Procaspase-Activating Compounds: *in vitro* Mechanism and *in vivo* Applications as a Personalized Anti-Cancer Therapy

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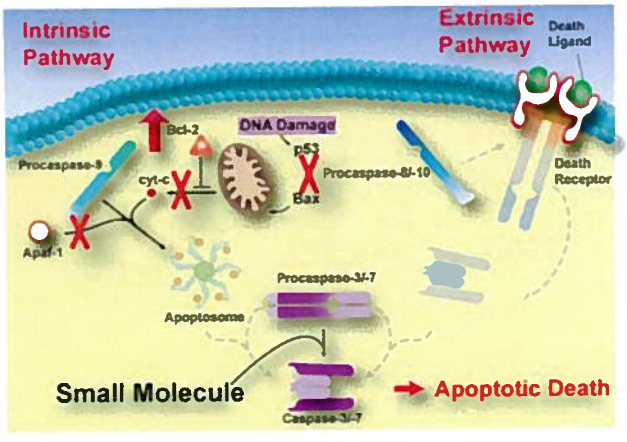
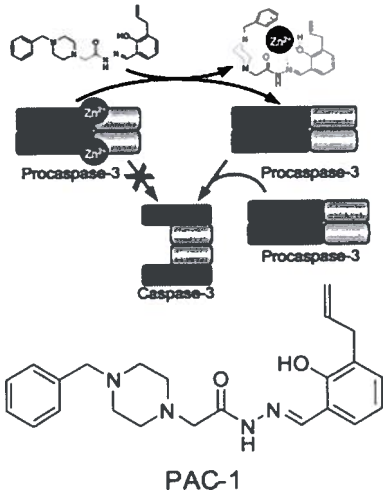
One key hallmark of cancer is its ability to evade apoptotic cell death. In normal cells, the apoptotic intrinsic and extrinsic pathways converge on the activation of executioner cysteine aspartate proteases caspases-3, -6, and -7. However, cancer cells are able to escape apoptosis through mutations which render the key apoptotic proteins inactive or aberrantly expressed. Paradoxically, procaspases are generally upregulated and rarely mutated in cancers. Therefore, a small molecule that directly activates procaspase-3 to caspase-3 to induce apoptosis in cancer cells by circumventing the defective apoptotic machinery could serve as an effective personalized anti-cancer therapy.

To this aim, a procaspase-activating compound (PAC-1) was discovered from a high-throughput screening for procaspase-3 activators. PAC-1 activates procaspase-3/-7 *in vitro* through the chelation and sequestration of inhibitory zinc ions. Furthermore, PAC-1 is potently cytotoxic in a variety of cancer cell lines as well as in isolated cells from primary resected tumors. Confocal microscopy studies reveal that a fluorescent derivative of PAC-1 co-localizes with active caspase-3/-7, lending further evidence that PAC-1 activates caspases in the cell via zinc chelation.

The *in vivo* safety, pharmacokinetics, and efficacy of PAC-1 and its derivatives have been assessed in mice and client-owned dogs with lymphoma. One derivative, s-PAC-1 has been found to reduce or stabilize the tumor progression in dogs with a continuous i.v. treatment over 24h. Some PAC-1 derivatives have the unique ability to induce a strong cytotoxic response in cell culture with exposure times as short as 15 minutes. This "pulse" feature would allow for a more attractive and simplified dosing regimen *in vivo*, compared to a continuous administration of the drug. However, currently identified "pulse" derivatives are neurotoxic *in vivo*; therefore ~800 PAC-1 derivatives are being synthesized in parallel to identify optimal compounds that are safe, effective, and display the desired "pulse" phenotype. Described herein are the *in vitro* and cell death mechanisms of PAC-1, and the *in vivo* application of PAC-1 and its derivatives as cancer treatments.





PAC-1