

Review

# Restricting Apoptosis for Postmitotic Cell Survival and its Relevance to Cancer

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## ABSTRACT

The importance of apoptosis in multicellular organisms is signified by the high degree of genetic conservation in the core components of this pathway from *C. elegans* through mammals. However, as the cells which comprise these organisms have diversified and become more specialized, so have the mechanisms which regulate the apoptotic pathway. The complex regulatory mechanisms by which the apoptotic pathway is refined are perhaps most apparent in differentiated postmitotic cells such as neurons, cardiomyocytes, and skeletal myotubes. The lack of significant regenerative potential in postmitotic cells demands that they must persist long-term, often for the full lifespan of an organism. Recent studies have identified several diverse mechanisms by which postmitotic cells restrict their apoptotic potential. Importantly, these mechanisms may also be coopted by cancer cells in order to evade apoptosis.

The execution of apoptosis is accomplished by the activation of caspases, a family of cysteine proteases which normally reside in cells as inactive zymogens. Initiator caspases are auto-activated by recruitment to a multimeric complex, following which they cleave and activate the effector caspases.<sup>1,2</sup> Activated effector caspases rapidly orchestrate the demise of the cell by cleaving hundreds of cellular substrates, resulting in chromatin condensation, membrane blebbing, and other apoptotic characteristics.

In mammalian cells, the activation of caspases by the intrinsic pathway of apoptosis is triggered by the release of cytochrome *c* from the mitochondria, where it normally resides as a key component of the cell's metabolic machinery. This pathway of apoptosis can be triggered by diverse stimuli, including loss of adequate growth factors, accumulation of DNA damage, or as a result of a buildup of unfolded proteins in the endoplasmic reticulum.<sup>3-8</sup> These apoptotic stimuli converge at the mitochondria, where the anti-apoptotic and pro-apoptotic members of the Bcl-2 family directly regulate apoptosis by controlling the release of cytochrome *c*. Once released from the mitochondria, cytochrome *c* binds to the adaptor protein Apaf-1 and induces its oligomerization. The subsequent recruitment of the initiator caspase-9 results in the formation of the apoptosome complex.<sup>9</sup> Following its auto-activation on the apoptosome, caspase-9 cleaves the effector caspases-3 and -7 to induce rapid cell death.

Apoptosis in postmitotic cells such as neurons, cardiomyocytes, and skeletal myotubes is a normal component of development.<sup>10-14</sup> Due to their limited regenerative potential, the survival of these cells is critical for organismal homeostasis and aberrant activation of apoptosis in these cells is a contributor to the pathology of many degenerative diseases.<sup>10,15-17</sup> Even though the core components of the apoptotic pathway are highly conserved among various mammalian cells, a theme emerging from recent studies is that postmitotic cells have developed unique mechanisms of regulating apoptosis. In this review, we highlight some of the protective mechanisms by which the apoptotic pathway is selectively restricted in postmitotic cells to ensure their long-term survival.

## ELIMINATING BAX/BAK REDUNDANCY

The release of cytochrome *c* is regulated by the Bcl-2 family of proteins that can be subdivided into pro- and anti-apoptotic members. Among the proapoptotic members, Bax and Bak act directly on the mitochondria to induce cytochrome *c* release. In most cells, Bax and Bak are functionally redundant and deletion of both of these proteins is required for inhibition of mitochondrial cytochrome *c* release.<sup>18</sup> In contrast, in a variety of neurons (e.g., sympathetic,<sup>19-21</sup> cerebellar granule,<sup>22,23</sup> cortical,<sup>24</sup> motor,<sup>25-27</sup> dorsal root ganglion<sup>26,27</sup> and hippocampal<sup>28,29</sup> neurons), deletion of Bax alone is sufficient to completely block

cytochrome *c* release and apoptosis. Bax deficiency is also sufficient to protect against neuronal apoptosis in many *in vivo* models of neurodegeneration including hypoxia ischemia,<sup>29</sup> sciatic nerve injury,<sup>30</sup> exposure to ethanol<sup>31</sup> or MPTP,<sup>32</sup> glaucoma,<sup>33</sup> and spinal muscular atrophy.<sup>34</sup> Consistent with a dependency on Bax alone, neurons derived from Bak deficient mice exhibit no protection from apoptotic stimuli.<sup>35</sup> Interestingly, the dependency on Bax for cytochrome *c* release in neurons appears to be engaged only after postmitotic differentiation, as Bax deficient neural precursors still undergo apoptosis in response to DNA damage,<sup>36</sup> while deficiency in both Bax and Bak together are required to completely protect these neuronal precursor cells.<sup>37,38</sup>

