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Signalling by death receptors in the nervous system

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Summary

Cell death plays an important role both in shaping the developing nervous system and in neurological disease and traumatic injury. In spite of their name, death receptors can trigger either cell death or survival and growth. Recent studies implicate five death receptors — Fas/CD95, TNFR1 (tumor necrosis factor receptor-1), p75^{NTR} (p75 neurotrophin receptor), DR4 and DR5 (death receptors-4 and -5) — in different aspects of neural development or degeneration. Their roles may be neuroprotective in models of Parkinson's disease, or pro-apoptotic in ALS and stroke. Such different outcomes likely reflect the diversity of transcriptional and post-translational signaling pathways downstream of death receptors in neurons and glia.

Introduction

The term “death receptor” refers to a group of eight members of the TNFR superfamily of single-pass transmembrane proteins [1]. The death receptors are the only members of this family whose cytoplasmic tail contains a conserved 80-amino acid sequence referred to as the “death domain”. In the appropriate cellular context, activation of a death receptor leads to formation of a death-inducing signaling complex (DISC) at the death domain and subsequent triggering of downstream cascades leading to death of the cell which expresses the receptor [1,2•]. Physiologically, death receptor activation can occur in *cis*, by transmembrane ligand expressed in the same cell as the receptor, or in *trans*, by ligand expressed at the surface of other cells or released into the extracellular milieu.

This review will focus on five death receptors and their ligands for which new roles in the nervous system have been reported over the last two years. FasL/CD95L triggers cell death

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Conflicts of Interest

The authors declare that they have no conflict of interest.

through its receptor Fas/CD95 by recruiting the adapter protein FADD (Fas-associated death domain) and pro-caspase-8. This ligand-receptor couple will be referred to simply as FasL and Fas in the body of the review. TNF α (tumor necrosis factor alpha) acts as a soluble homotrimer through two TNFR family members: TNFR1, which has a death domain, and TNFR2, which does not. Death signaling by TNFR1 involves recruitment of adapter signaling molecules TRADD (TNFR-associated death domain), FADD and RIP (receptor interacting protein). Death receptors-4 and -5 (DR4 and DR5) share the same ligand - TRAIL (TNF-related apoptosis inducing ligand) - and signal similarly to Fas. Lastly, p75^{NTR} can signal cell death when bound by NGF (nerve growth factor) or other ligands. Like the other 30 members of the TNFR family, death receptors also play multiple roles in cell differentiation, growth and proliferation [2•]. We will highlight examples of these as a reminder that expression of a death receptor in a given tissue does not necessarily imply a role in degeneration or death.

Death receptors in nervous system development

Death receptors and their ligands are expressed in the developing nervous system, and many recent reports confirm their ability to trigger death of embryonic neurons and glia *in vitro* [3-15]. However, with the exception of earlier reports concerning p75^{NTR}, there is still relatively little evidence that death receptors play a role in nervous system development *in vivo*. This may be because they mostly regulate phenomena other than cell death at this stage. The spontaneous mouse mutants *lpr* and *gld*, which are partial loss-of-function mutants for Fas and FasL, respectively, have normal numbers of cortical, hippocampal and motor neurons at birth [16,17•]. However, they do show reduced dendritic branching of hippocampal and cortical neurons during the period at which synaptogenesis is occurring [17•]. At similar stages, mice deficient in TNF α , which is normally produced by glia, show defects in synaptic scaling and ocular dominance plasticity in the visual system [18,19]. However, a full understanding of the overall role of death receptors in nervous system development will require a more systematic approach in null mutant mice.

Involvement of death receptors in disease mechanism and therapeutic strategies in ALS

There is now a considerable body of data pointing to a role for death receptors in pathological situations. This provides new opportunities for therapeutic intervention, focused on the death receptors themselves, their ligands or intracellular relays. Many pharmacological compounds, recombinant proteins and genetic strategies have therefore been designed and tested in rodent models of disease. Recent reports are summarized in Table 1.

Currently the best evidence of a role for death receptors in neurological diseases comes from the study of amyotrophic lateral sclerosis (ALS). In patients with ALS, degeneration and death of motor neurons in the spinal cord lead to progressive and fatal muscle paralysis. Much of our understanding of the underlying mechanisms comes from studies of transgenic mice overexpressing toxic mutant forms of SOD1 (Cu, Zn-superoxide dismutase-1), which cause ALS in a subset of patients with a familial form of the disease. Mutant SOD1 triggers neurodegeneration in a cell-autonomous fashion in motor neurons, but also drives disease progression through non-autonomous mechanisms involving microglia and astrocytes [20-22]. Three death receptors — TNF α , p75^{NTR} and Fas/CD95 — have been implicated in cell autonomous and/or non-autonomous aspects of the disease.

