Tissue Culture as a Hostile Environment: Identifying Conditions for Breast Cancer Progression Studies

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The cell culture environment (substrate, atmosphere, and medium) can have a significant influence on the characteristics of cells that propagate from clinical samples. In this issue of *Cancer Cell*, Ince and colleagues report improved conditions for the culture of primary human breast epithelial cells. They demonstrate that, when cells cultured using the new conditions are experimentally transformed, they are more tumorigenic, form tumor xenografts that closely resemble human breast ductal adenocarcinoma, and are more metastatic compared to cells cultured under standard conditions similarly transformed. This suggests that pre-existing differences in cell culture can modulate the tumor phenotype.

One major need in mammalian cell culture is the ability to propagate diverse developmental lineages faithfully for prolonged periods of time. In spite of all the advances in cell and molecular biology over the past three decades, there have been only modest advances in methods for the long-term and robust culture of diverse normal human cell types (e.g., neural cells, cardiomyocytes, pancreatic islet cells, colonic epithelial cells, and hepatocytes). Some of the key issues are the composition of the culture medium, the substrate upon which the cells are grown, and oxygen tension (Shay and Wright, 2002). Oxygen tension in most tissues of the body ranges from 1% to 6%, much less than the approximately 21% oxygen present at sea level. As a consequence, cells being cultured under normal laboratory conditions are actually being exposed to a physiologically hyperoxic environment. Why do the vast majority of scientists culture their cells in 21% oxygen, in poorly defined medium containing undefined components (e.g., serum and bovine pituitary extract), or on standard hydrophobic negatively charged plastic culture substrates? We suspect the honest answer by most scientists is one or all the following: it is easier, it has always been done that way, and it really is not so important that the culture conditions are ideal as long as the cells keep dividing. Even though it would be beneficial to have improved conditions that more closely represent the in vivo conditions, imagine the reaction of reviewers responding to a grant application requesting funds to develop improved chemically defined medium for propagating normal human breast epithelial cells!

This brings us to the manuscript by Ince et al. (2007) in this issue of *Cancer Cell* reporting that the phenotypes of human breast epithelial cells transformed in vitro may largely depend on the initial characteristics of the cells obtained at the time of primary culture. The authors developed a serum-free completely defined medium (called WIT) and changed from using standard cell culture ware (hydrophobic polystyrene surface) to Primaria dishes (http://www.BDBiosciences.com/). These dishes incorporate a variety of nitrogen-containing functional groups into the surface in addition to the negatively charged oxygen-containing groups found on standard tissue culture surfaces.

The traditional cell culture methods usually induce a rapid increase in $p16^{INK4a}$ gene expression in primary cells that must be abrogated (either by spontaneous methylation or selection for pre-existing methylated variants) for cells to divide beyond this initial proliferation blockade (Romanov et al., 2001; Kiyono et al., 1998). As a consequence, human mammary epithelial cells (HMECs) obtained from using these methods usually do not express $p16^{INK4a}$. Importantly, the human breast primary epithelial cells (BPECs) cultured using the new conditions do not experience the premature growth arrest, and their $p16^{INK4a}$ does not become inappropriately methylated (Figure 1). This suggests that either the medium composition or the substrate, or both, produced less stressful conditions for the cells.

After establishing and characterizing the BPECs, the authors then proceeded to transform BPECs and HMECs using similar manipulations (e.g., hTERT, SV40 early region, and H-ras). Transformed BPECs made invasive breast adenocarcinomas (containing ductal and glandular morphology), whereas transformed HMECs, established from the same

patients, formed squamous tumors without ductal or glandular elements when transplanted into animals (Figure 1). Finally, the experimentally transformed BPECs appeared to contain a high percentage of tumor-initiating cells (e.g., 100 cells made robust and invasive tumors that were not observed in cells cultured in the standard ways) and the tumors were more metastatic, suggesting that BPECs have more cancer stem-like characteristics. The results reported by Ince et al. (2007) offer insights into some previously confusing reports in the literature as well as raising new questions.

A central question not completely answered in this study is whether the new conditions actually provide for the selection of different cell types from the original specimen or whether the cells that grow out represent changes due to exposures of the same cell types to different cell culture stresses (Sherr and DePinho, 2000; Ramirez et al., 2001).The authors present substantial data showing literally thousands of gene expression differences between BPECs (more luminal, new conditions) and HMECs (more myoepithelial, old conditions). They also demonstrated that, once established under one condition, switching the transformed cells to the other condition for 3 weeks was insufficient to make substantial changes in these patterns of gene expression. However, the current studies do not definitively resolve whether the new medium is selecting for a novel precursor cell or modulating gene expression of the same original cells.

It has been difficult to determine the cell type that gives rise to a particular tumor in clinical samples. In breast cancer there are over a dozen histological subclasses, and little is known about the cell or cells of origin of these histological subtypes.

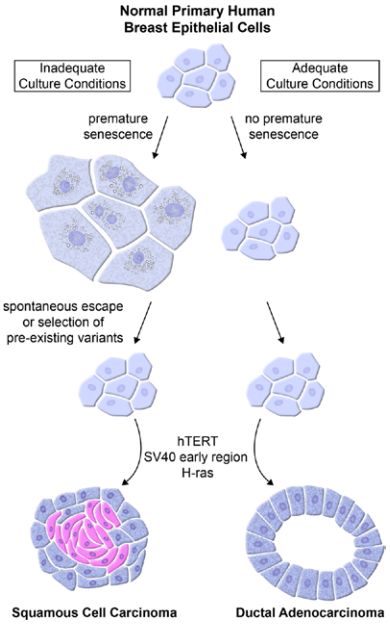

Figure 1. Culturing Human Breast Epithelial Cells in Standard versus the Improved Conditions

The cells cultured in inadequate conditions undergo a stress-induced premature growth arrest (left side), and those cells that escape this blockade will upon experimental transformation produce tumors that contain keratin pearls, a feature of squamous cell carcinoma. In contrast, the human breast epithelial cells cultured in the improved conditions (right side) and then experimentally transformed produce tumors with well-differentiated epithelial ductal structures with central lumens typical of ductal adenocarcinoma. Drawn by Angela Diehl.

Developing methods that permit a variety of epithelial precursor cell types to propagate from surgical specimens may permit the isolation of such target cell types to help separate the complexities of the behavior of the tumor phenotype from the influences of the tumor microenvironment (e.g., stromal cells and inflammatory responses).

One area that was not discussed in the present study was the importance of chronic oxygen toxicity and DNA damage on the cells. Many human and almost all murine cells are sensitive to oxidative damage when chronically cultured in room atmospheric conditions (Parrinello et al., 2003; Forsyth et al., 2003). Apparently, human keratinocytes and breast epithelial cells are more resistant to oxidative stresses, while other human types are more sensitive. Even though the currently reported conditions are adequate to observe the very interesting phenotypes observed in the present studies, one wonders if more luminal or other phenotypes might have appeared if more physiological oxygen levels were used. Given the increasing efforts being devoted to culturing a variety of normal cell types and stem cells, the take-home lesson from this study is that it seems very ill-advised to continue to grow cells under conditions known to produce DNA damage. Nevertheless, this is an important advance, and the hope is that others will now be encouraged to take the lessons from this and other studies to improve the mammalian cell culture field in order to be able to better study the biology of these cells.

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