PROC. N.S. INST. SCI. (2006)
Volume 43, Part 2, pp. 201-209

2005 NSIS Graduate Student Prize Winning Paper

CLEAR AS MUD: THE TYPE I/TYPE II MODEL FOR DEATH RECEPTOR-INDUCED APOPTOSIS

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Apoptosis is a highly organized form of cell death that plays an important regulatory role in many biological processes. The relationship between the two classical signalling pathways of apoptosis, the "death receptor" and "mitochondrial" pathways, was only vaguely appreciated until 1998, when death receptor pathway-mediated activation of the mitochondrial pathway was clearly demonstrated for the first time. The "type I/type II" model of death receptor-mediated apoptosis was proposed and subsequently adopted for use in categorizing cells according to the involvement of the mitochondrion during death receptor-induced apoptosis. Since that time, however, different interpretations of the type I/type II cell definition have appeared in the literature and, consequently, the meaning of type I and type II cells has become less clear.

L'apoptose est une forme de mort cellulaire très structurée qui joue un rôle important de régulation dans un grand nombre de processus biologiques. La relation entre les deux voies de signalisation traditionnelles de l'apoptose, la voie des « récepteurs de mort » et la voie mitochondriale, n'était connue que vaguement avant 1998, l'année où l'activation de la voie mitochondriale par l'intermédiaire de la voie des récepteurs de mort a été clairement démontrée pour la première fois. Le modèle « type I / type II » d'apoptose par l'intermédiaire des récepteurs de mort a été proposé puis adopté aux fins de catégorisation des cellules en fonction de la participation des mitochondries à cette apoptose. Depuis, différentes interprétations ont toutefois été formulées dans des ouvrages scientifiques quant à la définition des cellules de type I et de type II et, par conséquent, la signification de « cellules de type I » et de « cellules de type II » est devenue moins évidente.

INTRODUCTION

There are several different mechanisms by which a cell can die (Fiers et al. 1999). "Necrosis" is the familiar type of cell death often caused by injury or disease, and effectively refers to the bursting of a cell. The release of lytic enzymes from inside the dead cell can cause further tissue damage and activate non-specific immune responses, resulting in painful inflammation. Conversely, "programmed cell death" is regulated at the molecular level and does not cause the contents of dying cells to be exposed. Instead,

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the cell's deoxyribonucleic acid (DNA)[1] is cleaved into fragments and the contents of the cell are packaged into small membrane-bound vesicles that are engulfed and removed by specialized immune cells, phagocytes, without causing any inflammatory reactions. The morphological features of programmed cell death, such as DNA fragmentation and the production of small membrane-bound vesicles, are collectively referred to as "apoptosis." This term is Greek in origin and describes petals falling from a flower, in reference to the membrane-bound vesicles that are produced from a dying cell. The terms "apoptosis" and "programmed cell death" are used interchangeably in the literature; thus, for clarity "apoptosis" will be used in place of "programmed cell death" for the remainder of this essay.

The two classical pathways of apoptosis are the death receptor pathway and the mitochondrial pathway. The death receptor pathway is initiated by aggregation of so-called death receptors (e.g., Fas, tumor necrosis factor (TNF) receptors, and TNF-related apoptosis-inducing ligand (TRAIL) receptors) present on the cell surface. Death receptors are transmembrane proteins that belong to the TNF/nerve growth factor superfamily of cell surface receptors, of which Fas is one of the most thoroughly studied. The cytoplasmic tails of these receptors contain a specialized amino acid sequence that allows them to initiate apoptosis (Fulda & Debatin 2004). A number of stimuli can trigger receptor aggregation, including natural receptor ligands, receptor-specific antibodies (Scaffidi et al. 1998), ultraviolet irradiation (Aragane et al. 1998), and certain cytotoxic compounds (Bush et al. 2001). The mitochondrial pathway can be initiated by intracellular stimuli, such as oxidative stress or DNA damage. These two pathways eventually converge and cells dying via either pathway are morphologically identical. Because apoptosis is the mechanism by which chemotherapeutic drugs and radiotherapy eradicate human cancer cells, and some human cancer cells develop mechanisms to avoid apoptosis, a better understanding of apoptosis may lead to strategies that increase the effectiveness of cancer treatments.

