Separation Anxiety: Detachment from the Extracellular Matrix Induces Metabolic Changes that Can Stimulate Tumorigenesis

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One of the earliest stages of tumor progression involves the ability of cells to survive and proliferate when not attached to the extracellular matrix (ECM). New research using a physiologically relevant breast cancer model reveals how separation from the ECM stimulates metabolic changes characteristic of developing tumors.

The cellular microenvironment is a crucial regulator of tissue development, remodeling and homeostasis (Bissell et al., 2002; Nelson and Bissell, 2006). Cell–cell and cell–extracellular matrix (ECM) interactions provide critical signaling information that control cell survival. When non-malignant epithelial cells are separated from the ECM, they initiate programs that control apoptotic cell death; this prevents cells from proliferating when and where they should not. A key step in the progression of cells toward malignancy is the acquisition of the ability to survive and even to thrive when detached from adjacent cells and the ECM, a characteristic known as anchorage independence. This can be conferred in cultured cells by induced expression of activated oncogenes, but it is clear that simple expression of oncogenes in vivo is not sufficient to induce anchorage independence. Transgenic models that express oncogenes under control of tissue-specific promoters show a delay in tumor formation and outgrowth of only a few tumors (Vargo-Gogola and Rosen, 2007); for example, transgenic mice that express the Her2/Neu oncogene under control of the mouse mammary tumor virus promoter (MMTV-Her2/Neu) express the oncogene throughout the epithelial tissue of the mammary gland from the time of early development onwards, but only develop tumors after a latency of 6 months. Such studies point to intrinsic barriers present in cells contained within normal tissues that are not present in cells grown in culture; evidence that the normal tissue structure acts as a barrier to tumor progression is found in studies showing tumor outgrowth is accelerated when tissue structure is disrupted, such as occurs during chronic inflammation or tissue fibrosis.

Defining how loss of tissue structure is associated with tumor progression requires the use of experimentally tractable model systems that recapitulate the physiological conditions of normal tissues. Some of the most successful of these have used three-dimensional (3D) culture methods in which mammary epithelial cells are grown in Matrigel, a laminin-rich ECM (Hebner et al., 2008). Non-malignant mammary cells, cultured in 3D Matrigel, form cell structures that contain many of the structural and functional characteristics of the normal mammary gland, including the formation of hollow, acinar-like structures with apico-basal polarity and normal cell–cell junctions. By comparison, malignant breast cancer cells, grown in 3D ECM, continue to proliferate into amorphous masses. The processes that distinguish the non-malignant and malignant phenotypic responses have begun to be defined; one of the key processes that distinguish the different morphologies is the control of apoptosis. In non-malignant breast epithelial cells, attachment of cells to the ECM is necessary to prevent a specialized form of cell death known as anoikis. This effect can be visualized in the acinar structures formed by MCF10A breast epithelial cells grown in 3D Matrigel, as cells that are located in the central region of the acini (and are thus separated from the ECM) undergo anoikis. A series of publications from the Brugge laboratory have investigated how activation of oncogenic pathways found also in breast cancer, such as ErbB2 and Akt, suppresses luminal anoikis (Muthuswamy et al., 2001; Debnath et al., 2002, 2003).

A recent study from the Brugge laboratory has provided insight into a novel mechanism by which loss of attachment to the ECM may actually activate the genetic alterations associated with tumor progression (Schafer et al., 2009). Examination of MCF10A acini revealed greatly increased levels of reactive oxygen species (ROS) in cells located in the central lumen prior to activation of anoikis, and further showed that treatment with inhibitors of ROS such as N-acetyl cysteine or Trolox led to greatly increased proportion of MCF10A acinar structures that were completely filled with cells. These studies implicated elevated ROS levels in luminal cells as a critical step in the induction of anoikis (Figure 1A).

ROS are a byproduct of normal metabolism and although some ROS species act...
as critical signaling molecules, excess levels of ROS can damage cellular processes and damage DNA, leading to genetic alterations that can give rise to oncogenic transformations. Under normal conditions, ROS levels are contained by reducing equivalents produced by NADPH, but Schafer et al. found that NADPH levels were greatly reduced in the luminal cells. To mimic the cellular pathways activated by separation from ECM in the luminal cells, Schafer et al. assessed the effect of MCF10A cells plated either on normal tissue culture plastic (2D attached) or in plates that had been rendered non-adhesive by treatment with polyHEMA (detached; Figure 1B). They found that cell detachment led to increased ROS and consequent damage to metabolic pathways controlling fatty acid oxidation (FAO), and further showed that ROS-dependent inhibition of FAO caused decreased levels of cellular ATP, a signal for cell death processes that are involved in luminal clearing.

Schafer et al. further investigated why detached cells showed decreased NADPH. An important pathway for generation of cellular NADPH is the pentose phosphate pathway (PPP), which uses glucose as an energy source. They showed that detached cells had decreased glucose uptake, and decreased flux through the PPP. They found that activation of ErbB2 signaling in the detached cells, which was previously shown to increase luminal filling in MCF10A cells grown in 3D (Muthuswamy et al., 2001), led to increased glucose uptake, activation of PPP and decreased cellular ROS. They also found that inhibition of PPP, either by small molecule inhibitors or by downregulation of glucose-6-phosphate dehydrogenase led to increased ROS and decreased ATP in the ErbB2 activated cells. These experiments identified a signaling pathway in which cell detachment leads to decreased uptake of glucose and consequent decreased NADPH, increased ROS, and decreased ATP.

The experimental model systems described by Schafer et al. can be used to dissect how alterations in metabolic processes induced by other oncogenes affect the luminal filling phenotype. Previous studies showing that expression of Akt suppressed detachment induced cell death led to speculation that Akt could be acting to protect cells from apoptosis in part through increased glucose uptake and glycolysis, and thus improved metabolism and mitochondrial homeostasis (Debnath et al., 2002). In their current study, Shafer et al. evaluated this hypothesis, finding that activation of PI3K-Akt was both necessary and sufficient for rescue of ATP levels in matrix detached cells. Furthermore, the inhibition of cell death in cells expressing ErbB2 was shown to occur through activation of PI3K-Akt. These findings correlate well with previous studies showing that Akt-induced glucose uptake and glycolysis is accompanied by increased malignancy in vivo (Elstrom et al., 2004).

Although the study from Schafer et al. provides an important advance in our understanding of how detachment of non-malignant breast epithelial cells, either by separation from the ECM in 3D structures or from the substratum in 2D culture, leads to alterations in cellular metabolism and increased ROS, it also raises additional important questions about the fundamental processes underlying the control of cellular metabolism as cells progress toward malignancy. Investigations dating to 1973 show that there are significant differences in glucose metabolism between normal and tumor cells (Bissell...
Using a steady-state apparatus and radioactive labeling, Bissell et al. investigated metabolic dynamics in normal and Rous sarcoma virus transfected chick embryo cells, and found that the transformed cells had higher levels of glycolytic intermediates compared with the normal cells, though there was no difference in glucose carbon flow through the tricarboxylic acid cycle and amino acids. Rather, the transformed cells showed increased glycogen synthesis as a consequence of elevated glucose carbon flow through the PPP, allowing the transformed cells to produce 10 times more glycogen as compared with the normal cells, independent of their higher growth rate. The experimental models described by Schafer et al. may make it possible to define the relationship between the detachment-induced alterations in glucose metabolism and the sustained metabolic changes found in transformed cells as well, thus opening a potential new arena for development of new anticancer therapies that target the unique metabolic characteristics of tumor cells.

References


