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INDUCING APOPTOSIS IN CANCER THERAPY

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Every cell in a multicellular organism has the potential to die by apoptosis. Treatment of cancer cells with tumour necrosis factor -α can induce an apoptotic signal leading to the death of these cells; however, many malignancies are resistant to the effects of TNF-α. Overexpression of FLIP, a protein that blocks the caspase activity of FLICE, mediates the observed resistance. Thus, FLIP, which normally prevents inappropriate apoptosis, may become a tumour progression factor.
Cells of multicellular organisms have the inherent capacity to undergo inducible self-destruction by a highly organized process named programmed cell death, or apoptosis. Impairment of the apoptotic machinery of the cell or aberrant expression of anti-apoptotic proteins can contribute to the development of neoplasia and help tumour cells to escape from tumour surveillance by the immune system (Hanahan and Weinberg, 2000).

A significant part of the benefit achieved by chemotherapy relies on the induction of apoptosis in tumour cells. Therefore, the development of resistance to apoptosis is of considerable clinical relevance. The central event in apoptosis is the proteolytic activation of cysteine aspartyl-specific proteases (caspases) that cleave defined cellular target proteins and thereby induce the characteristic morphological hallmarks of apoptosis. These effector caspases are triggered by initiator caspases that, in turn, become activated by associating with the signaling complex of death receptors or a cytosolic multiprotein complex called the apoptosome (Shi, 2003).

Death receptors (e.g., Fas) are members of the of the tumour necrosis factor (TNF) receptor (TNFR) superfamily and comprise a subfamily that is characterized by an intracellular domain—the death domains (Sartorius et al., 2001). Death receptors are activated by their natural ligand, the TNF family. When death receptors interact with the DD-containing adapter protein FADD (Fas-associated death domain protein) through DD-DD association (Figure 1).

Death receptor-bound FADD in turn recruits the proenzyme form of caspase-8 (procaspase-8) thereby initiating the formation of the death-inducing signalling complex (DISC). Procaspase-8 is composed of an N-terminal regulatory prodomain consisting of two death effector domains, which are related to the DD, and a C-terminal caspase homology domain. In the DISC, several procaspase-8 molecules are located in close proximity, leading to their activation by dimerization (Donepudi et al., 2003, Boatright et al., 2003). Subsequently, the active caspase-8-dimer is stabilized by a two-step autoproteolytic process. First, procaspase-8 (p55/53) is cleaved between the p20 and p10 subunits of the caspase homology domain generating a FADD-bound p43/41 intermediate, which is still associated with the p10 subunit (Figure 1).

Proteolytic cleavage of the p43/41 intermediate between the prodomain and the p20 subunit results in the release of mature, active caspase-8 (composed of a p20/p10 heteromer) into the cytoplasm. The FADD-bound prodomain can be finally replaced by another procaspase-8 molecule to start a new cycle of caspase-8 activation. Although caspase-8 activation can occur under nonapoptotic conditions, it typically leads to apoptosis by two different pathways. In “Type I” cells, caspase-8 directly activates effector caspases, especially caspase-3, to an extent sufficient to ensure apoptotic cell death (Figure 2). In contrast, in “Type II” cells caspase-8-induced apoptosis relies on the stimulation of additional mitochondria-dependent amplification mechanisms (Sartorius et al., 2001; Boatright et al., 2003).

Recruitment of mitochondria in apoptosis is initiated by caspase-8-mediated cleavage of the Bcl-2 family member BID. Upon cleavage, a fragment of BID translocates to the mitochondria and induces the release of apoptogenic proteins, including cytochrome c, second mitochondria-derived activator of caspase/direct IAP-binding protein (Smac/Diablo) and the serine protease HtrA2/Omi (van Loo et al., 2001).

Cytosolic cytochrome c triggers the assembly of the caspase-9 activating apoptosome, which in turn is able to activate caspase-3. Because caspase-3 can process and activate caspase-8 and caspase-9, a self-amplifying circuit of caspase activation is established (Figure 2). Smac/Diablo and HtrA2/Omi block the caspase-inhibitory function of members of the inhibitor of apoptosis protein (IAP) family and thereby further facilitate the work of the effector caspases (van Loo et al., 2001).

Each step in death receptor-mediated apoptosis is well regulated; however, the control of apoptosis imparted by the isoforms of the caspase-8-related FLICE-inhibitory protein (FLIP)
is of particular interest. Although more than ten isoforms of FLIP mRNA have been described, only two of them, FLIPs and FLIPl, have been significantly studied at the protein level (Boatright et al., 2003). FLIPl consists of two N-terminal death effector domains and a C-terminal caspase homology domain devoid of enzymatic activity; whereas FLIPS is only composed of the N-terminal death effector domain and a short C-terminal stretch of amino acids not found in FLIPl.