Apoptosis research from the last several years has revealed that, although mutually exclusive in many situations, the death receptor and mitochondrial pathways of apoptosis are intimately linked. Specifically, death receptor signalling can inefficiently induce the death receptor pathway such that it requires amplification, which is achieved by activation of the mitochondrial pathway. Scaffidi et al. (1998) identified two cell types: those that succumb to death receptor-induced apoptosis independently of the mitochondrion (type I cells), and those that require signal amplification (type II cells) following death receptor aggregation. With this model, cells can be catego-

[1] The abbreviations used are: Apaf-1, apoptosis protease-activating factor 1; Bax, Bcl-2-associated protein X; Bcl-2, B cell lymphoma-2 gene; Bcl-X_L, long form of Bcl-X; Bid, BH-3 interacting DD protein; caspase, cysteine aspartate protease; DISC, death-inducing signalling complex; DNA, deoxyribonucleic acid; FADD, Fas-associated death domain; Fas, fibroblast-associated; FasL, Fas ligand; PKR, protein kinase R; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; tBid, truncated form of Bid

rized according to the pathway(s) of apoptosis induced by death receptor signalling. Unfortunately, in recent years the definition of type I and type II cells has been manipulated to the point of ambiguity. The recent apoptosis literature, highlighting the different interpretations and consequent distortions of the type I/type II cell model, is used in the following discussion to clarify the issues and make the definition more understandable. A clearer understanding of the implications for the type I and type II phenotype in the responsiveness of cancer cells to various apoptogenic anti-cancer therapies may lead to more effective treatment of cancer in the future.

TYPE I AND TYPE II CELLS

Caspases are proteolytic enzymes that play a major role in apoptosis. The name "caspase" is derived from cysteine aspartate protease, because caspases cleave substrates that possess cysteine-aspartate motifs. These enzymes are normally present in the cell in the inactive "procaspase" form, and are activated by autoproteolytic cleavage or cleavage by other caspases. When the mitochondrial apoptosis pathway is activated, pores form in the mitochondrial membrane. Pore formation is regulated by the Bcl-2 (B cell lymphoma-2 gene) protein family. Pro-apoptotic members of this family, such as Bax (Bcl-2-associated protein X), are thought to oligomerize and create these pores. Conversely, pro-survival Bcl-2 protein family members, such as Bcl-x_L (long form of Bcl-X) and Bcl-2 itself, protect against the action of proteins like Bax by binding to them, inhibiting oligomerization and subsequent pore formation. When present, these pores act as conduits through which cytochrome c is released from the mitochondrion. In the cytosol of the cell, cytochrome c binds to apoptosis protease-activating factor 1 (Apaf-1), and together they activate procaspase-9. In turn, caspase-9 activates procaspase-3, and caspase-3 mediates late apoptotic events such as DNA fragmentation (Liu et al. 1997). The death receptor pathway is initiated by aggregation of death receptors. Inside the cell, Fas-associated death domains (FADD) associate with the cytoplasmic tails of aggregated death receptors, and procaspase-8 associates with FADD. This complex of death receptor, FADD and procaspase-8 is referred to as the DISC, for death-inducing signalling complex. In the DISC, procaspase-8 is activated by autoproteolytic cleavage, and active caspase-8 then activates procaspase-3.

