Reactive oxygen species in cancer cells: Live by the sword, die by the sword

Reactive oxygen species and tumor biology are intertwined in a complex web, making it difficult to understand which came first, whether oxidants are required for tumor cell growth, and whether oxidant stress can be exploited therapeutically. Evidence suggests that transformed cells use ROS signals to drive proliferation and other events required for tumor progression. This confers a state of increased basal oxidative stress, making them vulnerable to chemotherapeutic agents that further augment ROS generation or that weaken antioxidant defenses of the cell. In this respect, it appears that tumor cells may die by the same systems they require.

Reactive oxygen species (ROS) and cellular oxidant stress have long been associated with cancer. However, the nature of this association is both complex and at times paradoxical, as it appears that (1) ROS and oxidant stress may induce cancer; (2) transformed cells appear to generate more ROS than do normal cells; (3) the thioredoxin antioxidant system is paradoxically amplified in malignant cells; (4) stimulation of cell cycle progression by growth factors, or by mutations that activate the receptor tyrosine kinase signaling pathway, involves an increase in ROS signaling; and (5) diverse cancer chemotherapeutic agents may be selectively toxic to tumor cells because they augment oxidant stress and push these already stressed cells beyond their limit. These connections are still not fully understood but are worth examining individually.

Induction of cancer by ROS

According to the mitochondrial paradigm of cancer (Wallace, 2005), mutations in nuclear or mitochondrial genes encoding components of the mitochondrial electron transport chain (ETC) can lead to an increase in ROS generation. This occurs when electron transfer is partially inhibited, leading to accumulation of electrons at sites along the ETC where they can be captured by O_2, leading to the formation of superoxide. This radical is rapidly dismuted by superoxide dismutase yielding hydrogen peroxide (H_2O_2), which can diffuse to the nucleus and attack DNA, thereby contributing to genetic instability. An example of this process has been described in prostate cancer, where mutations in mitochondrial DNA (which encodes specific subunits of the ETC) have been linked to increases in ROS production and tumor progression in prostate cancer models (Petros et al., 2005). Although the field is still emerging, mounting evidence supports the idea that chronic increases in ROS may trigger transformation and contribute to cancer progression by amplifying genomic instability.

Enhanced ROS generation by cancer cells

Previous studies suggest that cancer cells normally produce more ROS than do normal cells (Szatrowski and Nathan, 1991). One of the difficulties in testing this hypothesis arises from a lack of a comparable "normal cell" to use as a control. Some cell types have higher metabolic activities than do others, and these differences could easily translate into higher rates of mitochondrial ROS formation. So comparing a rapidly proliferating cancer cell to a normal quiescent cell may yield differences in ROS attributable to differences in their metabolic rates rather than to the oncogenic transformation. Addressing this problem requires a genetically comparable control cell line, preferably one that replicates some but not all of the genetic defects in the tumor cell line. This was precisely the approach used by Trachootham et al. in a report in this issue of *Cancer Cell* (Trachootham et al., 2006). Using an immortalized epithelial cell line (T72 cells) that was incapable of forming tumors as controls, they then introduced an oncogenic version of RAS by transfecting them with H-RASV^{12}, which conferred an ability to form tumors in a mouse xenograft model. In parallel experiments they overexpressed BCR-ABL as a means of inducing oncogenic transformation, using a tet-inducible system. This permitted a comparison of multiple cell lines differing with respect to these oncogenes (Figure 1). Their data reveal that both of the transformed cell lines exhibited significantly greater levels of intracellular oxidative stress,

