

Review

All in the family: evolutionary and functional relationships among death receptors

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Abstract

Over the last decade, significant progress has been made towards identifying the signaling pathways within mammalian cells that lead to apoptosis mediated by death receptors. The simultaneous expression of more than one death receptor in many, if not all, cell types suggests that functional innovation has driven the divergence of these receptors and their cognate ligands. To better understand the physiological divergence of the death receptors, a phylogenetic analysis of vertebrate death receptors was conducted based upon amino-acid sequences encoding the death domain regions of currently known and newly identified members of the family. Evidence is presented to indicate an ancient radiation of death receptors that predates the emergence of vertebrates, as well as ongoing divergence of additional receptors both within several receptor lineages as well as modern taxonomic lineages. We speculate that divergence among death receptors has led to their functional specialization. For instance, some receptors appear to be primarily involved in mediating the immune response, while others play critical roles during development and tissue differentiation. The following represents an evolutionary approach towards an understanding of the complex relationship among death receptors and their proposed physiological functions in vertebrate species.

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Abbreviations: EST, expressed sequence tag; TNF, tumor necrosis factor; TNFR, TNF receptor; *Dr*, *Danio rerio*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Gg*, *Gallus gallus*; *Xl*, *Xenopus laevis*; *St*, *Silurana tropicalis*; *Oi*, *Oryzias latipes*

Introduction

In all vertebrate cells, there exist multiple and often redundant apoptotic pathways which can be activated in response to a plethora of internal and external cellular signals. Internal pathways leading to apoptotic cell death may be initiated by stimuli including ultraviolet irradiation, growth factor withdrawal and chemotherapeutic agents, most of which induce mitochondrial perturbations often mediated via proapoptotic Bcl-2 family members. Affected mitochondria subsequently release cytochrome *c*, which combines with Apaf-1 to form the apoptosome complex, and/or Smac/Diablo, which inactivates Inhibitor of Apoptosis Proteins such as XIAP. Both of these factors ultimately promote a cascade of caspase enzyme activation leading to cell death.^{1,2}

Alternatively, extracellular factors, in particular those from the tumor necrosis factor (TNF) family of ligands, bind to cognate members of the death domain-containing subfamily (death receptors) from the larger tumor necrosis factor receptor superfamily (TNFRSF), which then indirectly initiate the caspase cascade. Intracellular death domains of activated death receptors recruit and bind cytoplasmic adaptor proteins via homophilic interactions, while adaptor proteins (eg. TNF receptor-1-associated protein, TRADD, and Fas associated via death domain FADD), in turn, interact with initiator caspases (e.g. caspase 8) through a death effector domain (DED). The processing of caspase-8 subsequently activates downstream caspases directly as well as initiates the mitochondrial signaling pathway described above, thus amplifying the initial signal and assuring eventual cell death.^{3–5} Importantly, the localization of TNF receptor family members to lipid rafts allows for the formation of these signaling platforms critical for the induction of downstream pathways. Also of interest, the death domain of TNFR1 has recently been shown to be required for the localization of this receptor to the lipid rafts.⁶

Notably, several death receptors also mediate downstream signaling unrelated to cell death. Specifically, death receptors employ TNF receptor-associated factors (TRAFs) as transducers of their signaling pathways, the activation of which may promote cell survival, proliferation, and/or differentiation. TRAF proteins are responsible for the TNFRSF activation of both nuclear factor kappa-B (NF- κ B) and mitogen-activated protein (MAP) kinase-mediated pathways.⁷ Alternatively, Toll-like membrane receptors, which are critical to the innate immune system (e.g. Toll-like receptor-2), can activate apoptotic pathways via adaptor proteins and TRAF family members, yet such receptors do not contain cytoplasmic death domains.^{8,9} Although involved in activating signaling pathways in common with death receptors, the Toll-like receptors are not considered to be directly related to death receptors, and will not be discussed further herein.

Common features of all death receptors include multiple cysteine-rich repeats within the extracellular ligand-binding domain, a type I (single pass) transmembrane domain, and a cytoplasmic death domain required for intracellular signaling.¹⁰ Accordingly, death receptors represent a unique family of proteins with this characteristic domain architecture that allows them to transfer external signals to the cell.