Scaffidi et al. (1998) examined death receptor-mediated apoptosis, induced by an anti-Fas antibody, in several different cell lines. In this study, two distinct death receptor pathways were identified: one involving rapid cleavage of caspase-8, and the other displaying delayed caspase-8 activation. It was subsequently determined that delay of caspase-8 activation occurs because activation of the mitochondrial pathway is required in order to amplify a weak death receptor signal. Activation of the mitochondrial pathway ultimately yields greater amounts of active caspase-8, enabling apoptosis to occur. From this study was born new terminology in the apoptosis litera-

ture. Type I cells were those that died following anti-Fas antibody treatment without involvement of the mitochondrion, whereas apoptosis in type II cells was mitochondrion-dependent (Fig 1). The molecular mechanism for

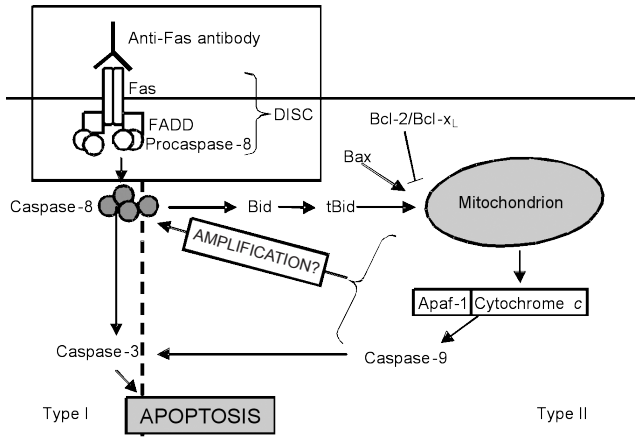

Fig 1 Anti-Fas antibody-induced apoptosis in type I and type II cells.
In type I cells, directly following anti-Fas antibody-induced aggregation of Fas, procaspase-8 is activated, yielding caspase-8, which activates procaspase-3. In type II cells, procaspase-3 is not immediately activated following Fas aggregation, due to insufficient generation of caspase-8. Instead, caspase-8 cleaves Bid, producing tBid, which activates the mitochondial pathway. Through an undetermined amplification mechanism, more caspase-8 is then generated and apoptosis ensues. Also shown are Apaf-1, cytochrome c, caspase-9 and Bcl-2 protein family members, which are involved in the mitochondrial apoptosis pathway.

activation of the mitochondrial pathway by death receptor-induced apoptosis was elucidated that same year. Luo et al. (1998) demonstrated that the mitochondrial pathway is triggered by the protein Bid (BH-3 interacting DD protein), and Li et al. (1998) showed that Bid is activated by caspase-8 in the death receptor pathway for type II cells. Caspase-8 cleaves Bid, yielding truncated Bid (tBid), which acts on the mitochondrion (Fig 1).

Unfortunately, one key aspect of this signalling model was unknown at the time and remains cryptic even today. Although it is well established that activation of the mitochondrial pathway amplifies caspase-8 activation, the molecular interactions that govern this process have yet to be identified. Recently, it was shown that caspase-3 might participate in a mitochondrial amplification loop (von Haefen et al. 2003, Klöpfer et al. 2004). This was inferred from the observation that caspase-8 is activated after caspase-3 activation, and that inhibition of caspase-3 activity prevents activation of caspase-8. In these studies, however, the authors failed to demonstrate directly that caspase-3 activates caspase-8. The possibility therefore remains that caspase-3 simply participates in a pathway that leads to

caspase-8 activation. For example, caspase-3 can mediate protein kinase R (PKR) activation (Suen et al. 2003) and PKR has been shown to participate in activation of caspase-8 (Gil & Esteban 2000, Page et al. 2002). Taken together, these observations suggest that PKR might complete the mitochondrial amplification pathway by mediating caspase-3-dependent activation of caspase-8. Currently, however, the caspase-8 amplification step is only vaguely understood, and the importance of PKR in type II cell death receptor-induced apoptosis remains unexplored.

