death, then demonstrated that blocking Ptc-induced cell death during chick neural development is sufficient to block the cell loss occurring in the Shh-depleted neural tube (fig. 4). Moreover, this mutant not only blocks cell death but actually allows development of a structure that resembles a neural tube [8]. Even though careful study is needed to determine the differentiation profile of the neuroepithelial cells in this putatively rescued neural tube, these initial

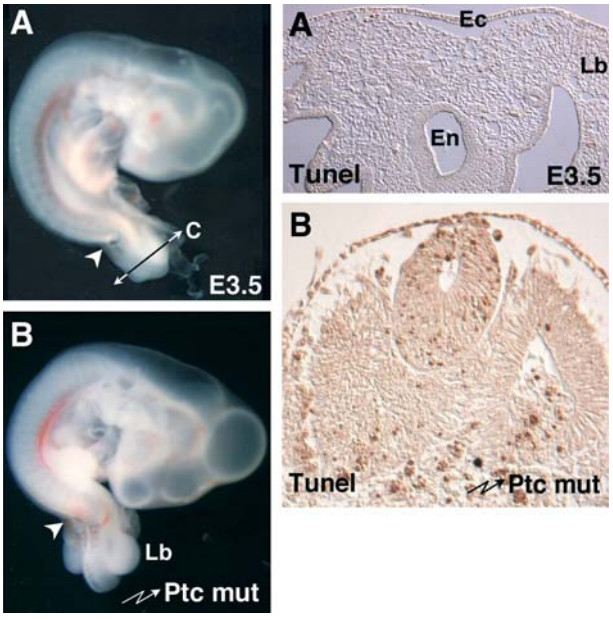

Figure 4. Ptc-induced cell death controls neural tube development. Experimental conditions leading to Shh absence (see [8]) drive massive cell death in the neural tube that frequently results in caudal destruction of the chick embryo (panel *A*, left) and to the complete disappearance of the neural tube (panel *A*, right). This has been classically explained as a loss of adequate differentiation information and consequently as death by default. However, we have shown that inhibition of Ptc-induced cell death by electroporating a dominant-negative mutant for pro-apoptotic activity (see [8]) in experimental conditions of Shh absence not only inhibits cell death but also allows development of what looks like a neural tube (panel *B*, left; partial caudal development, right; presence of a neural tube). Thus, Ptc-induced cell death may represent a regulatory mechanism that would eliminate neuroepithelial cells that growth in inadequate settings. Adapted from Thibert and colleagues [8] with permission of Science. (*A, left*) Whole E3.5 embryo electroporated with a mock construct showing the caudal destruction. (*A, right*) TUNEL staining on transverse section of *A* showing the absence of neural tube. (*B, left*) Whole E3.5 embryo electroporated with a Ptc dominant-negative (Ptc mut.) construct showing partial caudal development. (*B, right*) Ptc mut electroporation allows neural tube presence as seen by TUNEL staining. In *B* (*left*) the arrow shows the position of the inserted fragment of tantalium and indicates the site of node arrest. Ec, ectoderm; En, endoderm; Lb, limb bud.

studies argue that cell death is not simply a consequence of aberrant or inadequate differentiation but rather is an active process that shapes the neural tube. The studies also support the role of the dependence receptor Ptc in regulating cell fate during neural tube development.

A recent study by Tessier-Lavigne and colleagues led to the proposal that Shh is also a guidance cue for commissural neurons via its receptor, Ptc [99]. Thus, Ptc, like DCC, RET and UNC5H, appears to play a key role in neuronal pathfinding. Interestingly, other dependence receptors also appear to be closely related to neuronal migration and axonal guidance. Indeed, $p75^{NTR}$ was recently shown to be a coreceptor for Nogo (along with the Nogo receptor), and as such $p75^{NTR}$ appears to be involved in retraction of axons [100]. Similarly, integrins and their ligands (e.g. laminin or semaphorin) are likely to be involved in axonal guidance [101, 102]. This intriguing common trait of the dependence receptors as mediators of axon/neuronal guidance suggests a crosstalk between apoptosis regulation and neuronal pathfinding, but such crosstalk still needs to be apprehended.

Tumorigenesis and dependence receptors

The fact that the different dependence receptors trigger apoptosis in the absence of ligand suggests that they may all act as regulators of tumorigenesis. Indeed, the absence of ligand availability encountered because tumor cells intensively divide in a context of constant ligand concentration or because tumor cells metastasize may turn these receptors into killing machineries that consequently inhibit tumor progression by inducing tumor cell death. This model, which has yet to be demonstrated, is supported by multiple observations. First, the different dependence receptors seem to be implicated in cancer development and progression. For example, AR is associated with prostate cancer [77], and interestingly, epidemiological studies have shown that men bearing AR with short polyglutamine stretches, the domain responsible in large part for receptor pro-apoptotic activity, have a statistically significant higher likelihood of developing prostate cancer, especially metastatic prostatic cancer [103]. Similarly, Pflug and colleagues [104] found a progressive decrease in $p75^{NTR}$ expression associated with the development of prostate neoplasia, with cell lines from metastatic prostate carcinomas failing to express $p75^{NTR}$. Reexpression of $p75^{NTR}$ in PC3 prostate carcinoma cells restores a state of neurotrophin dependence, resulting in apoptosis if NGF is not supplied [83].

