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COMMENTARY

The Clinical Relevance of Cancer Cell Lines

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Although advances in genomics during the last decade have opened new avenues for translational research and allowed the direct evaluation of clinical samples, there is still a need for reliable preclinical models to test therapeutic strategies. Human cancer-derived cell lines are the most widely used models to study the biology of cancer and to test hypotheses to improve the efficacy of cancer treatment. Since the development of the first cancer cell line, the clinical relevance of these models has been continuously questioned. Based upon recent studies that have fueled the debate, we review the major events in the development of the in vitro models and the emergence of new technologies that have revealed important issues and limitations concerning human cancer cell lines as models. All cancer cell lines do not have equal value as tumor models. Some have been successful, whereas others have failed. However, the success stories should not obscure the growing body of data that motivates us to develop new in vitro preclinical models that would substantially increase the success rate of new in vitro–assessed cancer treatments.

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Human cancer-derived cell lines are fundamental models used in laboratories to study the biology of cancer, and to test the therapeutic efficacy of anticancer agents (1). HeLa was the first cultured cancer line. It was derived from cervical cancer cells taken from Henrietta Lacks in 1951 (2). Since then, hundreds of cancer cell lines have been established and propagated either in vitro as monolayer cultures or in vivo as xenografts in mice (3). No model is perfect and the caveats of these models are well known (4,5), but how closely do these cell lines actually resemble the tumors from which they were derived?

Cross-Contamination of Cancer Cell Lines

Nelson-Rees and colleagues were perhaps the first researchers, back in the 1970s, to question experimentally the relevance of these in vitro cancer models (6–9). Their cytogenetic studies revealed that many cancer cell lines used to model various types of cancers were in fact derived from the HeLa cervical cancer line and not from other cancers (6,8). A pertinent example in regard to the multidrug resistance (MDR) field is the KB cell line, which was used for some of the earliest in vitro anticancer drug screening and had been thought to be derived from an epidermoid carcinoma of the mouth but was actually derived from HeLa cells (10–13). Yet more disturbing was their discovery of contaminations with 2 cell lines, HBC and BrCa5, which were thought to be derived from malignant human breast tissue but were actually of rat and HeLa origin, respectively (7). Besides problems of origins related either to tissue or species of origin, Nelson-Rees and colleagues also demonstrated that the number of passages a cell line undergoes can lead to such extensive modifications in its characteristics that it no longer reflects the tumor from which it was derived (9).

Development of Cancer Cell Line Panels

An important paradigm shift occurred in the late 1980s in response to the limited success in the clinic of compounds identified through screens using transplantable murine neoplasms for solid tumors (14). Consequently, development of an in vitro human-based tool for drug discovery to increase the translational success of newly identified anticancer compounds was sought. So the idea arose to develop a panel of cell lines that would recapitulate the variability of the chemotherapy response observed in the clinic for a particular tumor type. At that time, the observed response rate of many tumors to conventional chemotherapy ranged from 25% to 70%. Therefore, it was assumed that six to nine cell lines per tumor type would be sufficient to capture this variability. In the United States, the National Cancer Institute 60 (NCI-60) panel of cancer cell lines, which included 60 cancer cell lines representing nine different cancer types, was launched in 1990 (15). A few years later, the Japanese Foundation for Cancer Research developed its own panel of 39 cancer cell lines, which also represented nine cancer types (16). Although that panel included 30 cell lines in common with the NCI-60, it also provided a subpanel of six gastric cancer cell lines owing to the prevalence of stomach cancer in the Japanese population. Those platforms led to the generation of a wealth of information but also led to further confusion as to the origin of some cell lines and to the development of new analytical methodologies to integrate high-throughput data (15,17). Nonetheless, recent efforts have been carried out by the American Type Culture Collection Standards Development Organization Workgroup ASN-0002 to develop a standardized protocol and a publicly searchable database for the authentication of human cell lines using short tandem repeat profiling (18–20). This is an important step to minimize, if not to eradicate, cell line misidentification.

