Reactive Oxygen Species in Cancer: A Dance with the Devil

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Reactive oxygen species (ROS) can initiate cancer, but oxidant generation in tumors leaves them vulnerable to further stresses. In this issue of *Cancer Cell*, Harris and colleagues show that augmenting oxidant stress in normal cells limits tumor initiation and progression. Hence, strategic targeting of antioxidant systems may undermine survival of new tumor cells.

Low levels of reactive oxygen species (ROS) can act as cellular signaling messengers by reversibly oxidizing protein thiol groups, thereby modifying protein structure and function. Higher levels of ROS disrupt cellular processes by nonspecifically attacking proteins, lipids, and DNA. Cellular antioxidant systems help to limit the damage by detoxifying ROS, while other antioxidant systems act by reversing oxidant-mediated modifications (Figure 1).

Oxidant stress and redox signaling have been implicated in the genesis of cancer, and ROS can affect the phenotypic behavior of cancer cells and their responsiveness to therapeutic interventions (Sabharwal and Schumacker, 2014). Oxidative damage to DNA can certainly promote cancer-causing mutations. Oncogenic transformation of fibroblasts is associated with increases in basal levels of oxidative signaling that may drive proliferation and promote further mutations. The importance of ROS in driving cancer progression was demonstrated by Gao et al. (2007), who administered the antioxidant N-acetyl cysteine (NAC) to mice carrying tumor xenografts. They observed a decrease in tumor growth that was traced to a redox-mediated attenuation in levels of the transcription factor, Hypoxia-Inducible Factor-1 (HIF-1) (Gao et al., 2007). Thus, HIF-1 activation by oxidant signals enhances the survival and progression of tumors by upregulating genes regulating glycolysis, angiogenesis, and cell metabolism.

The idea that ROS-driven oxidant stress initiates cancer and drives progression has fueled a long-standing interest in the use of antioxidants to prevent cancer. Yet a large-scale prospective randomized clinical trial detected an increase in prostate cancer in healthy men taking vitamin E supplements, suggesting that some oxidant stress may act as a brake on tumorigenesis. Moreover, in K-ras and B-raf-driven genetic models of lung cancer, Sayin et al. (2014) found that NAC and vitamin E increased tumor cell proliferation by attenuating ROS, DNA damage, and p53 expression. These findings suggest that increases in ROS generation that develop as a cell becomes cancerous represent a potentially toxic byproduct of metabolic reprogramming and antioxidant defenses—or exogenous antioxidants—may enhance survival/progression by protecting the cell against the antiproliferative effects of those oxidant stresses. The increases in oxidant stress that develop in newly formed tumor cells may render them vulnerable to therapeutic interventions that act by further augmenting oxidant generation (Trachootham et al., 2006; Schumacker, 2006). In that sense, tumor cells engage in a deadly dance where some oxidants contribute to mutation and growth while excessive stress slows proliferation and threatens survival.

In this issue of *Cancer Cell*, Harris et al. (2015) provide important new insight into this complex field. Using a combination of genetic and pharmacological tools to disrupt redox homeostasis, they assessed the consequences in terms of tumor initiation and progression. Their study focuses on glutathione (GSH), a tripeptide that plays a key role in antioxidant defenses. GSH synthesis requires L-cysteine, L-glycine, and L-glutamic acid and involves an enzyme complex (glutamate cysteine ligase, GSL) consisting of catalytic (GCLC) and amplifier (GCLM) subunits. While GSH itself can scavenge ROS, its primary function is to support enzymes that directly scavenge H_2O_2 or lipid hydroperoxides. Harris et al. (2015) employed oncogene-induced murine models of mammary cancer (MMTV-PyMT) in a genetic background lacking GCLM ($Gclm^{-/-}$). Deficiency in GCLM led to a 75% decrease in GSH levels, shifting the cells to a state of chronic oxidant stress. The effects on subsequent tumor development were then assessed.

Interestingly, increased oxidant stress led to fewer tumors that progressed more slowly than those in mice with normal GSH. This suggests that increased oxidant stress was detrimental to the process of tumorigenesis and progression toward an invasive phenotype. In related experiments, they used buthionine sulfoximine (BSO), an inhibitor of GSL, to suppress GSH synthesis. When administered continuously upon weaning, BSO depleted GSH levels, augmented DNA oxidation and conferred protection against tumorigenesis in a manner that mirrored the response in the GCLM-deficient mice. Curiously, the effects of BSO on DNA damage and the protection were lost when BSO was started later, after the appearance of tumors. They conclude that the oxidant stress mediated by GSH depletion inhibited tumorigenesis because, in the setting of oncogene-induced ROS generation, it pushed the tumor cells over the cliff as they emerged. They suggest that alternate antioxidant mechanisms may have protected established tumors from this double-hit stress. Of course an alternative possibility is that BSO did not affect DNA oxidation or progression in the established tumors because it was less able to access the tumor interiors.

