Review

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Surviving apoptosis: life–death signaling in single cells

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Tissue development and homeostasis are regulated by opposing pro-survival and pro-death signals. An interesting feature of the Tumor Necrosis Factor (TNF) family of ligands is that they simultaneously activate opposing signals within a single cell via the same ligand–receptor complex. The magnitude of pro-death events such as caspase activation and pro-survival events such as Nuclear Factor (NF)-κB activation vary not only from one cell type to the next but also among individual cells of the same type due to intrinsic and extrinsic noise. The molecules involved in these pro-survival and/or pro-death pathways, and the different phenotypes that result from their activities, have been recently reviewed. Here we focus on the impact of cell-to-cell variability in the strength of these opposing signals on shaping cell fate decisions.

Life–death decisions

During embryogenesis, development, and tissue turnover, some cells die by apoptosis while other cells avoid death and assume various cellular fates. What makes some cells die and others survive is not completely understood. In some cases, only specific cells receive the death signal, while in other cases, the signal is interpreted differently due to cell or context-specific cues. Such cell-to-cell variability, which has various origins, has recently been shown to play an important role in cell fate decisions [1–3].

Similarly, stress-response signaling often has a dual role, activating survival pathways to buffer and repair damage, and death pathways to kill cells when the damage is beyond repair. Examples include pathways regulating heat shock proteins, p53, autophagy, and inflammation, and here, too, individual cells often respond with variable outputs [4–6]. Thus, protective stress pathways and death signaling are tightly linked, and many cellular proteins have evolved to exert both functions, often in parallel [7,8].

Proteins that regulate cell death are also essential for normal cellular processes, including metabolism, proliferation, and differentiation [9,10]. In some cases, these proteins 'deviate' from their physiological function only when external cues point to cell death. In other cases, proteins exhibit both functions simultaneously (e.g., through interactions within different protein complexes), or have a survival role only when death is inhibited. Some of these proteins (e.g., caspases) belong to multiple signaling pathways (Box 1), while others, such as the TNF family of death ligands, activate parallel but opposing pathways through recruitment of different sets of signaling molecules. Thus, many proteins are essential for both life and death of cells, and the particular outcome may depend on cell type, exposure to external stimuli, or other context-dependent choices.

In this review, we examine how a 'death' signal can lead to a nondeath output, with a particular focus on the TNF family of death ligands. We also describe some nonapoptotic functions of 'death' proteins and discuss potential advantages of this convergence. Finally, we review how the interplay between death and survival signaling has been studied at the level of single cells, how variability in these signals contributes to variability in cell fate, and the implications of these studies for understanding the roles of life–death signaling in development and disease.

Integrating life–death signals

Cell-to-cell variability has been shown to have an important role in cell fate decisions [11]. This variability can result from differences in cellular state (genetic, epigenetic, phenotypic, or due to stochastic fluctuations) as well as from cell cycle differences or effects of the cellular microenvironment [1–3]. Cues external to the cell, such as death or survival stimuli, can be viewed as variable inputs acting on already variable cellular states. Together, these different sources of variability lead to downstream heterogeneity in phenotype.

The following simplified scenarios illustrate several ways in which competing pro-death and/or pro-survival signals can lead to variable cell fates. On the one hand, the relative strength of distinct and opposing stimuli may tip the balance in favor of survival or death, as in the case of a growth factor protecting cells from a death-inducing agent (Figure 1A). On the other hand, a single stimulus may induce both death and survival signals within a single cell; the internal state of the cell then determines which pathway is dominant at a given time (Figure 1B). For example, a block in apoptosis may unmask pro-survival signals triggered by a death ligand, or vice versa; this may be true at the cell population level, or may vary among individual cells (Figure 1Ci). Alternatively, pro-death and pro-survival signaling may actively compete to determine whether a cell

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Box 1. Nondeath roles for death proteins

Many proteins exhibit both pro- and antiapoptotic activities. For example, cytochrome c is important for both mitochondrial homeostasis and execution of cell death [125]. The first caspase to be discovered, Interleukin-1-beta-Converting-Enzyme (ICE/Caspase-1), is responsible for cytokine processing and represents a subgroup of inflammatory caspases with functions in immune signaling [126]. TNF was discovered for its role in tumor necrosis, but also acts as an inflammatory cytokine [127]. Subsequently, nonapoptotic roles have been uncovered for most proteins associated with apoptosis.