Given the variable nature of the cysteine-rich domains, and their probable coadaptation with specific ligands,¹¹ we chose to focus upon the conserved and functionally important death domain region to examine the phylogeny of the vertebrate death receptors.¹² The boundaries of functional death domains have previously been described using functional deletion and mutational analyses to determine residues and regions critical to their death-including capabilities.^{13–17}

Insights from non-mammalian model systems to study death receptor-mediated apoptosis

Apoptosis plays a critical role in the functional restructuring of many tissues during early development (for instance, neuronal and epidermal tissues). In adults, this process occurs most routinely in tissues that continue to actively differentiate such as those within the immune system, intestinal epithelium, testis, and ovary. For example, vertebrate females produce an excess of ovarian follicles during early ontogeny, the vast majority of which are subsequently lost via follicle atresia prior to reaching the preovulatory stage.¹⁸ Numerous studies have been devoted to understanding the precise regulation of atresia by both extra- and intra-follicular factors, and much of this work has focused upon interactions between the oocyte and the somatic granulosa layer.¹⁹

Our laboratory has taken a comparative approach to understanding this process by utilizing the chicken (*Gallus gallus*) ovary model system. Perhaps not surprisingly, results thus far demonstrate that there exists a striking conservation of intracellular survival and cell death-mediating pathways between mammalian and this avian species.²⁰ Thus, attention has recently been directed towards identifying extracellular signals that can induce selected follicles to undergo follicle atresia in the hen ovary. Specifically, ongoing studies are in progress to identify chicken orthologs from the TNF family of death domain-containing receptors, and to monitor death receptor expression and activation within the granulosa cell layer throughout the development of hen ovarian follicles. Significantly, all five of the currently identified *Gallus* death receptors (tumor necrosis factor receptor-I [TNFRI], Fas, TVB, p75 neurotrophin receptor [p75NTR], and death receptor 6 [DR6]) are known to be expressed in granulosa cells (Figure 1). A functional role for such receptors during atresia is suggested by the fact that four of the five receptors (TNFRI, Fas, DR6, TVB) are expressed at higher levels in atretic follicles compared to morphologically and functionally normal follicles (DR6²¹; Fas and TNFRI,²² TVB,²³ p75NTR, Johnson, unpublished).

In light of these observations, an obvious question arises as to why a single cell type should express so many

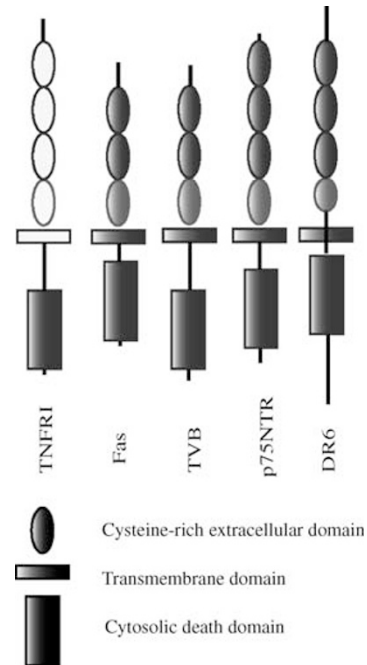


Figure 1 Representation of the major domains contained within the currently known *Gallus gallus* death receptors. The TNFRI extracellular and transmembrane domain architecture (unshaded) is predicted based upon the mouse and human TNFRI sequences

different members of this death receptor family. The answer will unquestionably prove to be complex, and will ultimately require a considerably greater understanding of the ligands that activate, and the cell signaling pathways activated by each individual receptor. The study of death receptor function will involve assessing several layers of complexity within each cell type. Specifically, it will be important to evaluate coexpression of decoy receptors,²⁴ as well as decipher crosstalk with nondeath domain-containing receptors.²⁵ In addition, it will be critical to characterize the complement of intracellular adapter proteins (e.g. TRAFs), and to monitor proteolytic cleavage (e.g. TNF- α -converting enzyme and matrix metalloproteinases) as well as post-translational processing (e.g. phosphorylation and glycosylation) of ligands and receptors to distinguish among signaling pathways mediated by death receptors. Evolution of death receptors has undoubtedly been influenced by these factors, as well as by their coevolution with both cognate ligands and nondeath receptor members of the TNFRSF. In spite of these complexities, examining the evolutionary context in which the death receptors have emerged within vertebrates can serve to establish a basis for understanding the divergence of individual functional specializations of each receptor. For instance, this evolutionary approach has previously provided evidence for both functional constraints and divergences among members of the caspase gene family.^{26,27} Moreover, from such insights we can offer predictions about the physiology of TNF death receptor family members in primary (untransformed) cells for which little, if any information is currently known.