The Rise of "-omics"

The emergence of "-omic" technologies in the first decade of the 2000s allowed the characterization of cancers at the molecular level, which revealed the genetic heterogeneity of the tumors along with numerous potential targets (21). As opposed to conventional chemotherapeutic agents, targeted therapy can only be applied to a small percentage of patients, pointing to the need for a larger panel of cancer cell lines to capture this genetic heterogeneity. The Center for Molecular Therapeutics at the Massachusetts General Hospital Cancer Center launched, in 2006, a panel of 1200 cell lines termed the CMT1000 platform (22). In addition to the biological limitations of the first two panels, this much larger panel now poses certain technical, analytical, and financial challenges. Such a large panel is not a reasonable alternative for most laboratories. Rather, it strongly underlines the need for systematic and centralized screens and cell repositories (23–25).

Superiority of TaqMan-Based Quantitative Reverse Transcription–Polymerase Chain Reaction as a Gene Expression Profiling Platform

The literature is replete with hundreds of predictive markers, yet clinical progress in improving cancer treatment has been incremental at best (26,27). Why is it so difficult to translate basic knowledge to the clinic? Two major issues can be raised: technical concerns—specificity and sensitivity of the gene expression profiling assays—and biological concerns—clinical relevance of the in vitro models used.

Multidrug-resistant cancer develops through multiple mechanisms, including reduced drug uptake, increased drug efflux, phase 1 and 2 metabolisms, DNA repair, evasion of drug-induced apoptosis, alteration of target proteins, and drug sequestration. These mechanisms can act individually or synergistically, leading to MDR, in which cells become resistant to a variety of structurally and mechanistically unrelated drugs (28). Pinpointing individual genes in families comprising highly homologous genes represents a major technological challenge (29). We and others have recently shown that the TaqMan-based quantitative reverse transcription–polymerase chain reaction assay provides the highest sensitivity and specificity in measuring ABC transporter gene expression profiles, a superfamily of 48 highly homologous members initially studied in the NCI-60 cancer cell line panel (30,31) (Figure 1). Further analysis has also demonstrated that the TaqMan-based quantitative reverse transcription–polymerase chain reaction assay provides greater predictive power for identifying transporter substrates when correlating gene expression patterns with patterns of drug resistance (30). The development of next-generation RNA sequencing platforms should provide even more specificity and molecular detail to the study of gene expression patterns associated with drug resistance, but this technique is still not practical as a clinical tool (32).

Failure of Cell Culture Models to Reflect Clinical MDR Gene Expression Patterns

The second issue that we would like to discuss is the clinical relevance of the in vitro models, especially cultured cancer cell lines, the most widely used models to find ways to reverse drug resistance and therefore improve the response of patients to treatment. Several recent studies have fueled the debate.

One should clearly distinguish between genomic and transcriptomic changes when comparing cell lines and primary tumors. It has been shown that at the genomic level, driver mutations are (nearly) always retained. In recent reviews of the relevance of lung cancer cell lines (33,34), Gazdar and colleagues pointed out that, "Perhaps the most important finding is that every important, recurrent genetic and epigenetic change including gene mutations, deletions, amplifications, translocations and methylation-induced gene silencing found in tumors has been identified in cell lines and vice versa" (33). Therefore, cell lines of various types having clinically actionable mutations retain their sensitivity to relevant targeted agents, and have proven to be of enormous clinical and biological value in identification and characterization of the target molecules and for studying and overcoming drug resistance. Lung cancer cell lines with *EGFR*, *BRAF*, and *ABL* mutations, ALK translocations, and HER2 amplifications remain exquisitely sensitive to their respective inhibitors in the absence of secondary resistance mechanisms. It has been shown that the sensitivity of all members of a large panel of lung cancer cell lines to tyrosine kinase inhibitors was tightly correlated to the presence of specific genomic changes (35).

Prolonged cell culture is more likely to induce the occurrence of secondary genomic changes, such as copy number variations as well as transcriptomic drifts. To investigate the latter, we searched the literature published during the past 30 years to identify the genes whose expression in cultured cancer cells was found to confer MDR. One should bear in mind that many of these genes mediate resistance to multiple drugs and that they are involved in many other cell functions. During the first phase of our research, we evaluated those genes in 80 samples of untreated primary ovarian serous carcinoma (36) and in 32 unpaired samples of ovarian serous carcinoma effusion obtained at diagnosis or at disease recurrence after chemotherapy (37). Our goal was to develop a model of ovarian cancer in which we could manipulate the gene signatures for intrinsic and acquired drug resistance highlighted through those translational studies. We compared the expression profile of 380 MDR-related genes of a panel of ovarian cancer cell lines, either drug sensitive, cisplatin resistant or multidrug resistant, to these clinical samples. Hierarchical clustering revealed two groups of samples—in vitro cultured cancer cells and clinical samples. Cells grown in three-dimensional culture, in vitro, or as xenografts in beige-nude-scid mice did not have any major differences in their MDR gene profile compared with two-dimensional cultures. However, when the eight additional cancer types of the NCI-60 panel were added to the analysis, we made the striking observation that all of the cell lines either grown in vitro or in vivo bore more resemblance to each other, regardless of the tissue of origin, than to the clinical samples that they are supposed to model (Figure 2) (38).