"TYPE I/TYPE II" IN THE LITERATURE

In 1999, the authors of the original type I/type II cell paper released a short article detailing several subsequent experiments that were performed in order to determine whether Fas ligand (FasL), the natural binding partner for Fas, induces apoptosis in the same fashion as anti-Fas antibodies (Schmitz et al. 1999). In response to anti-Fas antibodies and FasL, apoptosis appeared to be the same. In one experiment, the authors engineered their cells to overexpress pro-survival Bcl-2 protein family members and tested whether protection against FasL- and anti-Fas antibody-induced apoptosis was conferred on the genetically engineered cells. Indeed, overexpression of these pro-survival proteins did protect type II cells from FasL- and anti-Fas antibody-induced apoptosis, whereas a prototypic type I cell line remained vulnerable despite high Bcl-2 expression. The authors extended this observation to infer that such protection by pro-survival Bcl-2 protein family members is indicative of a type II cell. In addition, the authors suggested that a fundamental difference between type I and type II cells is that FADD recruitment to Fas is impaired in type II cells, which they demonstrated by visualizing the amount of FADD associated with Fas in either type of cell. The following year, a report was released that challenged the use of Bcl-2-conferred protection against death receptor-induced apoptosis for determining cell type, highlighting instead the importance of the nature of the apoptosis-inducing stimulus itself (Huang et al. 2000). Here the authors indicated that Bcl-2 family members provide no protection against apoptosis triggered by FasL in type II cells, and also that FADD-related differences are not distinct between the two cell types. This appears to be the first report questioning the validity of the type I/type II model, specifically suggesting that beyond cell and tissue type, the apoptosis-inducing stimulus might also dictate the nature of death receptor-induced apoptosis.

In hindsight, this original criticism of the type I/type II model, specifically concerning the importance of the nature of the death-inducing stimulus, now seems prophetic. In recent years numerous reports have surfaced where the type I/type II label has been liberally applied to cells as a result of apoptosis studies involving many different apoptosis-inducing stimuli. One that stands out is a report by members of the group that first described the type I/type II model. Here, however, 25 cell lines were categorized as type I or type II based on their response to soluble FasL, rather than anti-Fas

antibodies (Algeciras-Schmnich et al. 2003), in spite of the known differences between FasL- and anti-Fas antibody-induced apoptosis (Huang et al. 2000). Unfortunately, no studies on anti-Fas antibody-induced apoptosis in these same 25 cell lines have yet been performed to support or refute their findings.

Although the term "death receptor" refers to Fas as well as TRAIL and TNF receptors alike, the type I/type II model for death receptor-induced apoptosis was based only on Fas-mediated apoptosis. Since different Fas stimuli (e.g. anti-Fas antibodies vs. FasL) have different effects on apoptosis even in the same cell type (Huang et al. 2000), it is not surprising that a recent study on TRAIL receptor-mediated apoptosis was not consistent with the type I/type II model (Rudner et al. 2005). Here, the authors observed that the hallmark protective effect of Bcl-2 against death receptor-induced apoptosis in type II cells was limited only to lower doses of the death-inducing stimulus, which in this study was TRAIL. Although not based on anti-Fas antibody-induced apoptosis, this study was useful in demonstrating that in addition to the nature of the apoptosis-inducing stimulus, the dose of that stimulus may also dictate the way in which apoptosis occurs. To their credit, the authors refer to "type I/type II reactions", and not just "type I/type II cells" in this report. It is not immediately obvious whether this study truly contributes to the "type I/type II" field of apoptosis research. Here, the prototypic Fas receptor was not activated for induction of apoptosis. Instead, the type I/type II model was applied to TRAIL-mediated apoptosis, perhaps because Fas and TRAIL are both death receptors. However, multiple different TRAIL receptors can trigger cell death (Hoskin 2000), and may each transduce distinct apoptosis signals. Also, recruitment of FADD and caspase-8 for DISC formation is not common to all death receptors (Harper et al. 2003). It would therefore be unwise to assume that all death receptor family members are equal in terms of the apoptosis signals they transduce.

Certain cytotoxic drugs can induce the Fas apoptosis pathway. Consequently, apoptosis induced by these drugs has been studied in the context of the type I/type II model. In most of these studies, DISC formation and the involvement of caspases is clearly established. However, since anti-Fas antibodies are not the apoptosis-inducing stimulus, should the drug-induced form of Fas activation be an acceptable application of the type I/type II model? One example where the type I/type II model might apply to drug-induced apoptosis is that of ceramide. Originally, Fas-induced apoptosis was shown to involve intracellular production of the lipid, ceramide. Some believe that ceramide production is an essential step in death receptor-induced apoptosis, although this claim remains somewhat controversial. It has been observed that ceramide induces apoptosis in type II Jurkat T leukemia cells via a signalling pathway analogous to that of Fas-mediated apoptosis (Caricchio et al. 2002). Subsequently, sensitivity to ceramide has appeared in the literature as a criterion for type II cells (Barnhart et al. 2003). When ceramide-induced apoptosis in Jurkat cells was studied, normal cells as well as those rendered resistant to killing by FasL were