The molecular basis for why postmitotic neurons are dependent on Bax alone became clear when it was discovered that postmitotic neurons express a neuron-specific splice variant of Bak, named N-Bak.<sup>39</sup> Expression analysis indicates that N-Bak is exclusively expressed in postmitotic neurons and is the sole isoform of Bak expressed in sympathetic, cortical, cerebellar and hippocampal neurons.<sup>39,40</sup> The splicing of N-Bak involves a novel exon (exon N) which causes a frameshift resulting in the premature truncation of Bak. The resulting N-Bak protein contains only a single BH domain, and consistent with its homology to BH3-only proteins, overexpression of N-Bak results in Bax-dependent apoptosis in neurons.<sup>40</sup>

The singular dependence of neurons on Bax due to the loss of Bax/Bak redundancy provides these cells with a distinct survival advantage by restricting apoptosis at a point prior to the release of cytochrome *c*. The dependency on Bax alone for apoptosis also appears to extend to other postmitotic cells *in vivo*. For example, Bax deficiency protects cardiomyocytes against myocardial ischemia-reperfusion injury<sup>41</sup> and promotes cell survival in models of muscle degeneration.<sup>42,43</sup> However, since the status of Bax/Bak redundancy has not been specifically examined in pure populations of cardiomyocytes or myotubes, the mechanism by which Bax deficiency alone protects against apoptosis in these cells *in vivo* is unclear.

## STRICT CONTROL OF THE APOPTOSOME PATHWAY BY IAPs

The cytochrome *c*-mediated apoptosome pathway of caspase activation can be effectively regulated by the inhibitor of apoptosis proteins (IAPs), which can block apoptosis by directly binding to activated caspases.<sup>44</sup> The IAP family of proteins includes XIAP, cIAP-1, cIAP-2, and NAIP, all of which contain baculovirus IAP repeat domains and are ubiquitously expressed in cells.<sup>45</sup> While the addition of purified IAPs blocks caspase activation in cell free extracts and ectopic overexpression of IAPs potently inhibits apoptosis in cells,<sup>46-51</sup> the ability of endogenous IAPs to strictly regulate apoptosis in primary cells appears far more restricted. Recent studies show that the level of apoptosome activity is a key determinant for the effectiveness of endogenous IAPs in regulating caspase activation and apoptosis.<sup>52</sup> For example, in most mitotic cells where the levels of the apoptosome components are high, endogenous IAPs appear to be largely ineffective in regulating cytochrome *c*-mediated apoptosis. In contrast, in postmitotic cells such as neurons and cardiomyocytes, where the levels of Apaf-1 are markedly reduced<sup>52-54</sup> (likely resulting in fewer functional apoptosomes being formed), endogenous IAPs (XIAP in particular) are highly effective in controlling cytochrome *c*-mediated apoptosis.<sup>52,55,56</sup>

In postmitotic cells, the low levels of Apaf-1 enable the endogenous IAPs to effectively function as a safety brake that would prevent

against inappropriate caspase activation if cytochrome *c* is accidentally released from damaged mitochondria. Consistent with this hypothesized safety brake function of IAPs in postmitotic cells, the various IAP deficient mice generated do not exhibit any overt apoptosis phenotype,<sup>57-59</sup> as postmitotic cells are still dependent on cytochrome *c* release and therefore not expected to undergo spontaneous apoptosis in the absence of IAPs. However, as anticipated, postmitotic neurons where IAPs are inactivated appear more susceptible to apoptosis in acute injury situations.<sup>60,61</sup> Thus, increased effectiveness of IAPs allows apoptosis in postmitotic cells to be blocked even after the point of cytochrome *c* release.