Similar observations, with fewer total samples, were made for four other types of solid cancer—glioblastoma, colorectal cancer, breast cancer, and metastatic melanoma (microdissected from tissue sections). Our analysis of two types of leukemia—T acute lymphoblastic leukemia and acute myelogenous leukemia—revealed that differences between cell lines and cancer cells are not confined to solid tumors.

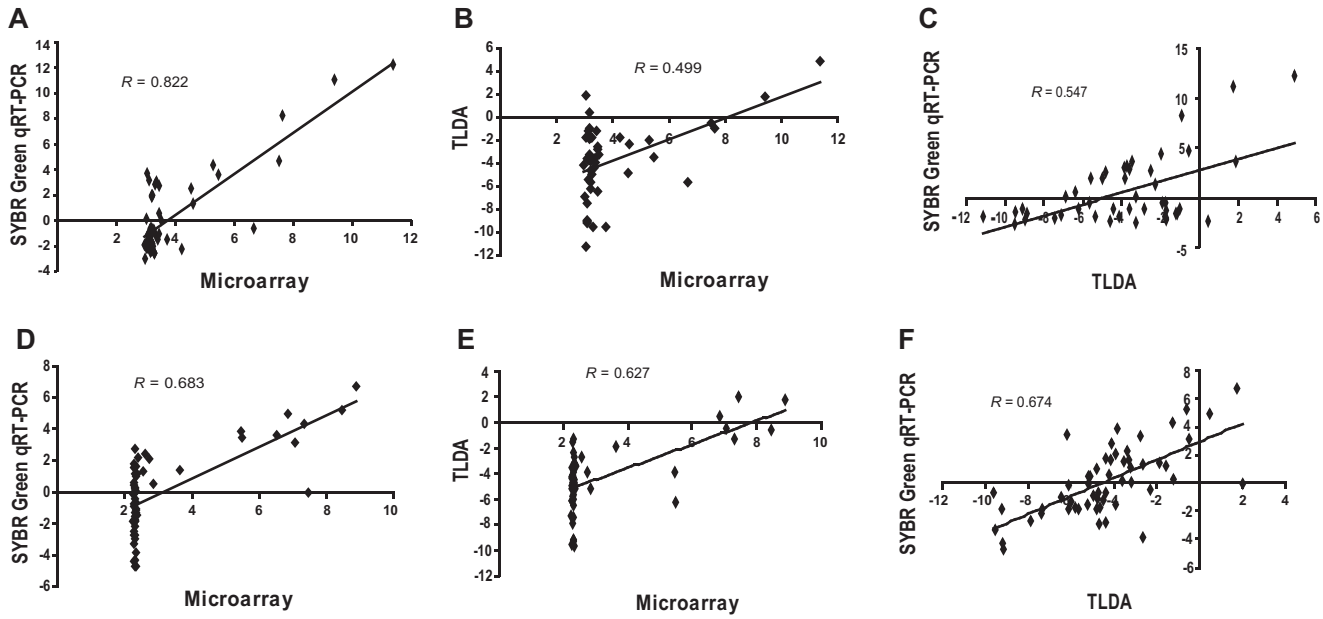

Figure 1. Correlation of gene expression data from three distinct platforms. Expression profiles for *ABCB1* across all 60 cell lines were compared between: SYBR Green and microarray (**A**); TaqMan low-density array (TLDA) and microarray (**B**); SYBR Green and TLDA (**C**); SYBR Green and microarray (**D**); TLDA and microarray (**E**); SYBR Green and TLDA (**F**). The data show that TLDA provides more sensitivity, yielding a larger dynamic range of measurement. The coefficient of correlation is given for each comparison. qRT-PCR = quantitivate reverse transcription–polymerase chain reaction. [Reprinted from (30), by permission.]

