Glutathione-Associated Enzymes In Anticancer Drug Resistance

Kenneth D. Tew

See related article by Tew, Cancer Res 1994;54:4313–20.

In the 1980s and 1990s, cancer drug resistance was a popular subdiscipline that permeated many therapeutic areas of oncology research. Numerous meetings and symposia were dedicated to the topic, wherein the proceedings and speakers were frequently heavily weighted towards the multidrug-resistant phenotype (MDR) and various allied topics surrounding the p-glycoprotein. A small contingent of us believed that there was more to resistance than membrane pumps, an opinion benignly tolerated (though not necessarily encouraged) by our friends and colleagues in the resistance field. As such, redox pathways were gamely presented as contributory determinants of drug response and these were exploited for the design and development of new drugs, particularly those that targeted glutathione pathways. Partly as a consequence, this perspective formed a platform for the (now) featured article that appeared in the August 15, 1994 issue of *Cancer Research*, "Glutathione-Associated Enzymes In Anticancer Drug-Resistance" (1). Scopus shows that it has been cited approximately 750 times. Since that time, the redox field has grown, spawning a number of dedicated journals and continuing to bring relevance to cancer and drug resistance. Indeed, partly because cancer cells grow and divide so rapidly, they are exposed to levels of reactive oxygen and nitrogen species (ROS and RNS, respectively) that are higher than their normal cell counterparts and it is generally recognized that aberrant redox homeostasis is an important property of the cancer phenotype. In this regard, metabolic pathways that influence oxygen and sulfur are influential in imprinting the cancer phenotype.

Life on Earth has evolved within our biosphere such that higher eukaryotes derive much of their energy requirements through oxidative metabolism, to date the most biologically efficient means of generating ATP. During the Precambrian epoch, oxygen was present at trace levels, but has increased and decreased at given points in an evolving geology, reaching a maximum of 35% during the Carboniferous period. Obviously, life has adapted (and presumably continues) to such significant deviations in oxygen availability. Given that oxygen is now an obligate requirement in mammals, paradoxically, its metabolism incurs considerable toxicities. Chemical and biological conversion of oxygen or nitrogen can lead to the production of ROS or RNS, families of chemically active molecules that contain free radicals that significantly impact cellular redox homeostasis (2). The yin and yang of oxygen and sulfur biochemistry influence many signaling pathways and can contribute to control the fate of mammalian cells. Co-opted during the evolutionary process, threshold effects of, for example, hydrogen peroxide can serve as critical growth signaling molecules performing through creation of various oxidation states of important cysteine residues in select target proteins (3). A variety of biochemical pathways are essential in maintaining cellular redox homeostasis and as a consequence of their biological importance, a great deal of functional complexity has evolved. An adequate balance between the formation and elimination of ROS/RNS is maintained in cells via pro-oxidant and antioxidant enzyme pathways. Certain endogenous factors regulate generation of ROS/RNS, which, in turn, contribute to cell physiology by influencing such events as proliferation, differentiation, proteostasis, apoptosis, autophagy, and senescence. The difference between too much and too little ROS will be subtle and yet can determine the fate of many pathways critical to cell survival. Many of the complex pathways that underlie redox homeostasis are evolutionarily well conserved, occurring in organisms from most representative phyla. The variable valence and nucleophilic nature of sulfur provides valuable biological flexibility, particularly when sulfur appears in the thiol groups of cysteine. There are approximately 200,000 cysteines encoded by the human genome, making it one of the least commonly used amino acids (4). However, this sparing usage does not infer restricted functionality, but instead implies evolutionary importance and, indeed, cladistics analyses of total cellular cysteine contents yield correlations with a degree of biological complexity (5). The flexibility of the thiol group of cysteine predicates that a number (more than a dozen) of different types of posttranslational modification can occur on this residue (6). In recent years, *S*-glutathionylation of cysteine has been described as a cyclical posttranslational modification, shown to be important in a number of biological settings, especially cancer. It is worth reflecting (particularly in the context of essentially equivalent millimolar levels of both phosphate and glutathione in cells) that this cycle has the potential to provide cells flexibility in a number of signaling pathways and might bear comparison with phosphorylation/dephosphorylation. In fact, a significant number of both kinases and phosphatases are themselves subject to redox-dependent regulation by *S*-glutathionylation (7). As our understanding of this posttranslational modification matures, bioinformatics approaches are now beginning to help in predicting predisposition of individual proteins to *S*-glutathionylation (8).

A further emphasis of the earlier *Cancer Research* article was glutathione *S*-transferases (GST), part of a family of phase II detoxification enzymes, the understanding of which has grown substantially over the last two decades. GST isozymes were originally described for their roles in detoxification and linkage of their expression levels with elevated cancer drug resistance was thought to reflect enhanced capabilities in breaking down drugs to noncytotoxic metabolites (9, 10). One specific isozyme, GSTP, is ubiquitous in mammals and is expressed at high levels in many types of tumor and drug-resistant cancers (7). In the two decades following the publication, much work has ascribed other functions to this protein, including a glutathionylase activity for the

Medical University of South Carolina, Charleston, South Carolina.