Both proteins can be recruited to the DISC but they function differently: FLIPs prevents the initial cleavage step of caspase-8 activation between the p20 and the p10 subunit of the caspase homology domain; FLIPl inhibits the final cleavage between the prodomain and the p20 subunit of the p43/41 intermediate (Figure 2).

There is growing evidence that FLIP can act as a tumour-progress factor (Tome and Tschopp, 2001; Igney and Krammer, 2002). For example, FLIP expression correlates with resistance against death receptor-induced apoptosis in a variety of B-cell lymphomas, and FLIP-transfected tumour cell lines develop more aggressive tumours in vivo (Tome and Tschopp, 2001; Igney and Krammer, 2002). Conversely, administering chemotherapeutic drugs to sensitize cells that are resistant to death receptor–induced apoptosis often correlates with decreased expression of FLIP (Wajant et al., 2002).

Additionally, FLIP is a target of the major anti-apoptotic pathways involved in carcinogenesis, namely the NFk-B, Akt/PKB, and MAPK pathways. The particular relevance of FLIP for apoptosis-resistance has been pinpointed in recent reports showing that decreased expression of FLIP is sufficient to confer sensitivity against death receptor–induced apoptosis. In the human ovarian epithelial cancer cell line Ov2008 transfection with a FLIP–specific antisense construct interferes with TNF-mediated, NFk–B-dependent increases in FLIP expression and confers sensitivity to the cytotoxic action of TNF, which is mediated by the death receptor TNF–R1 (Xiao et al., 2003).

Moreover, FLIP antisense oligonucleotides have been successfully used in three other studies to sensitize resistant prostate cancer cells (Hyer et al., 2002), multiple myeloma cells (Mitsiades et al., 2002), and chronic lymphocytic leukemia cells (Pedersen et al., 2002) to death receptor–induced apoptosis. A further report demonstrates that small interfering RNAs can be used for FLIP downregulation, too (Siegmund et al., 2002).

Inhibition of FLIP expression in tumour cells might be of particular importance for TNF-related apoptosis–inducing ligand (TRAIL–based cancer therapies. TRAIL is the ligand of two death receptors, TRAIL–R1 and TRAIL–R2, and has attracted considerable attention in recent years as a potential anti-cancer reagent because of TRAIL’s ability to induce apoptosis preferentially in tumour cells. It is worth noting that TRAIL alone has little, if any, apoptotic effect and therefore requires cotreatment with conventional chemotherapeutic drugs that sensitize tumour cells, but unfortunately also some normal cells, for death receptor–induced apoptosis.

Chemotherapy has often–pleiotropic effects, including the inhibition of anti-apoptotic pathways that typically regulate a whole battery of effector molecules. For example, the TNFk–B pathway induces, in a cell–type specific way, almost a dozen anti–apoptotic proteins including FLIP, and in animal models, interfering with this TNF receptors resulted in extensive ischemic and hemorrhagic lesions leading to septic shock and fulminant hepatic failure (Kreuz et al., 2001; Micheau et al., 2001, Ghobrial et al., 2005).

Therefore, it seems conceivable that chemotherapeutic drugs sensitize normal and cancer cells by targeting different cell–type specific effector proteins. Indeed, it has been recently shown that proteasome inhibitors sensitize primary keratinocytes to TRAIL–induced apoptosis by blocking the maturation and activity of caspase–3, most likely by interfering with the function of the inhibitory xIAP protein, an E3 ligase that drives the proteasomal degradation of caspase–3 and Smac/Diablo (Levrkus et al., 2003).

However, proteasome inhibitors can also sensitize tumour cells for death receptor–induced apoptosis by blocking NF–B–dependent increased expression of FLIP. This example illustra-
tes that selective sensitizers of apoptosis might broaden the applicability of anti-cancer strategies related to death-receptor activation. Future studies must now show whether selective decreases in FLIP expression allow for differential sensitization of tumour cells and normal cells for death receptor-induced apoptosis.

REFERENCES


**TÉCNICA PARA OBTENÇÃO DO PONTO DE CONTACTO EM RESTAURAÇÕES DE CLASSE II COM COMPÓSITO**

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A criação de um bom ponto de contacto em classes II com compósito, implica a escolha clínica do sistema matriz / cunha mais eficaz para cada caso.

Para as situações em que a quantidade de estrutura remanescente está diminuída, os autores desenvolveram uma técnica que facilita a inserção e fixação da matriz para realizar o contorno proximal da restauração. São utilizados os compósitos microhíbridos em conjunto com os condensáveis, para maximizar as propriedades ideais de cada um e assim obter um melhor ponto de contacto.