Editorial

Competition for growth factors: a lot more death with a little less Aktion

CJ Kearney¹ and SJ Martin^{*1}

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Apoptosis is frequently seen in response to cytotoxic drugs or other stimuli that provoke cell damage or stress. However, the vast majority of homeostatic cell deaths *in vivo* occur due to physiological cues that permit the selection of the fittest cells within a tissue, by weeding out the aged and the superfluous through competition. One such fitness test is competition for growth factors. Raff championed the idea of social control of cell death by predicting that all cells continuously require signals (i.e., growth factors) provided by other cells to maintain viability.¹ The failure to compete successfully for growth factors not only prevents cell division but also can lead to the generation of signals that eliminate cells via apoptosis. However, despite the importance of this topic, it still remains unclear precisely how growth factor receptor engagement, or lack thereof, interfaces with the cell death machinery. This is also a topic of much importance because transformed cell populations frequently overexpress their own growth factors or deregulate the signaling pathways acting downstream of growth factor receptors. In addition to promoting proliferation, deregulated growth factor receptor signaling can also result in the suppression of apoptosis.² Thus, learning how growth factor-induced pro-survival signaling operates may reveal key steps for therapeutic manipulation to enhance the sensitivity of transformed cells to chemotherapeutic drugs. So how does the failure to obtain sufficient growth factor lead to the engagement of the cell death machinery? This is the question that a paper by Ekert and colleagues,³ published in this issue of *CDD*, addresses.

The PI3-kinase (PI3K)/Akt pathway has long been linked with promotion of cell proliferation and suppression of apoptosis.⁴ PI3K is activated downstream of practically all growth factor receptors and acts upon the cell death machinery, as well as numerous other cellular functions, by activating the downstream kinase Akt (PKB). As a consequence of its potent pro-survival and growth-promoting properties, the PI3K/Akt pathway is frequently deregulated in cancer, through loss of PTEN, gain-of-function mutations in PI3K or activating mutations in Akt.^{5,6}

So how does Akt/PKB suppress cell death? Akt phosphorylates numerous substrates that can, at least in theory, raise the threshold for apoptosis (Figure 1). For example, Bad was one of the first cell death-associated Akt substrates to be identified, and phosphorylation of this BH3-only protein by the

latter kinase allows it to be sequestered through binding to 14-3-3 proteins, thereby taking it out of active service.⁷ Similarly, Akt-dependent phosphorylation of Bim also permits binding to 14-3-3 proteins.⁸ However, complete loss of Bad does not have a significant impact on the threshold for apoptosis induced by cytokine deprivation. Similarly, Akt has been reported to phosphorylate and inactivate caspase-9,⁹ but this is not a conserved event between human and mouse, making this phosphorylation event unlikely to represent a key Akt-dependent cell survival mechanism. Moreover, caspase-9 acts downstream of apoptosis-associated mitochondrial permeabilization and cytochrome-c release, a point of no return for the majority of proliferating cell types, beyond which intervention is highly unlikely to result in cell survival.¹⁰ FoxO transcription factors have also been implicated as important Akt targets in the context of apoptosis suppression, as the latter can transcriptionally upregulate Bim¹¹ (Figure 1). Akt-mediated phosphorylation of FoxO leads to its exclusion from the nucleus through binding to 14-3-3, followed by degradation, thereby suppressing FoxO-mediated Bim expression.¹² Another possible route to Akt-dependent cell survival involves the pro-survival Bcl-2 family protein Mcl-1, which is protected from GSK-3-dependent phosphorylation leading to proteasome-mediated destruction, as a consequence of Akt-mediated inactivation of GSK-3.¹³

Numerous additional Akt targets have been identified over the years, including MDM2 (leading to p53 degradation), IKK α (leading to NF κ B activation, which also has pro-survival function) and others.^{14–16} However, it remains unclear which, if any, of these Akt substrates have the dominant role in propagating cell death signals upon withdrawal of growth factors and loss of Akt activity. Clearly, one possibility is that all of these Akt targets have overlapping and partly redundant roles in growth factor deprivation-associated apoptosis. Alternatively, specific Akt substrates may have dominant roles in a cell type or growth factor-specific manner. To complicate matters further, three Akt isoforms exist, encoded by distinct genes, and it is not known whether these are also redundant for cell survival.