Both initiator and effector caspases exhibit nonapoptotic functions [9]. Caspase-8 promotes cell migration [128–130], T cell proliferation [131], wound healing [132], stem cell reprogramming [133], and macrophage differentiation [134]. Caspase-3 has a role in shaping cell morphology [135] and in differentiation of red blood cells, lens epithelial cells, and skeletal muscle cells, processes that involve degradation of intracellular organelles or substrates ('incomplete apoptosis') [136–140]. Caspases are required for spermatid differentiation, oogenesis, and wing development in *Drosophila* [141–143], and also have a role in neuronal sculpting, synaptic plasticity, and neural development [117,144–146].

Bcl-2 family members also have functions unrelated to apoptosis, such as regulation of mitochondrial homeostasis and glucose metabolism [147,148]. Mcl-1, an antiapoptotic Bcl-2 family member, was first discovered as a differentiation marker for myeloid cells, and is required for embryonic development and immune system function [149]. In addition, Bcl-2 members allow rapid switching between states that favor life versus death. For example, alternatively spliced isoforms and cleavage products of these proteins can promote either survival or death, and the fast degradation rate of Mcl-1 in particular allows cells to rapidly undergo cell death under conditions of stress [108].

DISC proteins such as c-FLIP, FADD, and RIP also promote death or survival, and combinatorial regulation of these proteins may determine cell fate [150,151]. c-FLIP can be pro- or antiapoptotic, depending on levels and the particular isoforms expressed [27,152,153], and cleaved FLIP (p43) regulates activation of survival pathways via NF-κB [154]. FLIP and FADD are both required for embryonic development and T cell proliferation [155], and FADD has a role in cell cycle progression, differentiation, and innate immunity [151]. Moreover, FADD, caspase-8, and FLIP appear to promote cell survival during development through inhibition of necroptosis, inducing apoptosis only in response to certain stimuli [35]. RIP1 can activate survival, apoptosis, or necroptosis, depending on its post-translational modifications [150]. Finally, kinases associated with the DISC can have pro- or antiapoptotic activity: p38, JNK, Protein Kinase C (PKC), and ERK either promote or inhibit apoptosis induced by death ligands, depending on context [59].

lives or dies, leading to cell-to-cell variability in response (Figure 1Cii). Finally, in cells exposed to a death-inducing agent, counter-balancing adaptive pathways may become activated to varying degrees in individual cells in a population, protecting against a future death stimulus (Figure 1D). Thus, the choice of a cell between life and death can be a function of both external context (e.g., signals from other ligands or cells) and its own internal state.

Death ligands and death receptors

Evasion of apoptosis is a hallmark of cancer cells and contributes to both cancer progression and resistance to chemotherapeutic drugs. Traditional chemotherapy targets the 'intrinsic' pathway of cell death, activating apoptosis from within cells through induction of DNA damage or other cellular stresses. By contrast, the 'extrinsic' apoptosis pathway is mediated by death ligands that bind to

