

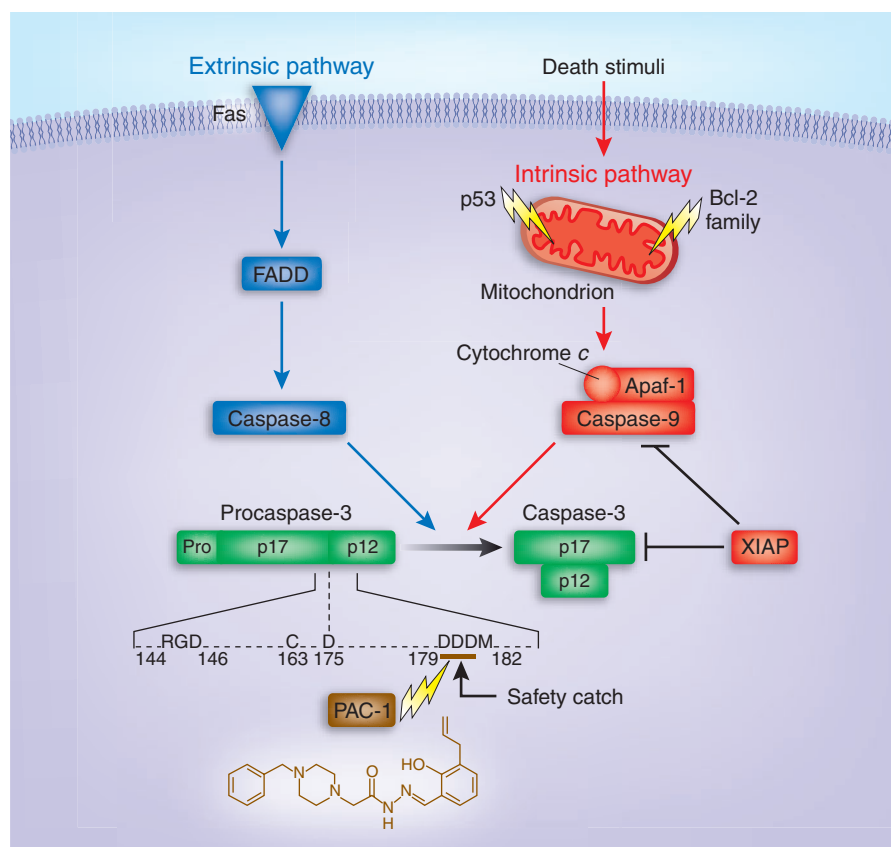
# Flipping the safety catch of procaspase-3

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**Caspase-3 is a central player in the orchestration of apoptotic cell death. A newly identified compound selectively activates caspase-3, has proapoptotic activity against transformed cells and retards the growth of procaspase-3-rich tumors.**

Selective induction of apoptosis in diverse tumor types using targeted ‘magic bullets’ is an important but elusive goal of cancer therapy. The ideal magic bullet is an agent that selectively destroys or is specifically delivered to a tumor but does not cause side effects by damaging normal tissues. Typically, it might target an important component of a signal transduction pathway that is defective in cancer. A paper in this issue of *Nature Chemical Biology* by Putt *et al.* reports a significant advance and a new approach toward this end<sup>1</sup>. This report identifies the first synthetic organic molecule (called PAC-1) that induces the proteolytic processing of dormant procaspase-3 to active caspase-3, the main executioner protease in apoptotic cell death<sup>2,3</sup> (Fig. 1). Procaspase-3 normally relies on a “safety catch” that prevents it from being inadvertently activated in living cells<sup>4</sup>; PAC-1 seems to both flip off the safety catch and pull the trigger<sup>1</sup>.