IL-3 is a major determinant of proliferation and viability in myeloid progenitor cells. To explore the requirement for specific Akt isoforms in protecting from IL-3-deprivation-induced apoptosis, Ekert and colleagues³ generated

¹Molecular Cell Biology Laboratory, Department of Genetics, The Smurfit Institute, Trinity College, Dublin, Ireland

^{*}Corresponding author: S Martin, Molecular Cell Biology Laboratory, Department of Genetics, The Smurfit Institute, Trinity College, Dublin D2, Ireland. Tel: +353 1 8961289; Fax: +353 1 679 8558; E-mail: martinjs@tcd.ie

Figure 1 The diversity of pathways leading to Akt-mediated suppression of cell death signals. Akt/PKB activation downstream of growth factor receptor engagement has been implicated in suppression of apoptosis through phosphorylation of Mdm2, GSK-3, FoxO, Bad, Bim and other substrates

IL-3-dependent myeloid progenitor-derived cell lines from mice lacking one of each of the three Akt isoforms. The authors then explored which of the Akt isoform-deficient cell lines were most vulnerable to IL-3 withdrawal and found that loss of Akt1 had a much greater impact than either of the other Akt isoforms. Thus, Akt1-deficient cells exhibited much greater levels of apoptosis under limiting IL-3 concentrations.³ To explore the possible targets of Akt1 in cell survival, the authors used a panel of cell lines deficient in the BH3-only proteins Bad, Bim or Puma, as well as their downstream targets Bax and Bak. Although cells doubly deficient in Bax and Bak were completely protected from IL-3-deprivation-induced apoptosis, loss of Bim or Bad had little effect. In contrast, loss of Puma phenocopied the effects of Akt1 overexpression in delaying the onset of cell death in response to IL-3 withdrawal, suggesting that Puma is a key modulator of IL-3-deprivation-associated apoptosis. However, it should be noted that loss of Bax and Bak was considerably more protective than loss of Puma, or indeed than overexpression of Akt1, suggesting that a degree of redundancy exists between Puma and other upstream regulators of the Bax/Bak channel in this context.

So how is Puma inactivated by Akt1? Puma has not been reported to be a direct target of Akt, but is well known to be

transcriptionally upregulated in a p53-dependent manner in response to a number of stimuli, including γ -irradiation and growth factor deprivation.¹⁷ Thus, Green *et al.*³ also explored the role of p53 in apoptosis induced by IL-3 deprivation and found that loss of this transcription factor robustly protected from loss of growth factor signaling. Moreover, IL-3-deprivation-induced upregulation of Puma was suppressed in p53-deficient cells. Thus, competition for IL-3 in myeloid progenitor cells may result in apoptosis as a consequence of a drop in Akt-dependent Mdm2 phosphorylation, leading to exclusion of the latter from the nucleus,¹⁴ followed by accumulation of p53 and transcriptional upregulation of Puma. Interestingly, studies by Rathmell and colleagues¹⁸ have also implicated p53-dependent upregulation of Puma in cell death invoked by growth factor withdrawal. However, there are clearly other mechanisms at play here, as loss of Bax/Bak was profoundly more protective than loss of p53 or Puma.³ Moreover, Puma-deficient cells were considerably more sensitive to undergoing apoptosis in response to IL-3 deprivation combined with an Akt inhibitor than were p53-deficient cells.³ This strongly suggests that p53 transcriptional targets, other than Puma, are also important in this context.

In summary, the present study suggests that loss of Akt1 signaling leading to the accumulation of p53 and upregulation of the BH3-only protein Puma are instrumental in culling myeloid progenitor cells that fail to obtain sufficient IL-3. Thus, loss of p53 function, as occurs in many transformed cell populations, may endow such cells with a competitive advantage over their neighbors in conditions where growth factors are limiting.

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