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Commentary

Making Every Cell Like HeLa

A Giant Step For Cell Culture

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In the 1950s, it took George Gey hundreds of attempts to come up with a method to grow cells from patient tumors.[1,2] His first success was derived from a cervical cancer explant from Henrietta Lacks (HeLa). Other researchers later followed in his footsteps in generating additional cultured lines, with cell culture ultimately becoming an important laboratory model for cancer research. It has not only provided a way to understand tumor biology but has also made important contributions in drug target identification and drug development. Since the establishment of HeLa cells, approximately 70,000 peer-reviewed papers have been published using them. That number is dwarfed by the thousands of papers describing the use of other cell lines that were developed following the HeLa cell line for use as model systems for understanding cancer. The majority of these lines was established from high-grade or metastatic tumors,[3] and many proved to be genetically different from the tumor from which they were derived. To date no commercial normal-cancer paired cell lines are available, although there are some cell lines used as models for normal cells (MCF10A).[4] Most primary cell cultures, regardless of the numerous methods used to sustain them, suffer from limited lifespan due to lack of understanding of the requirements for long-term stem cell maintenance and the inability to recapitulate an essential stem cell niche *in vitro*. These factors lead to gradual decrease in proliferation rate and cellular senescence.[5] Even today, one of the biggest challenges in cancer biology research is the development of a method to generate stable cancer cell lines from primary tumors. In this issue of *The American Journal of Pathology*, Liu et al[6] describe a breakthrough in cell culture that may be the answer to that challenge.

Overcoming Cellular Senescence

Cellular senescence, the eventual loss of proliferative ability, has been the main barrier to long-term culture. Several different approaches have been used to overcome cellular senescence in primary cell cultures. For example, irradiated or non-dividing mitomycin-treated mouse fibroblast cells were used as a feeder cell layer in the 1970s by several groups.[7] By slowing down the onset of cellular senescence, this method allowed establishment of primary cell cultures from both normal and cancer cells. Instead of occurring at five to six passages, feeder cell methods support 30 to 50 passages.

Perhaps the most common method of immortalizing cells has been transformation with viral oncogenes.[8] Specifically, transformation with SV40 large T antigen can essentially immortalize many cell types.[9] However, this genetic manipulation leads to genomic instability with the result that after a few passages, the cultured transformed cells lose the properties of the cells from which they were derived.[10] This progressive accumulation of genomic variation has limited the value of this model system since the characteristics of the immortalized cells is a function of the passage number.

Another approach to immortalization was based on the discovery that cells with ectopic expression of telomerase can escape senescence, as the progressive shortening of chromosome ends ultimately leads to cell death.[11] The use of exogenous expression of hTERT (the catalytic subunit of telomerase) in primary cells can prevent chromosome shortening and lead to immortalization of some cells types. Overexpression of hTERT does not lead to a tumorigenic cellular transformation of normal cells and thus is the viable method of choice for immortalizing primary cell cultures. However, it requires some technical expertise, and the resultant lines, similar to transformed cells, begin to show abnormal cell properties when grown for many passages.[12]

An additional approach to preventing cellular senescence is to prevent cellular differentiation through the use of Rho kinase (ROCK) inhibitors.[13] This approach has been recently used to keep embryonic and somatic cells growing in culture and to maintain induced pluripotent

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stem (iPS) cells in an undifferentiated state. It is thought to work by preventing dissociation-induced apoptosis.[14] However, none of these approaches have produced stable cell cultures that can be propagated, frozen, and grown without the transformation by oncogenes that change the inherent properties of the cultured cell.

A Novel Approach to Normal and Cancer Cell Line Generation

The method described by Liu et al[6] (hereafter referred to as the Georgetown method) combines the use of irradiated mouse fibroblasts as a feeder cell layer with the use of a ROCK inhibitor. The authors call these cells conditionally reprogrammed cells (CRCs) since they can derive them from both normal and cancer tissues and then grow them indefinitely under these conditions. Like hTERT immortalization, this method does not transform normal cells and maintains the normal diploid karyotype for as long as 33 passages from normal prostate cells. However, unlike hTERT or viral transformation methods, cells propagated by the Georgetown method maintain a normal phenotype.