The apparent emergence of death receptors in vertebrates

Evolutionarily, the death receptors identified to date are unique to the vertebrate lineage. Among mammals, the TNFR superfamily encompasses at least eight death domain-containing members, including Fas, TNFR1, p75NTR, ectodysplasin-A receptor (EDAR), death receptor (DR) 3, DR4, DR5, and DR6²⁸ (Table 1). Although previous studies have isolated several proteins containing death domains from invertebrates, there are no identifiable TNF receptor orthologs containing death domains found within the recently sequenced *Drosophila* genome,^{29,30} and none yet isolated from *Caenorhabditis elegans*, or *Porifera* despite concerted efforts.^{31–33} Recent sequence information (including genomic, complementary DNA and EST) from a variety of nonmammalian species now provides the opportunity to examine evolutionary relationships within the death receptor family, and to speculate upon potential functional divergence or specialization among the members.

A search of all available databases for sequences with homology to the death domains from the eight human death receptors revealed representatives from various non-mammalian vertebrate species, including fish (*Danio* and *Oryzias*), frog (*Xenopus* and *Silurana*) and bird (*Gallus*) (Table 1). These non-mammalian vertebrate death domains were also used to search the database in order to identify death receptors from as many taxonomic lineages as available. A minimum BLAST similarity value of $E < 0.01$ from our searches was used to identify putative death receptor homologs. Only one known adapter protein was identified using these criteria (*Xenopus* MyD88; $E = 0.002$ compared to *Xenopus* fullback death domain³⁴), and was not included in

our analysis. Amino-acid sequences from putative death receptors were deduced from identified nucleic acid sequences, and regions predicted to encode the death domain were isolated prior to analysis (Figure 2, ClustalX alignment³⁵). Across all species and death receptors, the death domain was bounded by two well-conserved blocks of amino acids and also contained an internal region characterized by several insertions and/or deletions and generally lower sequence conservation.

Death receptors diverged early during vertebrate evolution

The death receptor phylogeny (Figure 3) reveals a family of divergent proteins that predate the radiation of the vertebrates (estimated at 993–833 million years ago^{36,37}). Each death receptor family member comprises a well-supported clade including representatives from the different classes of vertebrate taxa sampled. As a result, previously unidentified EST sequences (e.g. *Dr* BF717854=Fas; *Gg* AW355430=TNFR1; *Xe* BI477064=TNFR1) as well as previously identified death receptors that remain unclassified (zebrafish hematopoietic death receptor, *Dr* ZH-DR, and ovarian TNF receptor, *Dr* OTR, within the DR5 clade) can be placed with certainty within established lineages (Figure 3).

Several observations and interesting patterns emerge from these phylogenetic data. For instance, remarkably different rates of divergence can be ascribed to individual members within the death receptor family. Some lineages are highly constrained, such as EDAR and DR6, both of which are remarkably conserved among animals as divergent as fish (EDAR, 87% identity of DD compared to the human) and