examined (Caricchio et al. 2002). Ceramide sensitivity was evident in the former cell type, while in the latter resistance to FasL killing also conferred protection against ceramide-induced apoptosis. This report provides evidence that ceramide is a critical signalling intermediate in the Fas pathway of cell death. However, FasL- and anti-Fas antibody-induced cell death are not equivalent (Huang et al. 2000), and the type I/type II model is based on anti-Fas antibody-induced apoptosis (Scaffidi et al. 1998). How relevant to the type I/type II model is a study on FasL-resistant cells? A further study using anti-Fas antibody-resistant cells would settle this issue, and more accurately determine how the type I/type II model might apply to ceramide-induced apoptosis.

Other stimuli also induce Fas-dependent apoptosis, such as ultraviolet light, which can induce aggregation of Fas (Aragane et al. 1998). Curcumin, a component of the spice turmeric, has also been shown to induce Fas aggregation, as well as caspase-8-dependent apoptosis (Bush et al. 2001), and both curcumin and ultraviolet light can induce apoptosis independently of FasL. Under these conditions Fas expression may be increased in response to the cytotoxic stimulus (Müller et al. 1998), and Fas aggregation might occur due to elevated numbers of Fas molecules on the cell. Still other drugs, like the anti-cancer drug doxorubicin, cause Fas-dependent apoptosis by inducing expression of FasL (Friesen et al. 1996). In one report, doxorubicin was used as the apoptosis-inducing stimulus and apoptosis was studied in the context of type I/type II cells (Fulda et al. 2001). Certain molecular events in doxorubicin-induced apoptosis agreed with the type I/type II model; DISC formation was far greater in type I cells than in type II cells, and apoptosis in type II cells was dependent on the mitochondrial pathway. In contrast, the authors found that doxorubicin induces Bid cleavage in both type I and type II cells, suggesting that the type I/type II model should not extend to drug-induced apoptosis. Indeed, it may be naïve to assume that a drug would have no other effect on the cell, beyond Fas aggregation, that could affect apoptosis.

SUMMARY

The type I/type II model for death receptor-induced apoptosis has been a valuable tool for understanding apoptosis under very specific conditions, and may someday help researchers predict chemosensitivity or resistance in tumours. Unfortunately, the type I/type II model has been altered and applied to irrelevant experimental systems by several research groups. Labelling a cell as type I or type II should be carefully considered, and carried out only after meticulous examination of anti-Fas antibody-induced apoptosis; however, even this rule may be too vague. Evidence suggests that the amount of anti-Fas antibody used to induce apoptosis may inherently alter the apoptotic response. Unfortunately, in the original type I/type II report a single agonistic anti-Fas antibody clone was used, and, in the majority of experiments, only at a single dose (1μg/ml) (Scaffidi et al. 1998). In only

one experiment did the authors examine the effects of different doses of the anti-Fas antibody on type I/type II cell apoptosis, and the concentration of that antibody never exceeded 1 μg/ml.

Fortunately, the type I/type II model is slowly evolving within the literature. Researchers are beginning to use terms like “type I/type II pathway”, “type I/type II apoptosis” and “type I/type II reactions” (Amanullah et al. 2002, Bond et al. 2002, Arechavaleta-Velasco et al. 2002, Rudner et al. 2005). Perhaps “type I and type II apoptosis” will someday be universally accepted, simply referring to caspase-8-mediated apoptosis that is independent of and dependent on the mitochondrion, respectively. Such a model of type I/type II apoptosis would be more versatile, as it could be applied in the context of any cell death stimulus. As yet, however, this area of apoptosis research remains controversial. Hopefully these issues will be resolved in coming years.

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