Caspase-3 is crucial in apoptosis because it cleaves and destroys (or modifies the functions of) hundreds of cellular protein substrates<sup>2</sup> and is an important point of convergence of the extrinsic (receptor-initiated) and intrinsic (mitochondrial) apoptosis pathways<sup>3</sup> (Fig. 1). Procaspase-3 is held dormant by an Asp-Asp-Asp (DDD) safety catch contained within a flexible loop near the junction of the large p17 and small p12 subunits<sup>4</sup> (Fig. 1). Removal or modification of the safety catch results in both autocatalytic maturation (self-cleavage at Asp175 to



**Figure 1** Summary of cell death pathways that converge on the proteolytic activation of caspase-3. Caspase-3 activation via tumor necrosis factor (TNF) family receptors (for example, Fas), FADD (Fas-activated death domain protein) and caspase-8 represents the extrinsic pathway (blue), whereas caspase-3 activation via the mitochondrial release of cytochrome c and Apaf-1-mediated processing of caspase-9 represents the intrinsic pathway (red)<sup>3</sup>. For clarity, not all of the players are shown. Procaspase-3 is shown as a PAC-1-sensitive dormant single-chain precursor with an N-terminal prodomain (Pro). During apoptosis, caspase-3 assembles as an active p17-p12 heterotetramer after proteolytic processing between the p17 and p12 subunits (at Asp175) and removal of the prodomain<sup>2</sup>. PAC-1 is proposed to regulate the Asp-Asp-Asp (DDD) safety catch at amino acids 179–181 in procaspase-3, consequently inducing a conformational change that leads to proteolytic processing into the active p17 and p12 subunits<sup>1</sup>. Cys163 is the catalytic cysteine in the active site of caspase-3; the sequence shown illustrates its proximity to the DDD safety catch and DDM motif. Although caspase-7 (not shown) is believed to be a downstream caspase, its position relative to caspase-3 in apoptosis pathways is unclear.

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generate the active p17 and p12 subunits) and increased propensity to proteolytic activation by upstream proteases in the apoptosis cascade<sup>2,4</sup>. Putt *et al.* envisioned that a small molecule capable of disabling this safety catch might have an impact on cancer therapy. They accordingly screened a chemical library to find compounds that can convert procaspase-3 to caspase-3 and discovered that PAC-1 apparently targets the safety catch, though they did not explicitly demonstrate an interaction of this region and PAC-1 (ref. 1). The related procaspase-7 has only two aspartate residues in a putative safety catch, but PAC-1 activated caspase-7 greater than ten-fold less effectively than it did caspase-3. Other caspases were not tested, but because they lack DDD safety catches<sup>4</sup> and their mode of activation may be generally different<sup>2</sup>, PAC-1 is anticipated to be specific for procaspase-3.

Procasase-3 is also synthesized in normal cells and is important in mammalian development<sup>2</sup>, so why is it considered to be such an attractive cancer drug target? Surprisingly, procaspase-3 concentrations in colon cancer tissue and many transformed cell lines are much higher than those in normal tissue and untransformed cells, respectively<sup>1</sup>. PAC-1 induces rapid apoptosis of diverse cancer cell types in culture. Tumor cells were much more sensitive to PAC-1-mediated killing than were normal cells, and the amount of apoptosis induced by PAC-1 strongly correlated with procaspase-3 concentrations, which suggests that procaspase-3 is the target of PAC-1 in cells. Notably, PAC-1 caused impressive retardation in the growth of experimental tumors in three distinct mouse models of cancer (one renal and two lung), including one in which PAC-1 was administered orally<sup>1</sup>. There was no evidence of gross toxicity, and plasma concentrations of PAC-1 were very low.