## ALTERED POINT OF COMMITMENT TO DEATH

In most mammalian cells, direct inhibition of caspases may only serve to prolong the inevitable, as the release of cytochrome *c* from the mitochondria represents a commitment point to death.<sup>62-64</sup> Therefore, even if apoptosis is prevented in these cells by pharmacological inhibition or genetic deletion of caspases, the rapid loss of mitochondrial membrane potential ( $\Delta\Psi_m$ ) that accompanies cytochrome *c* release results in metabolic failure from which these cells are unable to recover, causing their eventual death, albeit by nonapoptotic mechanisms. However, studies in sympathetic neurons have shown that NGF deprived neurons where the apoptotic pathway is arrested even after the point of cytochrome *c* release (e.g., with a caspase inhibitor or caspase-9 deficiency), retain the capacity for recovery and long term survival upon readdition of NGF.<sup>65,66</sup> Under these conditions, the ability of neurons to recover with NGF readdition correlates with the ability of these neurons to maintain  $\Delta\Psi_m$  even after cytochrome *c* release.<sup>66</sup> The ability of NGF deprived, caspase-inhibitor saved sympathetic neurons to retain  $\Delta\Psi_m$  is critically dependent on the production of ATP by glycolysis,<sup>67</sup> and  $\Delta\Psi_m$  can be preserved and recoverability extended with the addition of the mitochondrial permeability transition pore inhibitor cyclosporin A.<sup>68</sup>

Even though cytochrome *c* release precedes the loss of mitochondrial membrane potential in other neurons as well,<sup>69-71</sup> the ability of neurons to recover from that point may be stimulus dependent. For example, caspase inhibitors fail to promote the long term survival of potassium deprived cerebellar granule neurons or camptothecin treated cortical neurons.<sup>22,70,72</sup> The ability of cardiomyocytes and myotubes to recover after cytochrome *c* release has not been directly examined. We anticipate however, that the existence of evolutionarily conserved postmitochondrial mechanisms that control apoptosis (e.g., IAPs) are indicative of their likely functional importance in promoting the long-term survival of these postmitotic cells. Consistent with this model, XIAP overexpression or caspase-3 inhibition confers significant long-term protection in models of brain ischemia<sup>73,74</sup> and cardiac injury.<sup>75</sup>

## SELECTIVE EXPRESSION OF APOPTOSIS MODULATORS

Various proteins that interact with and modulate the activity of the core apoptotic components have been identified and shown to have restricted patterns of expression.<sup>76-83</sup> The expression pattern of these proteins during development and in specific tissues provides a mechanism by which the apoptotic pathway could be selectively modulated in specific cell types.

The pro-apoptotic protein ASC/TMS1 (apoptosis-associated speck-like protein/target of methylation-mediated silencing) is a CARD containing protein that is enriched in epithelial cells and

leukocytes. However, its expression is virtually absent in brain, heart, and skeletal muscle.<sup>82</sup> While ASC/TMS1 plays an important role in regulating inflammation, it also induces apoptosis through both the intrinsic and extrinsic pathways. ASC/TMS1 is significantly upregulated by p53 expression, and knockdown of ASC/TMS1 by siRNA was shown to suppress translocation of Bax to the mitochondria and subsequent apoptosis following DNA damage.<sup>84</sup> Furthermore, overexpression of ASC/TMS1 can directly bind and activate caspase-8 to induce apoptosis.<sup>85,86</sup> Thus, it is reasonable to hypothesize that the lack of significant ASC/TMS1 expression may impart neurons, cardiomyocytes and skeletal myotubes with increased resistance to apoptotic stimuli through both the intrinsic and extrinsic pathways.

Another apoptotic regulatory protein with an intriguing pattern of selective expression is ARC (apoptosis repressor with a CARD). ARC was originally identified by its homology to the CARD domain of caspase-9, and in contrast to ACS/TMS, its expression is highly enriched in neurons, heart, and skeletal muscle.<sup>87</sup> Recent studies have shown that ARC can inhibit apoptosis by blocking both the extrinsic and intrinsic arms of the apoptotic pathway.<sup>88,89</sup> ARC inhibits the death receptor pathway by disrupting the assembly of a functional DISC complex by interacting specifically with Fas, FADD, and procaspase-8. ARC also inhibits the intrinsic apoptotic pathway by directly binding to Bax and preventing its translocation to the mitochondria.<sup>88,89</sup> Consistent with its protective role in postmitotic cells, ARC inactivation via its degradation is important for apoptosis to proceed during ischemic heart<sup>88</sup> and brain<sup>90</sup> injury. Likewise, ARC deficient mice develop accelerated cardiomyopathy in response to biomechanical stress and are more sensitive to ischemia-reperfusion injury.<sup>91</sup> Thus, with the presence of anti-apoptotic- and the concomitant absence of proapoptotic-modulators, postmitotic cells are able to effectively control their apoptotic pathway.