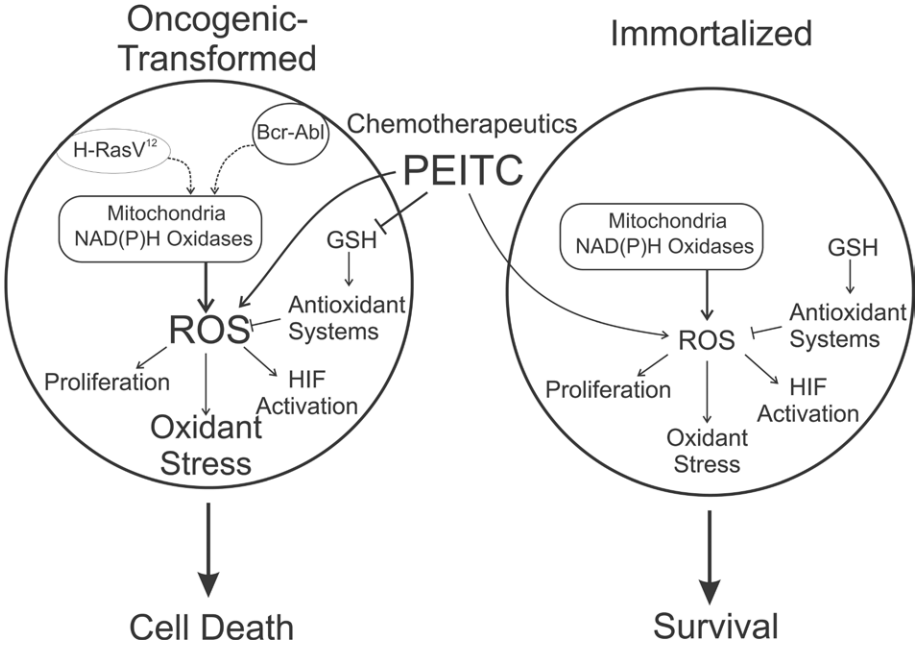

Figure 1. Oncogenic transformation, oxidant stress, and cell survival in response to chemotherapeutic agents that augment ROS production

as detected by two separate ROS-sensitive probes. These results support the hypothesis that neoplastic transformation is associated with an increase in the basal level of oxidant stress.

In normal cells, proliferation resulting from growth factor stimulation requires ROS signaling. This is consistent with the idea that constitutive activation of this pathway in cancer cells is associated with a basal increase in oxidant signaling. Of course, cancer can result from the activation, mutation, or suppression of a large number of genes, so these studies are not sufficient to conclude that all forms of cancer are necessarily associated with an increase in ROS signaling.

Thioredoxin reductase is overexpressed in malignant cells

The degree of oxidant stress in a cell reflects a balance between the rate of ROS production and the activity of scavenging systems that detoxify them. Thioredoxin reductase-1 (TR1) is a selenoprotein that functions in the thioredoxin antioxidant system. A critical function of that system involves its translocation to the nucleus in response to oxidative stress, where it acts to maintain a reducing environment required for effective DNA binding of transcription factors and subsequent gene expression (Hirota et al., 1999). Paradoxically, TR1 is overexpressed in a variety of malignant tumors, and a loss of TR1 is associated with a reversal of tumor phenotype and a decrease in tumorigenicity (Yoo et al., 2006). This increase in thioredoxin activity, in cells with exaggerated oxidant stress, may relate to its essential role in facilitating transcription in an environment where increased cytosolic oxidant stress signaling is required for stimulating cell proliferation.

Diverse chemotherapeutic agents can kill tumor cells by amplifying oxidant stress

Recent studies implicate increased oxidative stress in the cell death induced by diverse chemotherapeutic agents. Histone deacetylase inhibitors (Adachi et al., 2004), proteasome inhibitors (Perez-Galan et al., 2006), and redox cycling agents (Lecane et al., 2005) all appear to increase oxidant stress in cells, although the mechanism responsible for the increase has not been established. This common effect suggests that neoplastic cells may be more vulnerable to oxidant stress because they function with a heightened basal level of ROS-mediated signaling, which is required for the increased rate of growth. If this idea is correct, then addition of an agent that increases ROS generation, or that decreases ROS scavenging capacity, may push a tumor cell beyond the breaking point in terms of lipid peroxidation, DNA damage, and protein oxidation. The study of Trachootham et al. supports this idea by showing that β-phenylethyl isothiocyanate (PEITC) increases oxidant stress in transformed cells, possibly by generating ROS, but also by undermining the ability of the cells to detoxify oxidants. PEITC does this by depleting cellular levels of reduced glutathione (GSH), and by inhibiting the activity of glutathione peroxidase, a key cellular enzyme involved in the degradation of hydrogen peroxide. These effects were manifested by an increase in oxidation of cardiolipin, a lipid component of the mitochondrial inner membrane, and by a decrease in mitochondrial potential. It also inhibited H-RAS activity, without affecting H-RAS protein levels. By contrast, control cells exhibited a smaller increase in oxidant stress because their baseline levels of oxidant signaling were smaller, so the depletion of GSH presumably had less severe consequences for the cellular redox environment.

To the extent that ROS toxicity induced by certain chemotherapeutic agents can be an effective means of selectively eradicating malignant cells, it is useful to consider the most effective way to exploit this strategy. Trachootham and colleagues find that PEITC augments oxidant stress by decreasing oxidant scavenging, whereas other agents appear to increase ROS generation. Conceivably, combinations of agents with complementary mechanisms of action could prove to be more effective than single agents. Moreover, to the extent that different agents may induce ROS production or alter redox conditions in specific subcellular compartments such as the mitochondria, it is possible that significant synergy could be achieved by combining complementary agents, while still minimizing the effects on normal cells. Future studies that identify the specific subcellular compartments affected by novel oxidant stress-mediated chemotherapeutic agents are needed to address this possibility.

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Selected reading

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