It is well established that the tumor microenvironment plays a key role in tumor progression, metastasis, and tumor suppression.[15] Barceloos-Hoff et al[16] have shown recently that irradiation of mammary gland stroma promoted the tumorigenic potential of unirradiated premalignant breast epithelial cells both *in vitro* (cell culture) and *in vivo* (mouse). In the Georgetown method, the irradiated non-proliferative fibroblasts induce and maintain hTERT expression in the epithelial cells.[6] These effects may be achieved by direct cell-cell interaction or by secretion of diffusible growth factors and cytokines (eg, IL-6, HGF, TGF-β, etc). The effects may also be mediated by provision of an insoluble extracellular matrix (ECM) or through secretion of ECM remodeling proteins (eg, MMP-9 and MMP-3).[17,18] This undefined combination of secreted growth factors, cytokines, and ECM remodeling proteins by the irradiated non-proliferative senescent fibroblasts appears to be critical in maintaining the unrestricted growth potential of the epithelial cells in co-culture. Finally, another variable potentially impacting the unrestricted growth is the fact that mouse fibroblasts are used as the feeder layer. Although the factors secreted by the mouse cells may be similar to those produced by human cells, the impact of the non-human microenvironment on the human epithelial cells is not completely understood.

Another key component of the protocol for the Georgetown method is the use of a ROCK inhibitor to help maintain the undifferentiated and proliferative state of the epithelial cells. As discussed above, the use of ROCK inhibitors is not novel, but rather the combination with feeder cells appears to be critical in preventing transformation or senescence of the cultured cells.[19] To verify this, Liu et al[6] demonstrated through karyotyping that normal cells derived from normal prostate and breast tissues remained diploid, and these cells failed to develop tumors after injection into mice. Thus Liu et al[6] demonstrate that the combination of both irradiated murine fibroblasts and a ROCK inhibitor are essential for both initial survival and unlimited expansion. Removal of either of these factors leads to differentiation of the epithelial cells and ultimately cellular senescence.

Limitations

The Georgetown method has great potential for rapidly generating patient-derived primary cells for a wide variety of uses including biobanking, basic tumor biology, drug target identification, and drug discovery. Thus, further validation of this culture system is well warranted as scientists begin to use this method as a model system for such applications. Liu et al[6] show a strong initial characterization of these patient-derived cell lines, but more work remains. For example, in the future, a complete genomic comparison between the original frozen tissue samples and high-passage cultured cells would further validate this technique. Likewise, comparison studies including karyotyping, translocation assessment, specific gene mutations analysis, and gene expression profiling will increase the credibility of this new method. Studies of protein expression and posttranslational modifications comparing the original tumor with the cultured cell lines would also provide important confirmation of maintenance of the original phenotype. Finally, although establishment of xenografts in mice is commonly successful with explanted tumor cells, it would be important to establish that tumor cells derived by the Georgetown method exhibit this property.

Future Implications

Unlike previous efforts in cell culture, the new Georgetown method allows us to envision applications that were impossible using existing techniques. One important new opportunity will be the ability to develop cell lines from normal and tumor tissue from the same patient. Another opportunity will be the ability to amplify very small specimens. This will impact both tissue banking and, subsequently, our understanding of the etiology of tumors. A central problem in studies related to early cancers or pre-cancerous lesions is that the tumor material is often exhausted by the diagnostic technique. Since patient safety (ie, assigning the diagnosis) is always the foremost consideration, tiny specimens like microinvasive breast carcinoma or radial phase melanoma have been challenging to assess using standard genomic methods. Since the Georgetown method could allow expansion of cells from minimal starting material (a few hundred cells), new data on early stage tumors should be forthcoming. Finally, and perhaps more importantly, this method may allow assessment of drug resistance and the screening of large libraries in a 96-well high throughput format. The testing of multiple antibiotics on growing microorganisms is a common practice in microbiology, but it has been unsuccessfully mimicked in oncology, largely due to the lack of a good cell culture model. The Georgetown method, once it is more robustly validated, may represent a breakthrough for measuring tumor cell drug resistance.

Thus, this method has the potential to be a valuable tool for understanding tumor biology, as well being used as a new clinical tool. It will be interesting to watch as investigators move forward with this method whereby cells from nearly every tumor could result in lines reminiscent of those generated from the tumor of Henrietta Lacks.

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