Table 1 Vertebrate death receptors

Common name used in the analysis	Standardized family member designations	Human <i>Homo sapiens</i>	Mouse <i>Mus musculus</i>	Chicken <i>Gallus gallus</i>	Frog <i>Xenopus laevis</i>	Frog <i>Silurana tropicalis</i>	Fish <i>Danio rerio</i>	Fish <i>Oryzias latipes</i>
TNFR1	TNFRSF1A	XP_006950	NM_011609					
Fas	TNFRSF6	M67454	NM_007987	AF296874				
DR4	TNFRSF10A	4507558						
DR5	TNFRSF10B	AF012535	NM_020275					
DR3	TNFRSF12	NM_003790	NM_033042					
p75NTR	TNFRSF16	M14764	NM_033217	P18519	AF172399			
DR6	TNFRSF21	NM_014452	AF322069	AF349908				
EDAR	Unclassified	AF130988	AF160502					AF364814
TVB	Unclassified			AF161712				
Fullback (FB)	Unclassified				AF131890			
Death receptor (ZH-DR)	Unclassified						AF302789	
OTR	Unclassified						AF250042	
Unknown-EST	Unnamed			AW355430	BI477064	BG512043	BF717834	
Unknown-EST	Unnamed						AI629342	

[TNFR1 is also called CD120a, p55-R, TNFAR, TNFR60, TNF-r-I. Fas also known as CD95, APO-1, APT1. DR4 is Apo2, TRAIL-R1. DR5 is TRAIL-R2, KILLER, TRICK2A, TRICKB. DR3 is TRAMP, WSL-1, LARD, WSL-LR, DDR3, TR3, Apo-3. p75NTR is NGFR. DR6 is TR7. Standardized family member designations can be found at (<http://www.gene.ucl.ac.uk/nomenclature/genefamily/tnftop.html>). Death domain amino-acid sequences from each of the known human death receptors were isolated based upon the region corresponding to amino acids 370–438 of TNFR1. Corresponding amino-acid regions were used to search against the nr database and the EST databases with BLASTp and tBLASTn searches, respectively. Some of the sequences represent partial ESTs, and may not contain the entire predicted receptor coding region. Each of the sequences that were detected were subsequently used to search the protein database using BLASTx to verify that the most similar sequences to the unknown represented death receptors and not unrelated death domain-containing proteins]