Larger-Scale Studies

One could argue that cell lines capture only a small part of tumor heterogeneity and, therefore, a panel of cell lines would be more likely to reflect it. In the largest study of that sort conducted to date, Lukk and colleagues integrated microarray data from 5372 human samples to construct a global map of human gene expression (39). The samples were classified into 369 biological categories, each representing a particular cell or tissue type, disease state, or cell line. The data were gathered from 206 different studies generated in 163 different laboratories. Of interest, 96 of the biological entities contained at least 10 biological replicates, and approximately 14 000 genes were studied. The analysis revealed that most cell lines, both solid tissue and blood cells, cluster together rather than with their tissues of origin, with the exception of incompletely differentiated cell types (including smooth muscle, fibroblasts, and bone marrow mesenchymal stem cells), for which cell lines cluster with the primary cells. The differences observed in the gene expression profiles may be due to interlaboratory variation. However, the authors determined that the biological effects were stronger than those introduced by experimenters ($P < 2.2^{-16}$).

However, other studies came to the opposite conclusion. In a study that characterized a panel of 51 breast cancer cell lines, Neve and colleagues demonstrated that 72% of the genes found to be statistically deregulated in primary tumors (N = 145) were also in this 51-cell line panel (40). Interrogating the whole transcriptome expression profile, Wang et al. showed that 51 of 59 cell lines of the NCI-60 panel they analyzed represented their presumed tumors of origin (41). Barretina and colleagues published a Cancer Cell Line Encyclopedia (23) that consisted of a database that included the whole transcriptome expression profile, chromosomal copy number, and mutational profile of 947 human cancer cell lines. They observed a strong positive correlation between cell lines and primary tumors for all the three types of genetic information. The difference between cultured cell lines grown on plastic and primary tumors was suggested to be due to a background microenvironment-related gene expression profile that could simply be subtracted to leave meaningful clinically relevant gene signatures that could be manipulated (42).

Questioning the Encyclopedia

To test the hypothesis that cell lines derived from specific cancers retain their essential characteristics, we used a method similar to that of Barretina et al. to compare the gene expression of cell lines and primary tumors with TaqMan Low-Density Array expression data for 380 genes in 205 primary tumors and 90 cell lines of six different tissue types (breast, leukemia, colon, central nervous system, melanoma, and ovarian). We selected 255 genes with the highest variance in expression (variance >1) across all samples (primary tumors and cell lines). Then we computed the fold difference for each tissue separately in primary tumors and cell lines as the average fold difference between samples from that tissue and a random set of samples from all other tissues (n = 5 from each tissue). The correlation was calculated using the average fold change for the primary tumors and the average fold change for the cell lines. Our previous results (38) and Figure 3 show that even though cell lines have very different genomic signatures compared with primary tumors, cell line pairs derived from the same type of cancer and primary tumor pairs of the same type are more similar to each other than are pairs from different tissues. Cell lines from different tissues are closer to each other than any primary tumor, but among primary tumors, they are closest to the primary tumor from the same tissue