Targeting procaspase-3 makes perfect sense, because it lies well downstream in the cell death cascade<sup>1,3</sup>. Thus, PAC-1 should be effective against cancers with oncogenic defects rendering the cells resistant to apoptosis that involve proteins such as Apaf-1, Bcl-2, p53 and XIAP (all of which are upstream of procaspase-3)<sup>5</sup> (Fig. 1). Indeed, Putt *et al.*<sup>1</sup> showed that PAC-1 is effective in tumor cell

lines having faulty apoptosis pathways that are likely a result of reduced proapoptotic Apaf-1 concentrations or enhanced antiapoptotic Bcl-2 expression upstream of procaspase-3. Some tumors may actually downregulate procaspase-3 (ref. 6), and these are predicted to be resistant to PAC-1, as was shown for caspase-3-deficient MCF-7 breast carcinoma cells<sup>1</sup>. Therefore, it is crucial to select only those cancers having elevated concentrations of procaspase-3 for PAC-1-based therapy.

Previously, there have been other promising approaches to activating caspase-3 in tumors<sup>5</sup>. In one, researchers identified compounds that relieved the endogenous inhibition of already activated caspases in tumor cells<sup>7,8</sup>. Inhibitor of apoptosis proteins<sup>3,5</sup> (IAPs; for example, XIAP) normally bind to and restrain the activity of active caspase-3, caspase-7 and caspase-9 (Fig. 1). XIAP antagonists rapidly and selectively kill different tumor cells having higher concentrations of XIAP than untransformed cells<sup>7,8</sup>, but they seem to be less effective than PAC-1. Moreover, it is not clear in which scenarios other IAPs (for example, cIAP1 and cIAP2) might compensate for loss of XIAP function in the presence of these XIAP antagonists. PAC-1 offers a complementary approach to IAP inhibitors, which unlike PAC-1 seem to target a pool of already processed and activated caspase-3 in tumor cells<sup>7,8</sup>.

Research has shown that peptides containing the Arg-Gly-Asp (RGD) motif directly induce the processing of procaspase-3 to caspase-3 *in vitro*, much like PAC-1 (ref. 9). This motif is typically an integrin recognition signal in proteins of the cell's extracellular matrix, and Asp-Asp-Met (DDM) is one of the sequences in  $\beta$ -integrins that is recognized by RGD. Short RGD peptides have been widely used as inhibitors of integrin-extracellular matrix interactions to induce apoptosis in models of inflammation, angiogenesis and cancer metastases<sup>9</sup>. RGD peptides at high concentrations have been found to activate caspase-3 in cells independently of integrins<sup>9</sup>. As RGD and DDM are both present in procaspase-3 near the site of self-cleavage (Asp175) and the active site cysteine (Cys163; Fig. 1), it is possible that these RGD and DDM sequences are engaged in intramolecular interactions that normally

prevent autoactivation of procaspase-3. RGD peptides may block these interactions, causing a conformational change in procaspase-3 that exposes the nearby Asp175 to self-cleavage and thereby activates caspase-3 (ref. 9). Notably, the DDM sequence overlaps the DDD safety catch, which suggests that PAC-1 and RGD peptides might induce similar conformational changes in procaspase-3.

There is much to do and learn. An understanding of the molecular details of PAC-1 binding and activation of procaspase-3 will provide insight into this interaction and facilitate the design of even more potent PAC-1 derivatives. We need to know whether PAC-1 prolongs survival in addition to reducing tumor load. It is not clear why many tumor cells upregulate procaspase-3, a potential apoptosis mediator that carries the seeds of their own destruction<sup>1</sup>. In addition to its role in apoptosis, caspase-3 has roles in B cell proliferation and in the differentiation of many cell types<sup>10</sup>; will PAC-1 have side effects owing to the perturbation of these vital functions?

Despite these questions and uncertainties, PAC-1 has the attributes to become a potent weapon in the anticancer armory. PAC-1 seems to have advantages over other caspase-3-activating approaches: these include greater specificity for procaspase-3, excellent potency and tumor cell selectivity, and oral availability<sup>1</sup>. Because PAC-1 can induce efficient proteolytic processing of recombinant procaspase-3 in the absence of any other cellular components, PAC-1 may not be a magic bullet as such but a magic finger that both flips off the safety catch and pulls the caspase-3 trigger.

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