	10	20	30	40	50
Dr ZH-DR	Y H N R F F R L I G -	L S D N A I K S - -	A E S L F P E D	R V Y E L L K I W M E K	E G - L K A D F N S L I D
Dr OTR	Y H N K F F R S I G -	V S D N S I K L - -	A E T Q Q P M D	K V Y D L L R V W M Q K	E G - L R A N I N T L L Q
Hs DR4	S W D Q L M R Q L D -	L T K N E I D V V R -	A G T A G P G D	D A L Y A M L M K W V N K	T G - R N A S I H T L L D
Hs DR5	S W E P L M R K L G -	L M D N E I K V A K -	A E A A G H R D	T L Y T M L I K W V N K	T G - R S A S V H T L L D
Mm DR5	S W N R L M R Q L G -	L T D N Q I Q M V R -	A E T L V T R E	A L Y Q M L L K W R H Q	T G - R S A S I N H L L D
Gg TVB	E W K R F G R A L D -	L Q E N D L Y L A E Q H D R -	V S C E P F Y Q M L N T	W L N Q Q G - S K A S V N T L L E	
St BG512043	K W A Q F M R S L R -	L S D N T V W E A K E N N R D N A R E	Q N I A M L R A W Y Q Q	- - N P G D V N D L L A	
Gg AW355430R	W K E F V R R L G -	L T E N D L E R I E M E H -	R H L R D A Q Y E M V S L	W K L Q M G - H A A T V E H I S C	
XI B1477064	R W K E F A R R L G -	L S D N D I E R S E T D N -	R R Y R D A Q Y D M L L T	W K E R V G R S G A K R D A V C K	
Hs TNFRI	R W K E F V R R L G -	L S D H E I D R L E L Q N G R C L R E	A Q Y S M L A T W R R R	T P R H E A T L E L G R	
Mm TNFRI	R W K E F M R F M G -	L S E H E I E R L E M Q N G R C L R E	A Q Y S M L E A W R R R	T P R H E D T L E V V G L	
Hs DR3	R W K E F V R T L G -	L R E A E I E A V E V E I -	G R F R D Q Q Y E M L K R	W R Q Q Q P - - A G L G A V Y A	
Mm DR3	R W K E F V R T L G -	L R E A E I E A V E V E I -	C R F R D Q Q Y E M L K R	W R Q Q Q P - - A G L G A I Y A	
Hs Fas	Q V K G F V R K N G -	V N E A K I D E I K N D N V Q D T A E	Q K V Q L L R N W H Q L H G -	K K E A Y D T L I K	
Mm Fas	E A K K F A R E N N -	I K E G K I D E I M H D S I Q D T A E	Q K V Q L L L C W Y Q S H G -	K S D A Y Q D L I K	
Gg Fas	Q V M T F V R H H R -	L S E P T I E E T L D N S N N T S E	Q K I K L F Q K W Y Q K H G -	M G G A Y E T L I C	
Dr BF17834	T V K E V A R R S G -	M T A K D I E E Q E M N H P K D V R E	Q T F G L L E A W S Q R Q G -	L D K A Y R A L I T	
Hs DR6	Q W K D I Y Q F L C N A S E R E V A A F S -	N G Y T A D H E R A Y A A L Q H W T I R G P -	- - E A S L A Q L I S		
Mm DR6	Q W K D I Y Q F L C N A S E R E V A A F S -	N G Y T A D H E R A Y A A L Q H W T I R G P -	- - E A S L A Q L I S		
Gg DR6	Q W K D I Y Q F L C N A S E R E V A A F S -	N G Y A A D H E R A Y A A L Q H W T I R G P -	- - E A S L A Q L I S		
Mm EDAR	T W R H L A E S F G -	L K R D E I G G M T - D G - - - - -	- - - - - M Q L F D R I S T A - - -	G Y S I P E L L T	
Hs EDAR	T W R H L A E S F G -	L K R D E I G G M T - D G - - - - -	- - - - - M Q L F D R I S T A - - -	G Y S I P E L L T	
OI EDAR	T W R H L A E S F G -	L K R D E I G G M S - D G - - - - -	- - - - - L Q L F E R V S T A - - -	G Y S I P E L L T	
Gg p75NTR	T W R Q L A G E L G -	Y K E D L I D I C F T - R E E S P - - - -	A R A L L A D W S A K I E - - -	T A T L D A L L V	
XI p75NTR	T W K S L A G E L G -	Y Q D E I I D S F T - R E E F P - - - -	V R A M L S D W S S K D - - -	S A T T D A L L Y T	
Hs p75NTR	T W R H L A G E L G -	Y Q P E H I D S F T - H E A C P - - - -	V R A L L A S W A T Q D - - -	S A T L D A L L A	
Mm p75NTR	T W R H L A G E L G -	Y Q P E H I D S F T - H E A C P - - - -	V R A L L A S W G A Q D - - -	S A T L D A L L A	
Dr A1629342	S W K Q L A A T L G -	Y E Q E R V D V F G - R G Q D P - - - -	I H T L M T D W S Q Q E - - -	G S T L G L L C S	
XI FB	D W Q R L A S L L G -	Y E E E T I D T F G - R G E D P - - - -	V H T L L T D W S S K I E - - -	S S T L E V L C A	

Figure 2 Alignment of the death domain of all sequences presented in Table 1 using the program Clustal X v. 1.81.³⁵ We used the Gonnet protein similarity matrix,⁵⁸ a gap opening penalty of 10.00 and a gap extension penalty of 0.20. The death domain refers to the region described in Table 1. Dark and light gray boxes represent identical and similar amino acids, respectively. Hs=*Homo sapiens*, Mm=*Mus musculus*, Gg=*Gallus gallus*, XI=*Xenopus laevis*, St=*Silurana tropicalis*, Dr=*Daionio rerio*, OI=*Oryzias latipes*

chickens (DR6, 98% identity *versus* human). Clearly, we predict the existence of these receptors in other vertebrates, and accordingly expect to find conservation of critical functions that could illuminate these constraints on evolutionary divergence. Other family members demonstrate a high level of divergence in a fashion that largely recapitulates the splitting of the vertebrate lineage itself (e.g. Fas, see Figure 3). These observations enable us to predict that ligands that have coevolved with these receptors will show similar rates of divergence. This prediction currently holds true for the well-conserved mammalian EDAR ligand, ectodysplasin-A, and the highly divergent Fas ligand compared between human and mouse (94% identity compared to 75%, respectively). Unfortunately, many of these ligands across species and even within mammals (e.g. DR6) have yet to be identified.

