

Viewpoint

Rah, rah, ROS: metabolic changes caused by loss of adhesion induce cell death

Christian D Young and Steven M Anderson

Department of Pathology, Mailstop 8104, University of Colorado Denver, University of Colorado School of Medicine, P.O. Box 6511, Anschutz Medical Campus, Aurora, CO 80045, USA

Corresponding author: Steven M Anderson, steve.anderson@ucdenver.edu

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Abstract

The high rate of glucose utilization by cancer cells has been well characterized. Recent data suggest that when normal mammary epithelial cells are cultured under nonadherent conditions, glucose consumption decreases, ATP levels fall, and concentrations of reactive oxygen species rise. The rise in reactive oxygen species causes death of nonadherent cells, which can be suppressed with antioxidants. Nonadherent ErbB2-transformed mammary epithelial cells maintain glucose transport and antioxidant production; however, antioxidants appear to enhance anchorage-independent growth. These findings integrate aspects of glucose metabolism, anoikis suppression and antioxidant production in tumor cell biology and suggest that antioxidant therapy could stimulate tumor survival.

Reactive oxygen species (ROS) appear to play both positive and negative roles in cancer cells [1,2]. The major source of ROS is electron leakage from the mitochondrial electron transport chain, which then react with molecular oxygen forming ROS [2]. A number of oncogenes are known to increase ROS that can promote genetic instability and tumor aggressiveness [3-5]. Conversely, high levels of ROS are known to kill cancer cells [6]. Cancer cells that successfully manage levels of ROS could therefore gain a selective advantage to expand a tumor.

Anoikis refers to cell death that occurs following detachment of a cell from its native extracellular matrix [7]. A number of mechanisms that tumor cells use to bypass anoikis have been described [8], and this bypass must be accomplished in order for tumor cells to survive when proliferating and invading neighboring tissue. A recent article from the laboratory of Joan Brugge demonstrates a role for ROS and glucose metabolism in the context of mammary epithelial cells detached from the matrix [9].

Schafer and colleagues demonstrate a decrease in the amount of ATP in MCF10A mammary epithelial cells when

they are cultured under nonadherent conditions as opposed to adherent conditions [9]. The decrease in ATP levels is concomitant with a decrease in glucose consumption and an increase in ROS, which the authors suggest is due to a decrease in the flux of glucose through the pentose phosphate pathway. The pentose phosphate pathway is important because it produces nicotinamide adenine dinucleotide phosphate (NADPH), which is needed to reduce glutathione, which in turn eliminates ROS. ATP levels can be rescued in detached mammary epithelial cells by expressing the ErbB2 oncogene or by reducing ROS with supplemental antioxidants (which restores the catabolism of fatty acids as an energy source in the absence of glucose). Evaluation of MCF10A 3D acinar structures reveals that cells within the luminal space, not in contact with the extracellular matrix, have higher levels of ROS. Treatment of acinar structures with antioxidants prevents normal lumen clearing. Finally, it was demonstrated that colony formation by several cell lines that grow poorly in soft agar could be stimulated by treatment with antioxidants.

The authors conclude that detachment from the extracellular matrix causes normal cells to undergo cell death due to oxidative stress caused by reduced glucose metabolism, which can be suppressed by antioxidants. Further, transformed cells are better able to adapt to the nonadherent state due to their altered metabolism, and antioxidants aid in the survival and proliferation of transformed and pre-neoplastic cells [9].

Schafer and colleagues demonstrated mechanisms by which a transformed cell adapts its metabolism in order to survive in an extracellular environment where an untransformed cell would struggle to survive [9]. The link between glucose metabolism, antioxidant production and survival of detached cells is a remarkable discovery; however, a number of additional, unresolved questions come to mind.

NADPH = nicotinamide adenine dinucleotide phosphate; ROS = reactive oxygen species.

The use of detached cells in culture makes for a flexible, controllable model to study metabolic changes that occur in nonadherent cells. The relevance of this model to mammary duct lumen formation and mammary tumor cell detachment *in vivo*, however, must also be addressed. It would be interesting to know whether antioxidants would increase tumor cell survival and colonization of the lung or bone using intravenous or intracardiac xenograft mouse models [10], and whether antioxidants would stimulate the growth of primary tumors and/or metastatic tumors as observed in soft agar colony assays [9]. Of course, the ultimate question is the relevance to human breast cancer, which could be addressed by a large-scale clinical trial of antioxidants. Comprehensive epidemiologic data have been inconclusive regarding the effect of antioxidants on breast cancer progression [11], but many of these studies have focused upon ancillary or palliative aspects of treatment.

Schafer and colleagues focused upon glucose flux into the pentose phosphate pathway supporting NADPH production and glutathione reduction [9], but they also reported that malate (another source for NADPH production) could rescue detached cells from oxidative stress. In glioblastoma cells, glutamine flux through the tricarboxylic acid cycle to form malate and to reduce NADPH is as great as glucose flux through the pentose phosphate pathway [12]. These two mechanisms of NADPH production indicate the importance of this reducing agent; however, its eventual use is unclear. Schafer and colleagues suggest NADPH is used to reduce glutathione, to combat oxidative stress and to support the oxidation of fatty acids in detached cells [9]. Conversely, DeBerardinis and colleagues suggest that the reducing power of NADPH is used to drive fatty acid synthesis [12]. NADPH is probably used by a cancer cell to reduce glutathione and to combat oxidative stress as well as to drive the synthesis of fatty acids and nucleotides depending on the oxidative and/or bioenergetic stress that cell is experiencing. The culture conditions used by Schafer and colleagues have high concentrations of glutamine, but evaluation of glutamine usage by MCF10A cells was not reported.

Other questions raised by the findings of Schafer and colleagues' research [9] include the following. By what molecular mechanism do ROS impair oxidation of fatty acids? Does pyruvate rescue of ATP production in detached cells affect glutathione reduction? For how long will antioxidants rescue detached cells? Does altering the extracellular environment of a cancer cell have the same effect on metabolism and oxidative stress as detaching cells? Which attributes of apoptosis and autophagy are utilized during anoikis? What signaling pathways downstream of the epidermal growth factor receptor, phosphoinositide 3 kinase, and Akt are involved to modulate ATP, reducing glutathione and ROS levels? Resolution of these questions is likely to provide new insights into metabolism and tumor progression.

Competing interests

The authors declare that they have no competing interests.

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