Upregulation of TNF α and TNF receptors is observed in parallel with the appearance of symptoms in SOD1 mice [23,24] and increased levels of TNF α are found in plasma of ALS patients [25]. However, genetic ablation of TNF α has no beneficial effect on survival, motor axon degeneration or gliosis in either of two different lines of mutant SOD1 mice [26•]. Although application of recombinant TNF-binding protein-1 (rhTBP-1) had protective effects in *wobbler* mice - another model of motor neuron degeneration - the treatment did not in fact

reduce TNF α levels in the CNS [27]. The beneficial effects of treatments as diverse as thalidomide, folic acid, insulin-like growth factor-1, and metal chelators are all correlated in SOD1 mice with reductions in TNF α levels [28–31]. However, it does not seem likely that they all act through TNF α /TNFR directly to prevent degeneration; the reductions more probably reflect a decrease in inflammation secondary to neuroprotection by other mechanisms. Given the lack of evidence for a direct role in motor neuron death, an interesting possibility is that TNF α may trigger the redistribution of axonal mitochondria known to occur in ALS motor neurons *in vivo* [32].

Reactive astrocytes in the spinal cord of SOD1 mice express NGF and can trigger motor neuron death *in vitro* in a p75^{NTR}-dependent manner [33,34]. Moreover, NGF acting through p75^{NTR} in the absence of nitric oxide triggers the death of cultured motor neurons from SOD1 mice, but not from controls [8]. However, earlier pharmacological studies produced conflicting results as to the role of p75^{NTR} in disease progression *in vivo*. Genetic testing of the potential role of p75^{NTR} in ALS mice is therefore a crucial next step.

Probably the strongest evidence for death receptor involvement in ALS concerns Fas/CD95. Motor neurons can be triggered to die *in vitro* by activation of a motor neuron-specific pathway downstream of Fas (called the Fas/NO pathway), which includes an amplification feedback loop Fas-NO-FasL-Fas [10•,35]. Motor neurons cultured from SOD1 mutant embryos are hyper-sensitive to Fas activation [10•,35,36•]. In presymptomatic SOD1 mice *in vivo*, activation or misexpression of all intermediates in the Fas/NO pathway, together with enhancement of Fas-FADD interactions, is observed [10•,24,36•–39•]. Importantly, therapeutic benefit has been observed following inhibition of Fas/FasL function. Intrathecal infusion of siRNAs against Fas in mutant SOD1 mice almost completely blocks the pathological activation of caspase-8, p38 and other elements of the Fas/NO pathway, protects motor neurons and prolongs lifespan [36•]. Partial loss-of-function mutations for FasL confer a modest extension of survival in the same mice [40]. It will be important to repeat these experiments using true null mutants for Fas and FasL. Intriguingly, lithium, a drug widely used in mood disorders, was also reported to block the activation of Fas, FADD and caspases-8 and -3 [39•] and to provide neuroprotection, not only in mutant SOD1 mice [39•,41•] but also in a small cohort of ALS patients [41•]. It remains to be determined whether these data can be replicated in larger controlled studies and whether lithium acts by inhibiting apoptosis or by activating autophagy [41•].

More indirect evidence of a role for Fas comes from studies of the matrix metalloproteinases (MMPs), which are involved in the proteolytic processing of TNF α and FasL from precursor to soluble forms. Inhibition of MMPs with the broad spectrum inhibitor Ro28-2653 had some positive effects in mutant SOD1 mice [42]. However, genetic knockout of MMP-9 attenuated disease in one study [37] but exacerbated symptoms in another [43]. Tissue inhibitors of metalloproteinases can signal through MMP inhibition to enhance stabilization and activation of death receptors such as Fas. One member of this family, TIMP-3, is strongly upregulated in degenerating motor neurons in spinal cords of SOD1 mice [38].

Overall, therefore, there is intriguing presumptive evidence that Fas signaling may play a role in ALS, but more data from genetic studies in mice and human patients are required to support this hypothesis.

Death receptors in other diseases and degenerative processes in the nervous system

In models of Alzheimer's disease, death receptor signaling may potentially play a role both upstream and downstream of accumulation of the A β (amyloid-beta) peptide. TNF α stimulates expression of BACE1 (beta-site amyloid precursor protein-cleaving enzyme-1) and consequently production of A β [44]. In turn, cell death of septohippocampal neurons induced

by A β *in vitro* and *in vivo* requires the presence of p75^{NTR} [12]. Data from several different models of spinal cord injury, axotomy, deafferentation and ischemia provide strong evidence that Fas activation has a deleterious effect on survival of injured neurons [15,16,45-48]. In contrast, in the MPTP model of Parkinson's disease, Fas activation protects dopaminergic neurons from MPTP toxicity, providing the first *in vivo* evidence that death receptors can act to enhance neuronal survival [49]. In the same MPTP model, TNF α has opposite effects depending on the cell context: it is protective for hippocampal neurons but triggers degeneration of dopaminergic axons [50]. These results exemplify the importance of cellular context in determining whether death receptor signaling is pro- or anti-apoptotic.

