A mitochondrial Achilles' heel in cancer?

Mitochondria are principal actors in apoptosis as central hubs for diverse apoptotic signals. A new paper demonstrates the therapeutic potential of directly engaging these apoptotic pathways by identifying a mitochondrial toxin selective for tumor cells.

It is now well established that mitochondria are critical targets in many, if not most, examples of apoptotic cell death, sequestering factors (cytochrome c, AIF, Smac/Diablo) that are necessary for activating cytoplasmic caspase cascades and additional nuclear events of apoptosis (Olsen and Kornbluth, 2001). These previously bland organelles are also the principal site of action for the cell death and survival functions of the Bcl-2 protein family.

In this issue of *Cancer Cell*, Fantin et al. (2002) describe a mitochondrial toxin selective for tumor cells, known as F16, which they identified using high-throughput cell-based screening of a chemical library. The concept of a mitochondrial basis for killing tumor cells has been around since the early part of last century, built upon metabolic studies demonstrating general differences in metabolic control between cancers and normal cells. The most consistently reported metabolic disturbance in tumor cells is abnormally high rates of glycolysis, which is demonstrated now on a daily basis in the clinic with the use of fluorodeoxyglucose PET scanning to diagnose recurrent or residual malignant disease. The question of mitochondrial involvement in a metabolic phenotype of cancer has been more difficult to pin down.

Warburg (1956) initially posited that mitochondrial oxidative phosphorylation was defective in tumor cells, and that this initial insult led to gradual and compensatory increases in glycolytic ATP production as the central event of cell transformation. He predicted that treatments producing mitochondrial injury, of a general nature, would strike a greater blow against cancer cells due to their already compromised respiratory state, than against normal cells. Various defects in cancer mitochondria have been described, including reduced capacity for oxidation of NADH-linked substrates, reduced expression and/or activity of ATPase and OXPHOS complexes, and mtDNA mutations, without a general pattern. There is also the possibility that high rates of glycolysis may suppress respiration, known as the Crabtree effect, without implicating defective mitochondria (Sussman et al., 1980).

Interventions designed to attack metabolic susceptibilities of tumor cells have included glycolytic inhibitors (2-deoxyglucose), membrane pore activators (lonidamine), and thiol-active chemicals (arsenicals). More recently, screening of a combinatorial library of compounds based on natural benzopyran inhibitors of mitochondrial complex I yielded several novel compounds with pronounced cytostatic effects against a cancer cell line panel (Nicolaou et al., 2000). Polyketide inhibitors of F(0)F(1)-ATPase scored among the top 0.1% of 37,000 compounds tested for selective cytotoxicity in the NCI 60 cell line panel (Salomon et al., 2000). Unlike these studies, Fantin et al. (2002) employed cell-based screening without presupposing a mitochondrial target. Thus, the F16 compound was selected out of 16,000 small molecules in a Chembridge library for its selective inhibition of cell proliferation of a mouse mammary epithelial cell line expressing a constitutively active neu oncogene. This approach lends further credence to the prior observations that mitochondriotoxic agents can have anticancer activity.

The structure of F16 is characteristic of a delocalized lipophilic cation (DLC), many examples of which are concentrated in mitochondria. Fluorescent properties of several DLCs, including F16, make them useful probes of mitochondrial membrane potential. The archetypal DLC, the Kodak laser dye rhodamine-123, was initially reported in the early 1980s to accumulate selectively in carcinomas and chemically transformed cell lines (Davis et al., 1985) and newer generation rhodacyanine dyes have similar properties. Extended treatment of carcinoma cell lines with Rh-123 leads to eventual cytoplasmic redistribution of the dye, indicative of mitochondrial toxicity, and cell death, with sparing of normal epithelial cells.

Preferential killing of cancer cells with these compounds correlates well with their selective uptake in mitochondria within cancer cells. Thus, the width of the therapeutic window for these compounds as potential anticancer agents is likely to be controlled by factors affecting intramitochondrial and also intracellular drug levels. Partitioning of a cation across the inner mitochondrial membrane follows the Nernst equation