Interesting insight arises from their studies of PyMT;$Gclm^{-/-}$ primary

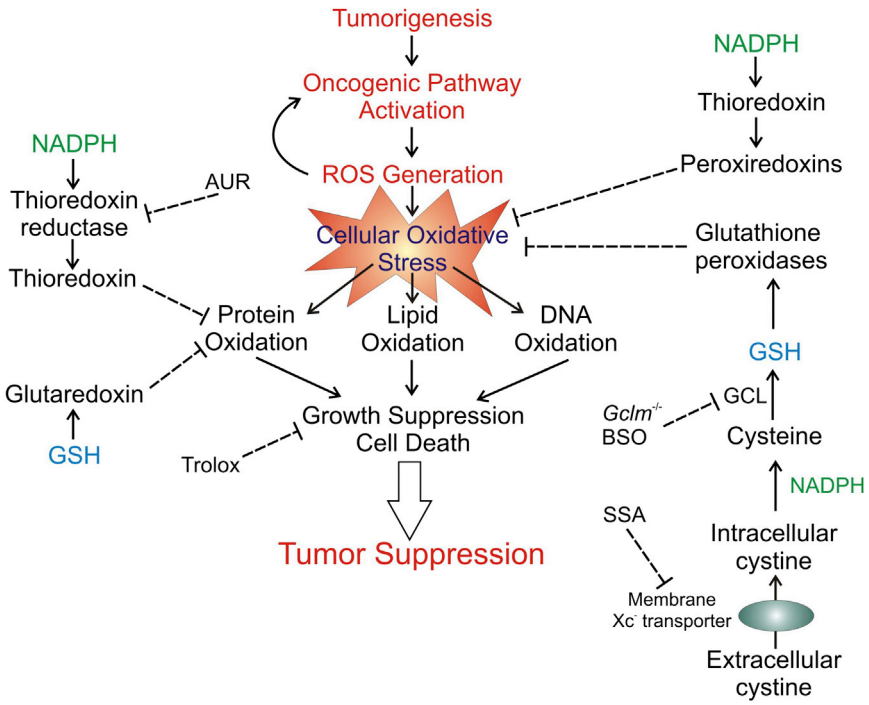

Figure 1. Cancer Cells Dance with the Devil ROS

Oncogenic transformation activates proliferative reprogramming pathways that generate ROS, particularly H_2O_2. This increases cellular oxidative stress, leading to protein, lipid, and DNA oxidation. Collectively these stresses oppose proliferation and threaten cancer cell survival. In response, cells rely on peroxiredoxins and glutathione peroxidases to scavenge hydroperoxides and thioredoxins or glutaredoxins to repair oxidized proteins. GSH is important for the function of some peroxidases and the protection of proteins from excessive oxidation. GSH synthesis requires glutamate-cysteine ligase (GCL) consisting of GCLC and GCLM subunits. Deletion of GCLM or inhibition of GCL by BSO depletes cellular GSH levels, augmenting oxidant stresses. In that setting, additional stresses induced by inhibiting thioredoxin reductase or preventing uptake of cystine by the Xc^- transporter pushes the oxidant stress into the toxic range. Importantly, in the setting of depleted GSH, the cells become highly dependent on the thioredoxin system, which is fueled by NADPH.

mammary epithelial cells, which exhibited oxidant stresses and decreased growth rates that were reversed by the antioxidant Trolox. The authors conclude that decreased GSH in normal epithelial cells suppresses growth through a mechanism involving impaired ROS detoxification. They also detected activation of Nuclear Factor (erythroid-derived 2)-like factor 2 (Nrf2), a transcription factor that regulates expression of antioxidant enzymes. Other redox-dependent transcription factors were probably also activated in response to the same oxidant stimulus.

GSH synthesis is facilitated by an amino acid transporter in the plasma membrane termed Xc-, which exports glutamate in exchange for cystine. Intracellular cystine is then reduced to cysteine using NADPH, making it available for protein synthesis. They found evidence of increased Xc- activity, which likely contributed to the increased expression of supplementary antioxidants such as thioredoxins.

Thioredoxins are small peptides that repair oxidized proteins through cysteine thiol disulfide exchange. Oxidized thioredoxins are reactivated by thioredoxin reductases, which rely on NADPH and are independent of GSH. Compared to cells or mice given BSO alone, cell growth and tumor progression were inhibited when cells depleted of GSH were also given inhibitors of either the Xc- transporter or thioredoxin reductase. Again, the effects of dual inhibition were rescued by the antioxidant Trolox. These findings suggest that tumor cells lacking GSH can still compensate by upregulating the thioredoxin system in an Xc–dependent manner.

What does this tell us about ROS in cancer? First, as cells become cancerous, they activate metabolic pathways that drive proliferation and survival. But these pathways also generate ROS, which strains the ability of the cells to handle further stress. To deal with this, many cancer cells reprogram glycolysis to augment flux through the pentose phosphate pathway to assure an adequate supply of NADPH, the proximal driver of the cellular antioxidant machinery (Sabharwal and Schumacker, 2014). Activation of redox-dependent transcription factors may also promote expression of supplementary antioxidant systems. Harris et al. (2015) demonstrate the importance of this stress by showing that tumorigenesis was suppressed when GSH levels were depleted by BSO or GCLM deficiency. The ability to reverse growth suppression with Trolox suggests that the oncogenic pathways themselves are critical for tumorigenesis and progression and the ROS they generate are a cost of doing business. The present findings also suggest that the excessive ROS undermine tumorigenesis through their effects on protein oxidation rather than non-specific damage to lipids and DNA. This conclusion is based on their finding that thioredoxin attenuated cell growth restriction in the context of depleted GSH. Thioredoxin reverses protein oxidation directly and also indirectly by maintaining glutaredoxin function. It also supports hydroperoxide clearance by reactivating peroxiredoxins, in a GSH-independent manner. Collectively, the studies of Harris et al. (2015) identify important tumor vulnerabilities that could be exploited therapeutically by inhibiting GSH homeostasis while simultaneously removing the safety net provided by thioredoxin function.

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