Signaling mechanisms downstream of death receptors in neural systems

The generality of the classical model involving activation of caspases and mitochondrial pro-apoptotic factors through formation of a DISC is currently questioned [2•]. It has been particularly challenged by two sets of results in the nervous system: (a) demonstrated requirements for novel signaling and transcriptional events in the execution of neuronal death; and (b) unexpected outcomes of death receptor activation such as cell survival and neurite outgrowth [2•]. These are summarized for dying and healthy neurons and glia in Figure 1.

Involvement of stress-activated protein (SAP) kinases represents a first layer of complexity superimposed on the prototypic death receptor pathway. Depending on the cellular context, different SAP kinases are involved. To kill motor neurons, Fas needs to selectively activate p38 kinase but not JNK (c-Jun N-terminal kinase) [35]. This in turn leads to obligate transcriptional upregulation of neuronal nitric oxide synthase [10•]. DR4 acts through JNK3 but not p38 kinase to trigger oligodendrocyte apoptosis [6], whereas TNFR1 in neuroblastoma cells signals through both JNK and p38 kinase [13]. p75^{NTR} death signalling also shows cell type-dependent complexities. Production of ceramide by neutral sphingomyelinase is an obligatory step for caspase activation in NGF-treated motor neurons [8] whereas in sensory neurons, p75^{NTR} elicits potassium efflux through phosphatidylinositol-4,5-bisphosphate-dependent activation of G protein-coupled inward rectifier potassium (GIRK) channels [4•].

In spite of their name, death receptors can activate non-apoptotic pathways in appropriate cellular contexts, and are indeed required for cell survival and tissue regeneration in several systems [2•,49]. Fas-induced branching of cortical and hippocampal and sensory neurons does not require caspase activation [17•]. In agreement with this, Fas-triggered axonal outgrowth involves activation of the MEK1/ERK1/p35 pathway in sensory neurons and of the Ezrin-Rac1 pathway in cortical neurons [51].

Control of death receptor signaling can occur at multiple levels. In neurons, both Fas and TNFR localize to lipid rafts, specialized microdomains of the plasma membrane, and such localization seems to be required for death signaling [5]. Neuronal cell death induced by p75^{NTR} requires palmitoylation, translocation to lipid rafts and metalloproteinase cleavage of the extracellular domain [14]. In agreement with the emerging importance of subcellular compartmentalization of death signaling pathways, proteomic analysis of reverse signalling through FasL reveals interactors associated with endocytosis and trafficking [52]. There are diverse alternative mechanisms for control of the outcome of death receptor activation. Regulation of Fas or TRAIL-R2/DR5 can occur at the transcriptional level in a p53-dependent manner [15,37,46]. In cancer cells, activation of DR4 and DR5 is regulated by O-glycosylation and by decoy receptors [1,53]. Intracellular inhibitory or decoy proteins such as FLIP (FLICE inhibitory protein), LFG (lifeguard), FAIM_L (Fas apoptosis inhibitory molecule, long form), survivin or GMEB1 (glucocorticoid modulatory element binding protein-1) can prevent caspase activation downstream of death receptors and thereby confer resistance to death signals, providing a novel mode of action for neurotrophic factors [3,5,7,11•,54].

Conclusions

Our vision of death receptor signaling has radically changed over the last decade, as the many data pointing to “positive” signaling outcomes such as growth or survival have been progressively taken into account. Cellular context strongly affects the choice of outcome in the nervous system but potential molecular mechanisms to explain this — alternative co-receptors, interaction with other signaling pathways — need to be further investigated. Nevertheless, the classical role of death receptors as triggers of degeneration and cell death seems to come into play in several forms of neurodegeneration, particularly ALS and stroke. New genetic and pharmacological tools will allow these hypotheses to be more fully tested *in vivo*.