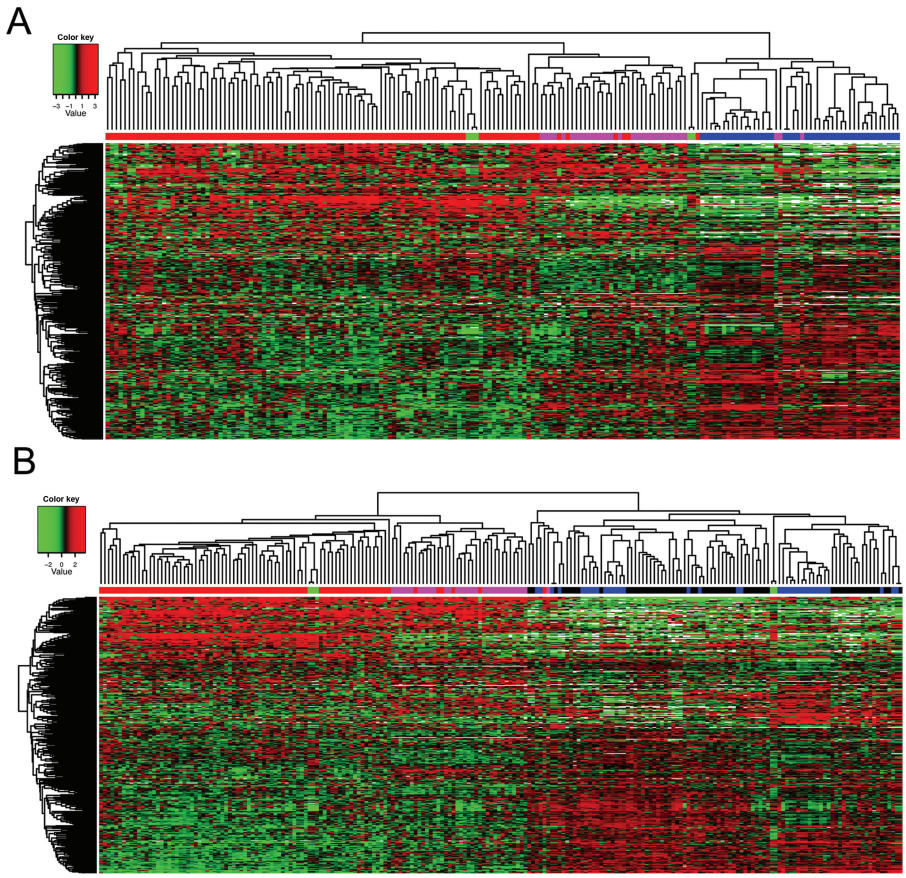

Figure 2. Hierarchical clustering using the average linkage algorithm and 1 – Pearson correlation as the distance measure of the ovarian cancer samples analyzed. **A**) The 380 multidrug resistance–linked gene expression profile (measured using TaqMan low-density array) of ovarian cancer models (in vitro and in vivo) is strikingly different from that of specimens of untreated ovarian primary serous carcinoma taken from 80 patients as well as 32 effusion samples originating from primary ovarian serous carcinoma. The X-axis shows clusters of samples. **Red** = primary ovarian serous carcinoma; **magenta** = effusion samples originating from primary ovarian serous carcinoma; **green** = normal ovarian tissue; **blue** = in vitro models of ovarian cancer, including xenograft models of ovarian cancer, ovarian cancer cell lines of the National Cancer Institute 60 (NCI-60) panel, and cisplatin-resistant cell lines. The Y-axis shows gene clustering. **B**) When adding the eight additional cancer types of the NCI-60 panel to the heatmap presented in panel A, the striking observation is made that all the cell lines either grown in vitro or in vivo bear more resemblance to each other, regardless of the tissue of origin, than to the clinical samples that they are supposed to model. Along the X-axis: **red** = primary ovarian serous carcinoma; **magenta** = effusion samples originating from primary ovarian serous carcinoma; **green** = normal ovarian tissue; **blue** = in vitro models of ovarian cancer; **black** = cancer cell lines of the eight additional cancer types of the NCI-60 panel. The Y-axis shows gene clustering. [Reprinted from (38).]

than any other tumors. It is unclear whether this is evidence of a tissue-specific signature or of a cancer-specific signature.

Do All Cancer Cell Lines Have Equal Value as Tumor Models?

Most of the available tumor cell lines, including those integrated into the above-mentioned very large cancer cell line panels, were established using culture methods similar to those used to generate HeLa. Does this mean all cancer cell lines have equal value as tumor models? Small-cell lung cancer cell lines were some of the first to be generated that showed dramatically different behavior, growing as floating spheroids in liquid medium (34). However, the three-dimensional growth characteristic of small-cell lung cancer lines has resulted in their exclusion from large tumor cell line panels growing in two-dimensional mode.

Some more recently established cell lines, genetically authenticated as derived from specific individual cancer patients, were banked at low passage and characterized to confirm expression of important features, such as drug sensitizing mutations or other characteristic genetic changes. These lines may have greater clinical relevance. In a recent study, the use of Ewing's sarcoma cell lines harboring the EWS–FLI1 gene translocation supported the identification of an apparently characteristic sensitivity of this tumor to PARP inhibitors (43).

Work in glioma performed by Lee and colleagues using modified medium has led to the generation of a cell population harboring similarities to neural stem cells that closely mimic the genotype, the transcriptome expression patterns, and the in vivo biology of primary glioblastomas (44). Such cell populations stand in contrast to the widely used, high-passage glioblastoma cell lines.