Additionally, in spite of the ancient divergence of the death receptor family, several members have continued to diversify within lineages, in particular, the related TRAIL receptors DR4 and DR5 in humans (there is currently no known ortholog of DR4 in the mouse). In humans, the origin of these receptors appears to be the result of a tandem duplication event, with both genes encoded on human chromosome 8 (DR4; 8p21, and DR5; 8p22–p21). Zebrafish also express a pair of closely related receptors, ZH-DR and OTR, most similar to the TRAIL receptors. Moreover, a third lineage containing two receptors within one species occurs in the p75NTR subgroup, with *Xenopus* expressing both p75NTR-like and a related receptor, fullback (FB; Genbank Accession AF131890), the function of which has not yet been described. The phylogeny of this p75NTR receptor clade predicts an older split within the vertebrate lineage, followed by the subsequent loss within

mammalian species (human EST Genbank search with fullback revealed only the currently identified p75NTR). Resolution of this branch awaits identification of a more complete set of vertebrate sequences.

Finally, several interesting higher-order evolutionary relationships among the TNF death receptor family are clearly seen. These consist of well-supported groupings of receptor clades that are closely related to one another, in particular the TNFRI/DR3 receptors group together as do the EDAR/p75NTR/DR6 receptors (Figure 3). The result is four distinct death receptor lineages, consistent with the observation that two rounds of genome duplication may have occurred around the time of the transition from invertebrates to vertebrates³⁸ (Figure 3).

Functional categories related to evolutionary subgroups

We propose that the well-supported branches of the phylogeny fall into two major functional categories (Figure 3, subgroups a and b). The first group includes both the EDAR and p75NTR receptors that have been characterized as being involved in signaling pathways mediating development. EDAR is required for the development of epithelial appendages in vertebrates (hair and teeth in mice and scales in fish³⁹) and has recently been shown to interact with Edaradd, a death domain-containing adapter molecular similar to TRADD.⁴⁰ Similarly, p75NTR is involved in mediating both survival and apoptosis during the development of neuronal cells.⁴¹

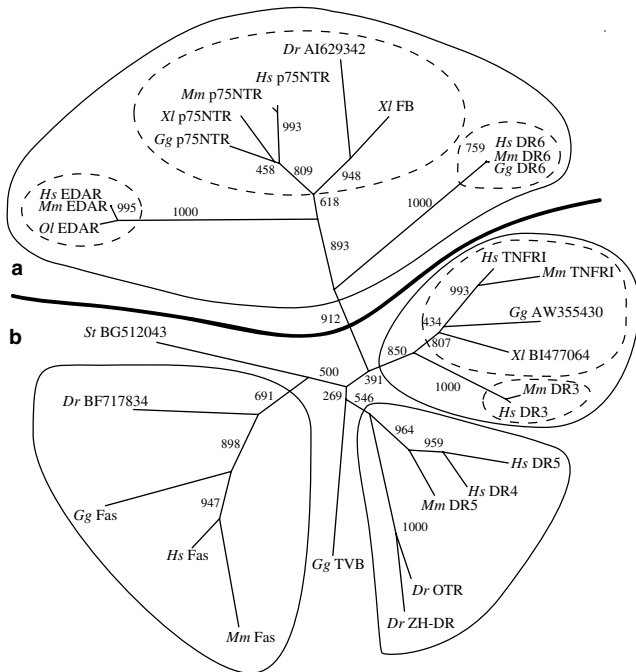


Figure 3 Phylogeny of the death receptor gene family based upon death domain homology. This tree was constructed using the neighbor-joining algorithm of the program NEIGHBOR in the PHYLIP (phylogeny inference package) program (Seattle, WA, USA). Distances were calculated using the Jones–Taylor–Thornton similarity matrix.⁵⁹ Bootstrap support (out of 1000) is indicated. A full maximum likelihood tree (estimated using PROML from the PHYLIP package) estimated the same topology. In addition to the phylogeny shown, we searched for all death domains found in other nonreceptor proteins using the tBLASTn search. Including these death domains, found in both vertebrate and invertebrate species, in our analysis did not alter the topology or increase the resolution of the phylogeny for the receptor proteins, nor did they provide evidence a single ancestral death domain (data not shown). (a) and (b) denote different functional categories. All sequence accession numbers are found in Table 1 and abbreviations in the legend of Figure 2.