DCC has also been proposed to be a tumor suppressor, because loss or reduction of DCC expression occurs in numerous tumors of many different origins, and in particular in colorectal cancer [37]. However, while some lines of evidence argue against DCC as a classical tumor suppressor [46], no mechanism has been proposed to explain the ability of DCC to inhibit tumor growth in vitro and in nude mice [42]. The pro-apoptotic activity of DCC in the absence of netrin ligand would be consistent with a role for the pair DCC/netrin-1 in homeostatic regulation of intestinal epithelium, and possibly in the suppression of tumorigenesis. Specifically, the observation that netrin-1 is produced mainly at the bases of the intestinal crypts, whereas DCC is expressed along the villi [L. Mazelin et al., unpublished data], might allow DCC and netrin-1 to play key roles in intestinal epithelium fate. Within the crypt, cells at the base of the crypt express DCC in an environment that is also rich in netrin-1, thus promoting survival. However, as the intestinal cells cease proliferation and move toward the tip of the villus, they will encounter progressively lower concentrations of netrin-1, placing them at increased risk for DCC-mediated apoptosis.

In the context of tumor development and progression, the loss of DCC appears to be a relatively late event in colorectal tumorigenesis [35, 105], suggesting that it may be linked in some fashion to the acquisition of invasive or metastatic properties. Thus, it is tempting to speculate that there might be a powerful selective advantage for a transformed colonic epithelial cell to lose DCC function, and thus netrin dependence (fig. 5). This speculation also fits well with several recent observations on the other netrin-1 receptors, UNC5H1, 2 and 3. Indeed, it has been found that expression of the UNC5H2 (UNC5B) receptor is regulated by the tumor suppressor p53, and UNC5H2/B plays a crucial role in p53-induced apoptosis [54]. Moreover, like DCC expression, UNC5H expression is lost or

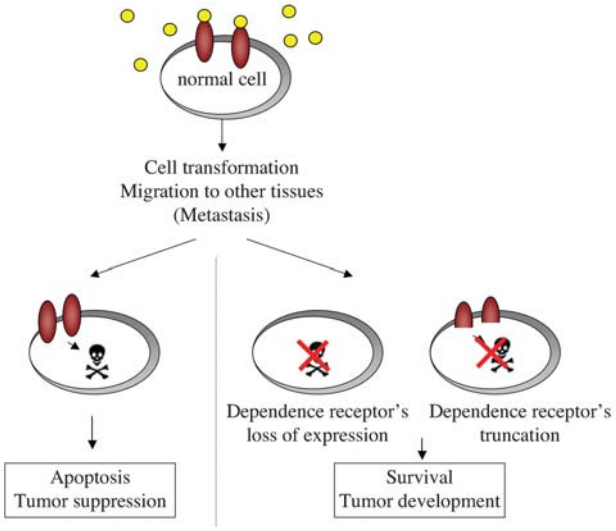

Figure 5. Dependence receptors and tumor suppression. Normal cells located in their normal environment express dependence receptors that are bound to ligands supplied by local sources. However, migration away from locally supplied ligands leads to apoptosis induced by unbound dependence receptors. Loss of expression of dependence receptors may thus allow metastasis due to the loss of requirement for ligand support.

reduced in the vast majority of colorectal tumors, and the loss/reduction is in part related to loss of heterozygosity (LOH) affecting the chromosome regions which harbor the respective UNC5H loci.

Several observations suggest that Ptc is a tumor suppressor. Premature protein termination and inactivating mutations of Ptc or loss of Ptc expression are frequently associated with various cancers such as basal cell carcinoma and medulloblastoma [75]. Moreover, Ptc expression inhibits the hallmarks of cell transformation in vitro [106], and Ptc heterozygous mutant mice developed spontaneous BCCs upon ultraviolet radiation [107]. We have recently shown that in vitro, Ptc expression inhibits anchorage-independent growth through the control of apoptosis. Indeed, Ptc expression inhibits growth in soft agar of transformed cells, an inhibition blocked by the presence of Shh, by general inhibitors of caspases or by a Ptc mutant resistant to caspase cleavage [8].

These data suggest that Ptc but also more generally dependence receptors may act as a tumor suppressor by controlling apoptosis. These results support the notion that dependence receptors may play a role in metastasis suppression by limiting tumor growth outside of ligand-expressing territories. However, more direct and in vivo evidence for such a role of the pro-apoptotic activity of these receptors in regulating tumor expansion is required to support this tempting hypothesis.

Conclusion

From a classical dogma presenting a receptor as an inert molecule anxiously waiting for a ligand to be switched on, the dependence receptor hypothesis proposes that more that 10 receptors show some intriguing similarities with respect to their pro-apoptotic functions when unliganded, their implication in migration/guidance during development and their frequent loss of expression or mutations in cancer. Data that have supported this notion of an active unbound receptor triggering cell death are mainly based on cell culture experiments. Only recently has some work shown that the bizarre receptor behavior observed in cell culture may be of great importance in vivo as was shown recently in the case of Ptc-induced cell death in neural tube development [8]. For sure, additional data will be required to demonstrate that some or all of these proteins do indeed demonstrate vital negative regulatory roles in developing tissues. Similarly, it remains to be demonstrated that their inactivation during tumorigenesis is intimately linked to more robust growth and survival of associated cancer cells. Nonetheless, evidence for a pro-apoptotic unbound receptor is beginning to emerge, and the major question now is to see whether these dependence receptors represent a restricted family of receptors or whether it is a more general phenomenon that can be applied to many receptors.

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