In our laboratory, Gillet and colleagues were able to successfully correlate hepatocellular carcinoma (HCC) cell lines with primary HCC clinical samples using a 45-gene signature (unpublished data). The similarity in gene expression profiles of normal hepatocytes and multidrug-resistant cells is indeed striking (45). Once a neoplasm

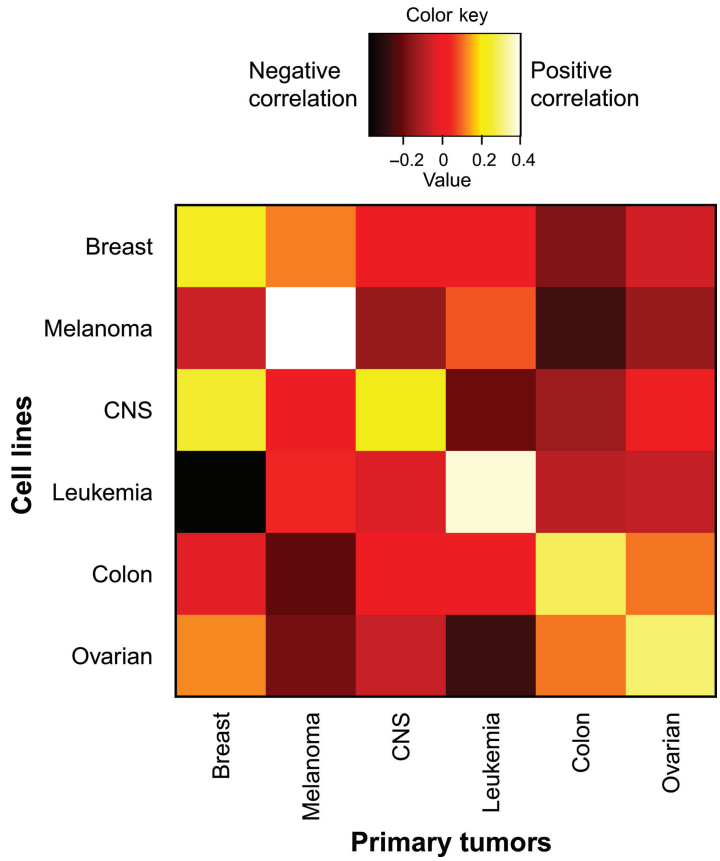

Figure 3. Comparison of mRNA expression profiles in cell lines and primary tumors of six different tissue types (CNS = central nervous system). We selected 255 genes with the highest variance in expression (variance >1) across all samples (primary tumors and cell lines). Then we computed the fold difference for each tissue separately in primary tumors and cell lines as the average fold difference between samples from that tissue and a random set of samples from all other tissues (n = 5 from each tissue). The correlation was calculated using the average fold change for the primary tumors and the average fold change for the cell lines. For each tumor type, the log fold change of the 5000 most variable genes was calculated between that tumor type and all others. Pearson correlations between tumor type fold changes from primary tumors and cell lines are shown as a heat map.

develops, its gene expression profile renders it intrinsically resistant to conventional chemotherapy. Using HCC cell lines and the connectivity map tool published by Lamb and colleagues (25), Gillet and colleagues were able to validate hypotheses developed from the use of a database built from gene expression profiling of drug-treated cell lines (unpublished data). It is tempting to speculate that this similarity of HCC cell lines and primary tumors reflects the expression in primary HCC of MDR genes characteristic of liver that enable survival of HCC cells in culture without any selection (45).

Need for Better Ex Vivo Models to Bridge the Bench-to-Bedside Gap

Cultured cancer cell lines are the most widely used in vitro models and have formed the basis for our current understanding of cancer biology. In spite of their limitations, they are still quite important to current research but should certainly not be considered as the unique, authoritative model. There is a growing body of data that should motivate us to direct more energy toward the development of in vitro models that would better predict the success or failure of chemotherapeutic agents.