Phylogenetic analysis provides strong support for placing DR6 within this same major subgroup of developmentally specialized receptors, and it will be of special interest to elucidate the physiological role of this recently identified and highly conserved receptor. Surprisingly, DR6-deficient mice develop normally, and the only observed phenotypes are enhanced CD4⁺ T-cell proliferation and upregulated Th2 cytokine production.⁴² DR6 is capable of initiating apoptosis in select transformed cell lines, as well as activating NF- κ B and c-Jun terminal kinase in others.⁴³ Accordingly, based on our phylogenetic classification, we predict that the physiological role of DR6 will be related to mediating developmental pathways, such as those that occur during T-cell differentiation. Moreover, given its widespread expression patterns, it is likely important in other tissue types as well.^{21,43}

The second major functional category encompasses the remaining death receptors representing three separate clades, all of which play a role in mediating the immune response (Figure 3, subgroup b). Notably, the ancient divergence of these receptors occurs coincident with the emergence of the acquired immune response in vertebrate species. Within this category, TNFRI and DR3 form a single clade, suggesting a more recent divergence. DR3 is ex-

pressed in tissues of immune system origin, specifically the spleen, thymus, and peripheral blood leukocytes with little or no expression in the other tissues examined.⁴⁴ On the other hand, TNFRI has a more ubiquitous distribution, yet is still most often implicated in mediating the inflammatory response.⁴⁵ These related receptors are also unique among the death receptors in that they can be phosphorylated on serine/threonine residues residing within the membrane proximal regions (not the death domains utilized to create this phylogeny) by the mitogen-activated protein kinase p42 (MAP/ERK).⁴⁶

The Fas receptor forms another clade within this second functional category, and often has been linked to the control of cellular homeostasis within the immune system.⁴⁷ Fas is expressed in a variety of normal cells, including ovarian granulosa cells, yet significantly the Fas ligand is reported to be limited to cells of immune system origin.⁴⁷ This selective expression pattern facilitates a critical communication between the immune system and nonimmune cell types. Of particular interest was the finding that the phylogeny of Fas shows a surprisingly high degree of differentiation among vertebrates, including mammals. This result was unexpected given that Fas is most often depicted as having a comparatively direct signaling pathway linking receptor activation with caspase activity.⁴⁸ The unexpectedly high rate of divergence among Fas receptors is consistent across the various vertebrates species, and continues within the mammalian lineage as demonstrated by only a 55% identity within the death domain of mouse Fas compared to human Fas. This lack of evolutionary constraint supports the proposal that Fas has a more diverse physiological role than was previously thought, including the potential to promote cell survival.^{47,49} Studies on the role of Fas in nonmammalian vertebrate species may shed additional light on this issue.

The remaining clade in this second functional category includes the human TRAIL receptors, DR4 and DR5 (Figure 3). Most research concerning TRAIL receptors has been directed towards the potential use of the ligand, TRAIL, as a chemotherapeutic agent, as carcinoma cell lines are often more susceptible to TRAIL-induced cell death than are primary cultures.⁵⁰ Thus, although TRAIL receptors in mammals are reported to be expressed almost ubiquitously,^{51,52} the role of TRAIL receptors in non-cancerous cells has not yet been clearly established.⁵³ The widespread pattern of expression suggests that the function of TRAIL receptors is highly dependent upon cell-specific regulatory mechanisms. Moreover, the existence of at least five receptors that bind the TRAIL ligand, including three receptors without death-inducing functions, accentuates the complexity involved in the regulation of this pathway.⁵³ Within the TRAIL receptor clade (Figure 3, surrounding DR4 and DR5) are included two *Danio* receptors ZH-DR and OTR, each with fairly limited tissue distributions. The death receptor, ZH-DR, is reported to be specific to haematopoietic cells,⁵⁴ while OTR is expressed in a variety of tissues, but most strongly in the ovary.⁵⁵ Based on our analysis we predict that ZH-DR and OTR represent duplicate genes that have evolved tissue-specific functions.

