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Combining 2-deoxy-D-glucose with electron transport chain blockers:

A double-edged sword

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Oxidative stress, originating from reactive oxygen species (ROS) and free radicals, provides a constant challenge to eukaryotic cell survival. Amongst many other biological roles, it is an important modulator of programmed-cell death, and any ability to augment or decrease its production might ameliorate diseases.[1,2] Scientists have successfully exploited the oxidative stress common in cancer cells to preferentially kill malignant cells. Most cancer cells exhibit overproduction of ROS,[3] which makes those cells more vulnerable to exogenous ROS-modulating agents that would not be toxic to normal cells that have a low ROS output.

In living cells, the major sources of endogenous ROS are the hydrogen peroxide (H_2O_2) and superoxide anions ($O_2^{\bullet -}$), which are generated as byproducts of cellular metabolism, primarily through mitochondrial respiration.[4] In mammalian cells, the mitochondrial electron transport chain (ETC) is a major site of cellular ROS production, where the electrons escaping from their transport complexes react with oxygen to form superoxide ($O_2^{\bullet -}$). In normal cells, and despite the great efficiency of electron transport, a small percentage of electrons are permanently leaked to oxygen, resulting in the formation of the toxic free-radical superoxide ($O_2^{\bullet -}$), which is greatly reactive either by itself or through its product, H_2O_2.[5] In malignant cells, increasing evidence has supported the hypothesis that some alterations occur in the mitochondrial ETC that lead to even higher production of the highly reactive oxygen species. What causes this altered ETC, whether structural changes in mitochondria or mutations in its DNA is not clear. Altered tumor cell metabolism may represent cancer's Achilles' heel.[6] Nevertheless, to counteract this phenomenon, tumor cells have developed a mechanism through which the abundance of ROS in their matrix is mitigated. Glucose has been hypothesized to provide the reducing equivalent necessary to either detoxify the H_2O_2 via the formation of pyruvate,[7] or through the regeneration of the

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reduced nicotinamide adenine dinucleotide phosphate (NADPH), an important electron source for the glutathione and peroxidase enzymes.[8]

Several studies have been conducted to evaluate the impact of glucose deprivation on the induction of oxidative stress and cell death in cancer cells vs. normal cells in a variety of cell lines including colon cancer.[9] In accord with this line of investigation, the extent of the oxidative stress and susceptibility to glucose deprivation were assessed following the disruption of the electron transport chain by using ETC blockers, which clearly enhance the cytotoxicity and glucose deprivation in multiple human carcinoma cell lines, such as those of colon, breast and glioblastoma vs. their normal cell counterparts.[10,11]

All colon cancer cell lines tested in a report by Fath and colleagues in this issue of *Cancer Biology & Therapy*, demonstrated higher steady-state levels of pro-oxidants, relative to normal colon epithelial cells.[13] A synergistic cytotoxic effect was observed when the clinically relevant inhibitor of glucose metabolism, 2-deoxy-D-glucose (2DG), was used in combination with the ETC blockers Antimycin A (Ant A) and rotenone (Rot). The level of intracellular pro-oxidants in these human colon cancer cells, as determined by oxidation sensitive probes, H_2DCFDA and DHE, was highly elevated when the HT29 and HCT116 cancer cells were treated with both 2DG and Ant A. This increase was considerably greater than that caused by the treatment with either of the drugs alone. Furthermore, the apoptotic effect of Ant A was ruled out as a contributing factor when substituted by its analog, the 2-methoxy-antimycin A (Meth A). This analog was found to no longer inhibit the electron transport. This is an important finding as Meth A is known to be unable to bind Bcl-2 or Bcl-x_L; this possibility has been recently reported as an alternative explanation for the cytotoxicity of Ant A.[12] Additionally, the findings presented here demonstrate that co-treatment with 2-DG combined with the ETC blockers disrupt the thiol metabolism by increasing the glutathione disulfide. Importantly, the treatment of tumor cells with both drugs together induced killing of up to 70% of the cells. To clinically prove the efficiency of those drugs, an in vivo test was performed on xenografted tumor cell-bearing mice. As anticipated, the tumor growth was significantly inhibited. These data robustly support the authors hypothesis that any disruption of both the ETC and glucose metabolism would severely impair tumor growth and represents a promising and novel antitumor strategy for cancer management.

This report and many similar studies have focused on the concept of killing tumor cells by increasing their ROS production, while attenuating their detoxification, which could be a promising therapeutic approach. An important next step would be to determine the mechanism of cell death under these circumstances. Mitochondria are a key target in cell death induced by many conventional therapies (radio- and chemotherapy) and disruption of the mitochondrial membrane potential has been suggested as an important step for cellular commitment to cell death. Elucidating how this, and the mitochondrial dynamics of tumor cells[14] in general, may determine the apoptotic response is of great interest. At the same time, normal cells have shown some evidence of ROS formation, particularly when treated with both 2DG and the ETC blockers. In this case, normal cells are expected to produce even more ROS, the impact of which on cell viability should not be underestimated. Thus, more clarification on the percentage of normal cell death would have given the reader a better comparison. Tumor cell targeting therapeutic regimens should only be considered successful when they show a minimal effect on the surrounding normal cells.

Because ETC blockers are widely known to increase the cells inner ROS generation and 2DG indirectly prevents their breakdown and increases their reactivity, the combination of targeting the ETC and glucose metabolism has been shown to be particularly effective in colon cancer cells. By demonstrating the efficacy of this particular drug combination, Fath

and colleagues have put forward a new concept that may pave the road to improved therapies for the treatment of different types of cancer.

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Abbreviations

Ant	antimycin A
2DG	2-deoxy-glucose
ETC	electrons transport chain
ROS	reactive oxygen species
Rot	rotenone

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