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Abbreviations and references in which they are mentioned

ASK-1, apoptosis signal-regulating kinase 1 [24,35]
 ERK, extracellular signal-regulated kinase [6]
 FADD, Fas-associated death domain [5,9,16,35,38,47,55•]
 FAIM, Fas apoptotic inhibitory molecule [11•]
 GIRK, G-protein-coupled inwardly rectifying potassium [4•]
 GMEB1, glucocorticoid modulatory element-binding protein 1 [54]
 JNK, c-Jun N-terminal kinase [6,9,13,24,27]
 LFG, lifeguard [3,5]
 MEKK1, mitogen-activated protein kinase/ERK kinase 1 (MEK1) kinase
 MKK, mitogen activated protein kinase kinase [6,24]
 MMP, matrix metalloproteinase [37,38,42,43]
 nNOS, neuronal nitric oxide synthase [9,35,46]
 nSMase, neutral sphingomyelinase [8]
 NTF-R, neurotrophic factor receptors
 PACSIN, protein kinase C and casein kinase substrate in neurons [52]
 SNX18, sorting nexin 18 [52]
 PIP2, phosphatidylinositol 4,5 biphosphate [4•]
 TACE, TNF α converting enzyme
 TIMP, tissue inhibitor of metalloproteinases [15,38]
 TRADD, tumor necrosis factor receptor-associated death domain [13]
 TRAF, tumor necrosis factor receptor 1-associated factor [39•,52]
 TRAIL-R, TNF-related apoptosis-inducing ligand (TRAIL) receptor

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Figure 1. Death receptor signaling pathways in neurons and glia

The two panels summarize novel aspects of death receptor signaling reviewed in the text. (A) Signaling events activated during cell death or degeneration. Once activated by their ligands (not shown, for clarity), death receptors cluster into multi-molecular assemblies in lipid rafts and trigger a series of post-translational and transcriptional events leading to caspase activation downstream of mitochondrial decision points. (B) Signaling events activated during cell survival or growth. Multiple control mechanisms act coordinately to prevent death receptor activation and inhibit downstream signaling. In addition, death receptors can stimulate signaling pathways directly associated with growth or survival.

Pharmacological and genetic interventions in death signalling pathways in the nervous system in vivo

The Table summarizes recent *in vivo* studies targeting the nervous system, grouping them by target — ligand, receptor or effector — and by the pathology that they most closely model. Genetic gain- or loss-of-function or pharmacological approaches have provided important validation (or invalidation) of mechanisms identified *in vitro*, and can also serve as proof-of-principle for subsequent therapeutic strategies. The principal outcomes of these interventions are described in the text. Abbreviations: MPTP (1-methyl-4-phenylpyridinium), SNA (sciatic nerve avulsion), (MCAO (middle cerebral artery occlusion), *lpr* (lymphoproliferative), *gld* (generalized lymphoproliferative disease), JEV (Japanese Encephalitis Virus).

Table 1

Ligand	Molecule/Gene	Pathology	Model	Approach	Delivery	Reference
TNFα	TNFα	ALS	SOD1 G93A, G37R mice	gene KO		[26•]
	TNFα	Parkinson	MPTP neurotoxicity	gene KO		[50]
	TNFα	synaptic scaling	Neuroplasticity	gene KO		[18,19]
	TNFα binding protein	ALS	wobbler mice	recombinant protein	subcutaneous	[27]
TNFα and FasL	Thalidomide, lenalidomide	ALS	SOD1 G93A mice	pharmacological	oral	[29•]
	MMP-9	ALS	SOD1 G93A mice	gene KO		[37,43]
TNFα & IFNγ	IFNγ receptor type 1	Alzheimer	APP mice	gene KO		[44]
	FasL	ALS	SOD1 G93A mice	<i>gld</i> mutation		[40]
FasL	FasL	motor neuron injury	SNA	<i>gld</i> mutation		[46]
	FasL	branching	Neural development	<i>gld</i> and <i>lpr</i> mutations		[17•]
	TIMP-3	stroke	MCAO	gene KO		[15]
Receptor complex	soluble Fas receptor	spinal cord injury		recombinant protein	intrathecal	[45]
TNFR	TNFR1/2	Parkinson	MPTP neurotoxicity	gene KO		[56]
	TNFR1/2	neuronal injury	Trimethyltin	gene KO		[55•]
	TRADD siRNA	Encephalitis	JEV virus	gene silencing	intracerebral	[13]
Fas	Fas siRNA	ALS	SOD1 G93A mice	gene silencing	intrathecal	[36•]

	Molecule/Gene	Pathology	Model	Approach	Delivery	Reference
	FLIP(L)	stroke	MCAO	transgenic expression		[47]
	Fas	motor neuron injury	Axotomy	<i>lpr</i> mutation		[16]
	Fas	Parkinson	MPTP neurotoxicity	<i>lpr</i> and <i>gld</i> mutations		[49]
FADD?	Lithium	ALS	SOD1 G93A mice, human patients	pharmacological	oral, intraperitoneal	[39,41•]
p75 ^{NTR}	p75 ^{NTR}	Alzheimer	A β neurotoxicity	gene KO		[12].
Effector						
DAXX	Daxx dominant negative	ALS	SOD1 G93A mice	transgenic expression		[9,10]
NO synthase	nNOS, iNOS	motor neuron injury	SNA	gene KO		[46]
Free radicals	Neu2000	ALS	SOD1 G93A mice	pharmacological	oral	[39•]