Inclusion of the chicken TVB within this clade is only very weakly supported, although the presence of a transmembrane domain and the similarity of N-terminal cysteine-rich domains

indicate that TVB is probably most closely related to human DR4 and 5. Interestingly, the chicken TVB receptor was originally isolated because of its ability to bind to envelope proteins of the cytopathic avian leukosis-sarcoma virus.⁵⁶ Although viruses may interact with the TVB receptor to mediate infection, the identification of a naturally occurring (as yet unidentified) ligand is anticipated. Its role in viral immune response is especially fascinating in the light of our results showing the TVB death domain to be relatively divergent from all other death receptors. More recently, it was reported that the human DR5 receptor also mediates viral cytotoxicity in colonic epithelial cells;⁵⁷ however, there is no evidence for direct binding of the receptor by viruses. Several of the death receptors have been implicated in mediating an immune response to viral infections, and no doubt this physiological function will be the subject of continuing research.

Finally, the *Silurana* (EST BG512043) sequence also appears within this functional category of death receptors involved in the immune response. The phylogeny places it closest to the Fas clade. The partial sequence from the EST database only contains information from the C-terminus through the death domain. In order to conclusively characterize this putative receptor, a more complete sequence identifying the presence of a transmembrane domain and a cysteine-rich region will be required. Such information, together with detailed studies of its expression and function, will help to elucidate why this death domain is so divergent from its neighbors.

Conclusions

The phylogenetic analysis presented herein indicates that members of the death receptor family represent an ancient divergence. Although ancient, several members of the death receptor family show strong phylogenetic relatedness to one another. Most interestingly, the evolution of death receptors has been quite dynamic, showing highly variable rates of constraint and divergence along the separate lineages and even showing continued differentiation through gene duplication within some vertebrate groups. Based on their phylogenetic relatedness, members of the death receptor family can be categorized into subgroups according to their predicted function. Significantly, our phylogenetic analysis demonstrates that the divergence of several death receptor clades involved in the immune response occurred concomitant with the initial appearance of adaptive immunity in vertebrates, while other clades appear to have become specialized in mediating development processes.

Finally, this review illuminates the critical need both for physiological studies in normal (non-transformed) cells to complement ongoing studies in transformed cell lines, as well as for additional comparative studies in non-mammalian vertebrates. Recall the model system discussed above in which hen granulosa cells express each of the five currently identified avian members of the death receptor family. We hypothesize that such receptors act in a highly pleiotropic fashion to mediate functions as diverse as communicating with the immune system, promoting differentiation, and signaling cell death. Moreover, we expect significant crosstalk

among the death receptors such that activation of one receptor will rapidly modulate the expression and function of related family members. Ultimately, an understanding of such interactions will enable us to better understand the dynamics that contribute to the viability or death of developing ovarian follicles.

Note Added in Proof

Moreno *et al.* (2002) recently published the characterization of Eiger, a unique TNF homolog in *Drosophila*. Their results elegantly demonstrate that Eiger signals through the DRONC/DARK pathway (homologous to caspase-9/Apaf-1 signaling in vertebrates) to initiate apoptosis, rather than through the death domain-dependent, adaptor-mediated activation of DREDD (homologous to caspase-8). This discovery suggests the evolutionarily important hypothesis that ancestral TNF receptors did not contain death domains. Accordingly, the death domain module may have been co-opted from the Toll-like receptor signaling pathway into the TNF receptor family linking these apoptosis initiating cytokines to multiple intracellular signaling pathways. These findings support our conclusion that death receptors are unique to the vertebrate lineage, and suggest a possible scenario in which TNF receptors became linked to death domain-mediated signaling pathways.

Moreno E, Yan M, and Basler K (2002) Evolution of TNF Signaling Mechanisms: JNK-Dependent Apoptosis Triggered by Eiger, the *Drosophila* Homolog of the TNF Superfamily. *Curr. Biol.* 12: 1263–1268.

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