$$\Delta\Psi = 60 \log \{[C+]_m / [C+]_c\},$$

and, as concluded by the authors, predicts that cells with higher mitochondrial membrane potentials will accumulate increased mitochondrial concentrations of the cation $[C+]_m$ (Rottenberg and Wu, 1998). Although an association of higher resting mitochondrial membrane potentials with cancer cells is often cited, this finding is clearly incompatible with the reports of structural and genetic abnormalities in cancer cell mitochondria. More likely, aerobic glycolysis (the Warburg effect) in cancer cells may compete for ADP and phosphate with oxidative phosphorylation, resulting in a significant increase in resting $\Delta\Psi m$ as mitochondria shift from phosphorylating to nonphosphorylating conditions. As shown by this group, addition of F16 to *normal* liver mitochondria causes cyclosporin A-dependent mitochondrial swelling, indicative of permeability transition pore (PTP) activation. Indeed, selectivity of Rh-123 has not been demonstrated with isolated mitochondria from cancer cells, suggesting that the mitochondrial susceptibility in cancer cells is interwoven with cellular metabolic control mechanisms. Mitochondrial uptake of F16 and other DLCs also reflects distribution across the plasma membrane, according to the plasma membrane potential, $\Delta\Psi_p$, which may vary between cancer and normal cell populations. It will be important and fascinating to examine how the individual oncogenes with unique F16 sensitivity profiles reported by Fantin et al. (2002) affect these metabolic parameters.

In the absence of a discrete enzyme or structural protein inhibited by F16 (chemical genetics), alternative approaches must be used to validate mitochondria as the critical targets of F16. Fantin et al. (2002) report that treatment of cancer cells with the uncoupler FCCP to depolarize mitochondria abolish-

es any additive toxicity from F16, consistent with a requirement for mitochondrial accumulation of the compound. Dose-response measurements at low concentrations of F16 (100 nM) should demonstrate concordance between the direct mitochondrial effects of F16 (uncoupling, increased O_2 consumption) and cell toxicity. Structure-activity relationships may demonstrate that cytotoxicity of a series of related compounds is correlated with relative mitochondrial uptake. Despite strong correlative evidence, nonmitochondrial sites of action can emerge to complicate conclusions about mechanism. MKT-077, a rhodacyanine DLC dye entered into Phase I clinical trials, has since been reported to have direct effects on additional targets, including telomerase inhibition, crosslinking of F actin, and reactivation of wild-type p53 via binding the hsp70 family member, mot-2 (mortalin) (Maruta et al., 1999; Naasani et al., 1999; Wadhwa et al., 2000).

The central importance of mitochondria in apoptotic cell death pathways offers up several molecular targets in mitochondria that are highly expressed in cancer cells. Foremost among these are the Bcl-2-related survival proteins, Bcl-2 and Bcl-x_L. There are now several examples of small molecules that bind to the hydrophobic groove of these proteins and either displace proapoptotic family members (Bax, Bak) from a heterodimeric complex or alter membrane topology of the survival proteins (Degterev et al., 2001; Tzung et al., 2001). Although the report by Fantin et al. (2002) does not examine the effect of Bcl-2 expression on F16 cytotoxicity, a separate group compared the ability of Bcl-2 to rescue L929 cells treated with neutral hydrophilic, anionic lipophilic, and cationic lipophilic photosensitizers (Klein et al., 2001). Overexpression of Bcl-2 sensitized L929 cells to the cationic drug, Victoria Blue BO, even in the dark, associated with increased mitochondrial uptake. Since the proposed functions of the Bcl-2 antiapoptotic proteins include regulation of mitochondrial membrane permeability, compounds with selective accumulation in mitochondria, including F16, may represent another approach to eradicate cancer cells expressing high levels of Bcl-2/Bcl-x_L and resistant to standard chemotherapy.

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Functions of p53 suppress critical consequences of damage and repair in the initiation of cancer

A pivotal study reveals a long-sought-after mechanism for gene amplification and provides important implications for oncogenesis.

One of the most frequent and mystifying types of abnormalities observed in human carcinomas is the amplification of large genomic regions associated with complex chromosomal rearrangements (complicons). These abnormalities are believed to be the functional basis for tumor progression and drug resistance both in vivo and in vitro (Kuehl and Bergsagel, 2002; Federspiel et al., 1984). In a recent publication by Zhu et al., the molecular dissection of these complicated structures in the experimental generation of lymphomas has illuminated a molecular mechanism of gene amplification and several factors that can modulate this process. As the result of a long series of fundamental observations, culminating in a recent elegant study (Zhu et al., 2002), the Alt laboratory has identified several of the major determinants that generate the intermediates in this process and has been able to propose a mechanism. They show that, in a setting where V(D)J recombination is initiated, deficiencies in both p53 function and nonhomologous end-joining abilities conspire to generate complex genomic rearrangements. In this system, the chromosomal rearrangement is initiated by RAG (recombination-activating genes)-mediated recombination that catalyzes a