Corresponding Author: Kenneth D. Tew, Medical University of South Carolina, 173 Ashley Avenue, P.O. Box 250505, Charleston, SC 29425. Phone: 843-792-2514; Fax: 843-792-2475; E-mail: tewk@musc.edu

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forward reaction of the *S*-glutathionylation cycle (11) and other noncatalytic activities such as chaperone-like properties that regulate kinases such as c-jun N-terminal kinase (JNK; ref. 12). To some degree, GSTP can be said to be acting as a switch in the control of stress mediated kinase signaling, providing a further link between redox and phosphorylation. On reflection, the ubiquitous expression of GSTP in non-hepatic tissues and its abundant levels in many cancer cells should have implied functionalities that are unrelated to detoxification. Indeed, growing evidence shows that GSTP forms complexes with a significant number of intracellular proteins, perhaps reflecting a required step in the forward *S*-glutathionylation reaction or as a reversible ligand binding temporarily shielding critical amino acid residues (7). Whatever the reasons for the affinity of GSTP for such proteins, there is likely to be important implications for the formation of such protein complexes in cancer.

Whereas cancer cells produce more ROS and generally express high levels of glutathione and associated enzymes, equally demonstrable is that certain normal tissues are intrinsically sensitive to changes in cellular redox potential. For example, rates of myeloproliferation through the bone marrow compartment are influenced by oxygen and redox gradients. Over a half-century ago, cystine and cysteine were shown to be important for the maintenance of the required balance between hematopoietic stem cell (HSC) quiescence, self-renewal, and lineage commitment in the human bone marrow compartment (13). HSCs give rise to all differentiated hematopoietic cell lineages and normal hematopoiesis operates optimally when the marrow compartment is between 1% and 3% oxygen (14), but hypoxic niches and gradients are required for HSC maintenance. A hypoxic environment in the osteoblastic niche encourages quiescence in HSCs and movement to the more oxygenated vascular niche promotes HSC differentiation (15). Regions of low oxygen may protect quiescent HSCs from toxic oxygen radicals and ROS gradients may synchronize HSC–niche interactions. Redox active drugs that modulate thiol pathways impact myeloproliferation. For example, *N*-acetyl cysteine (NAC) reverses ROS suppression of N-cadherin–mediated HSC adhesion to osteoblasts, inducing cell migration within the marrow compartment (16). Gradients of ROS affect elements of the niche structure and serve to regulate HSCs within this microenvironment. Redox modulation can serve as a regulator of cell self-renewal and differentiation in glial cells, where cysteine pro-drugs alter extracellular redox and enhance self-renewal of progenitor cells, whereas buthionine sulfoximine, by depleting glutathione, promotes differentiation. These types of observations support the principle that redox gradients and their pharmacological manipulation have a role in influencing HSC migration, differentiation, and myeloproliferation. It seems likely that pharmacological manipulation of redox conditions might be harnessed in influencing myeloproliferative pathways and clinical testing of a variety of redox active agents has been attempted. In fact, Telintra is a GSTP inhibitor and is presently in clinical trial for the treatment of myelodysplastic syndrome (17). Details of such drugs are contained in a variety of reviews (18) and undoubtedly future opportunities remain for further drug discovery and development.

Discussion of redox homeostasis would not be complete without reference to antioxidants. In various guises, antioxidants have a distinct following as dietary supplements and have also been quite extensively studied in chemoprevention settings. Most Western diets are replete with a sufficiency of antioxidants and supplementation could be viewed as rarely advantageous. Although not all antioxidants are equal, a variety of cancer chemoprevention clinical trials (with, e.g., selenium or vitamin E supplementation) have uniformly yielded negative results. Such trials are notoriously difficult to design and implement because endpoints require long-term follow-up. Perhaps there are ways to redesign and revisit prevention trials; however, somewhat contentiously there is growing evidence that antioxidants may in fact worsen cancer prognosis and survival. In this regard, animal studies using a genetically engineered mouse model that mimics early non–small cell lung cancer showed that antioxidants were harmful, not beneficial (19). With either NAC or derivatives of vitamin E, they reported that these antioxidants increased cancer burden and mortality in a dose-dependent manner. There are even reports that antioxidants can accelerate metastatic spread in mouse models of melanoma (20). Thus, with an existing burden of cancer cells, it may be detrimental to provide additional sources of antioxidant molecules and perhaps this is not the same in precancerous situations. Nevertheless, an explanation for such results may lie in the earlier observations that cancer cells frequently deviate from the normal redox threshold and as such their buffering capacities are anomalous. Provision of additional reducing equivalents could serve to stimulate growth and encourage survival. In perspective, care should be demonstrated in the simple interpretation that antioxidants are uniformly advantageous.

In conclusion, perhaps this earlier *Cancer Research* article appealed to an audience who were interested in the importance of glutathione pathways in a cancer setting. Even today, redox anomalies appear to be associated with a range of human pathologies and there is every reason to believe that their continued appreciation will present research opportunities that will further our understanding of the cancer phenotype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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