## POSTMITOTIC CELLS AND CANCERS: COMMON MODES OF APOPTOSIS CONTROL?

While increased restriction of the apoptotic pathway is physiologically important for maintaining the long-term survival of postmitotic cells, it is conceivable that these same mechanisms could be coopted in mitotic cells to achieve the apoptotic resistance required for cancer progression. Indeed, striking parallels between the survival mechanisms described above for postmitotic cells have been documented in many cancers.

While they are not considered to be classical oncogenes, IAPs have been detected at elevated levels in a variety of cancers. For example, ML-IAP has been shown to be specifically upregulated in most melanomas, while its expression is virtually undetectable in normal adult tissues.<sup>92</sup> Other IAPs have been detected at elevated levels in nonsmall cell lung cancer, prostate cancer, and several types of lymphomas and leukemias.<sup>93-96</sup> Importantly, since IAP effectiveness is coupled to apoptosome activity in primary cells, IAP-mediated apoptotic resistance in tumor cells may be acquired without the overexpression of IAPs. Indeed, there are several studies which have identified cancers with diminished apoptosome function,<sup>97-100</sup> although the contribution of IAPs to the apoptotic resistance of these specific cells has not been established. Further evidence supporting a role for IAP-mediated apoptotic resistance in cancer comes from recent studies exploring the therapeutic potential of IAP antagonists. Small peptide mimics of the endogenous IAP inhibitor Smac<sup>101-103</sup> and nonpeptidic compounds identified in a small molecule library screen<sup>104</sup> are effective in sensitizing several tumor derived cell lines to various apoptotic stimuli by antagonizing IAP function.

Recent studies have also demonstrated that the dysregulation of apoptotic regulatory proteins such as ASC/TMS1 and ARC may contribute to apoptotic resistance in tumor cells. As discussed earlier, ASC/TMS1 is a pro-apoptotic regulatory protein that is highly expressed in several mitotic epithelial cell populations, but is excluded from adult neurons, cardiomyocytes, and skeletal muscle. Interestingly, ASC/TMS1 was identified as a target of methylation-induced silencing in breast cancer<sup>82</sup> and subsequent studies have identified methylation induced silencing of ASC/TMS1 in several other cancers, including gastric carcinomas, lung carcinomas, malignant melanomas, and glioblastomas.<sup>105</sup> Since roles for ASC/TMS1 have been described in the apoptotic, inflammatory, and NF- $\kappa$ B signaling pathways, the specific mechanisms by which loss of ASC/TMS1 contributes to cancer development is still unclear.

In contrast to ASC/TMS1, ARC is selectively expressed in postmitotic neurons, cardiomyocytes and skeletal muscle where it can exert its anti-apoptotic function through both the extrinsic and intrinsic pathways. Recently, ARC has been found to be highly expressed in a variety of cancer cell lines, and overexpression of ARC conferred breast cancer derived cells with resistance to chemically and radiation induced apoptosis.<sup>106,107</sup>

Whether cancer cells utilize the other postmitotic cellular mechanisms highlighted in this review to restrict apoptosis is still unclear. However, future studies that investigate the regulation of apoptosis in postmitotic and cancer cells could test this prediction.

## CONCLUDING REMARKS

The ability of cells to activate apoptosis and die is important during development and essential for the maintenance of homeostasis in organisms. Apoptosis is also a major tumor suppressor mechanism in situations where cells lose their proliferative control. While the core components of the apoptotic pathway are common to most cell types, the diverse mechanisms by which this pathway is regulated in primary cells reflects the differential demand for apoptosis in specific cellular populations. While mitotic cells are at continual risk of becoming cancerous, this risk does not exist in terminally-differentiated postmitotic cells since these cells have permanently exited the cell cycle. In fact, our ability to maintain the long-term survival of postmitotic cells that largely comprise the brain, heart and skeletal muscle is critical for normal physiological functions. Therefore, cells must efficiently balance the need for having a primed apoptotic pathway versus the risks associated with it.

In this review, we have highlighted a few of the diverse mechanisms by which apoptosis is selectively controlled in postmitotic cells. Undoubtedly, additional novel mechanisms that strictly control the major control points in the apoptotic pathway exist in these cells. Identification of these mechanisms has an exciting future as it opens the possibility of selectively modulating apoptosis in specific cell types for therapeutic goals.

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