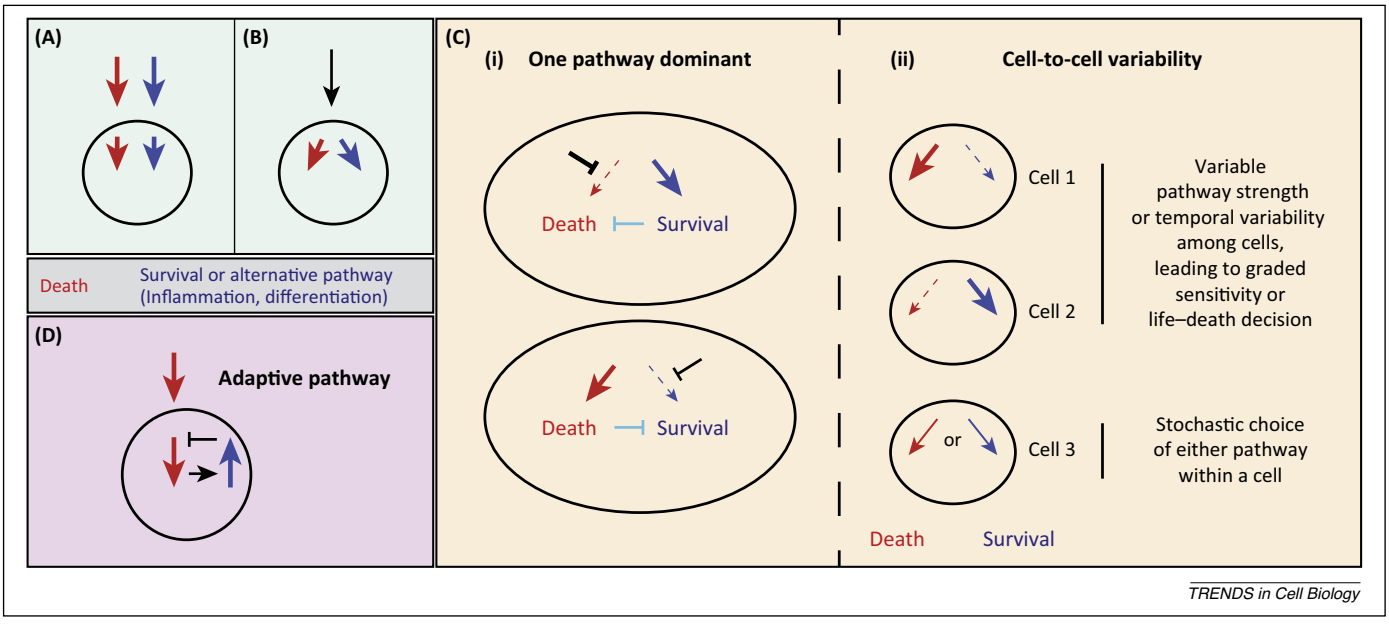

Figure 1. Scenarios illustrating how parallel or competing death and survival signals may influence cell fate decisions in individual cells. **(A)** Separate death and survival signals (e.g., an inhibitor drug and growth factor ligand) activate parallel pathways within a cell. **(B)** A single stimulus, such as a death ligand, activates both pro-death and pro-survival pathways. **(C)** In the case of a single stimulus activating two pathways, one pathway might be dominant within a cell population, for example if the other pathway is not functioning (as in very high expression of an antiapoptotic protein; bold black inhibition arrow), or if the first pathway negatively regulates the second pathway (aqua inhibition arrows), leading to activation of only one of the pathways within the cell population. In this case, the strength of a single pathway would vary among individual cells, leading to variability in cell fate (i). Alternatively, both pathways may be activated within cells, and cell-to-cell variability affects the relative strength or duration of each pathway, further leading to differences in cell fate (ii). In cells 1 and 2, variable pathway strength, or temporal variability among cells, leads to graded sensitivity within the cell population and variable life-death fate outcomes. In cell 3, one pathway or another is activated stochastically within a cell, also leading to variable life-death fate outcomes among cells. **(D)** A drug treatment that can induce cell death leads to activation of a compensatory adaptive pathway that mediates resistance to the death signal in cells that escape death initially. The difference between (B,C) and (D) is that in (B,C), the death signal directly activates an alternative pathway alongside the death pathway (as in the case of death ligands), whereas in (D), the adaptive pathway is indirectly activated in response to the death signal via a separate pathway. It should be noted that these two outcomes are not necessarily mutually exclusive.