In both the Gillet et al. (38) and Lukk et al. (39) articles, gene ontology studies revealed the upregulation across all cancer types of a group of genes that are necessary for the cell lines to grow and thrive in their in vitro environment (eg, cell cycle and primary metabolic processes). Moreover, a critical aspect of tumors is that they do not proliferate at the same rate as cultured cells. In fact, cultured cells are selected for rapid growth with doubling times much shorter than those of cancer cells in vivo. Most cancer cell lines have been maintained for decades in growth-promoting cocktails, in monolayer as opposed to three-dimensional culture, and in high oxygen tension (21%, whereas physiological oxygen tension ranges from 2% to 5%). These conditions clearly affect the cells' characteristics and undoubtedly select for subpopulations of cells that differ dramatically from the predominant cells of a primary cancer (46–48). Therefore, one should not be surprised that cell lines are so different from the primary tumors from which they originated.

Besides interindividual tumor heterogeneity, which is a challenge in itself, intratumor genetic heterogeneity has been demonstrated (49). This presents a major challenge not only to individualized therapy but also to the development of ex vivo models. Cancer cell lines most likely represent a cell subpopulation of the tumors they are purported to model, and efforts should be made to develop new (heterogeneous) cell lines that exhibit the genomic and transcriptomic heterogeneity of the original tumor.

Studies in our own laboratory have shown that established cancer cell lines are highly selected for expression of genes associated with MDR (38). The microenvironment plays a major role in the initiation and maintenance of this phenomenon. Many of the genes that are involved in drug resistance are also involved in various cell functions (eg, cell cycle, growth promotion, apoptosis). Therefore, our work raises concerns about using cancer cell lines not only for studies on drug resistance but also for more basic research on the physiology of cancer.

We believe that for the study of drug resistance and other characteristics of cancer cells, more effort should be directed toward the development of new ex vivo models that more closely mimic the in vivo cancer microenvironment so as to avoid radical changes in cellular characteristics brought on by extended periods of cell culture and culture conditions. Primary tumors maintained for a relatively short period of time (several months) in a redefined culture system (ie, oxygen tension, pH, glucose concentration, three-dimensional matrix, medium composition) may mitigate some of the aberrations associated with cancer cell line–based models. This type of model could also allow the evaluation of therapeutic efficacy in a context that would include intratumor heterogeneity (49), which is not possible with individual cell lines. In vivo, genetically engineered mouse models of cancer may recapitulate many, but not all, aspects of human cancer (50,51). Furthermore, primary xenografts (52) and orthotopic grafting with or without humanized stromal cells (53) show some promise because the cells have not adapted to tissue culture and are grown in a context that more closely mimics the in vivo cancer microenvironment. However, xenografts can also be frequently contaminated by highly infectious human tropic mouse viruses (eg, xenotropic murine leukemia viruses), which can bias any further experiments and thus underlines the need to test xenografts for such contaminations (54).

Summary

Human cancer-derived cell lines are the most widely used models to study the biology of cancer and to test hypotheses to improve cancer treatment. Since the development of the first cancer cell line, the clinical relevance of those models has been continuously questioned, supported by the emergence of new technologies, from G-banding to the "-omics." Cancer cell lines have been marked by both success and failure. Cell line misidentification arose as the first problem, which should now be minimized by the launch of a standardized protocol for the authentication of human cell lines using short tandem repeat profiling. The limited success at translating bench work with cell lines to the bedside was the major shortcoming of the in vitro models. This led to a paradigm shift in the late 1980s with the development of the NCI-60 panel, a human cancer cell line panel that helped to reproduce the variability of response to chemotherapy observed in the clinic. Perhaps the most notable success obtained from the use of this new model system is the development of the proteasome inhibitor bortezomib (Velcade) for the treatment of patients with multiple myeloma.

The rise of "-omics" along with the development of new high-throughput analytical methods allowed interrogation in depth of the clinical relevance of human cancer-derived cell lines. Although it appears that at the genomic level driver mutations are retained, several studies reveal a drift at the transcriptomic level, leading to the conclusion that cancer cell lines bear more resemblance to each other, regardless of the tissue of origin, than to the clinical samples that they are supposed to model. However, other studies have come to the opposite conclusion, suggesting the need for larger human cancer cell line panels, such as CMT1000 or the Cancer Cell Line Encyclopedia, to fully capture tumor heterogeneity.

One point is clear: the limited number of success stories from the use of cancer cell lines should not obscure the growing body of data that could motivate us to develop new in vitro preclinical models that would substantially increase the success rate of new in vitro–assessed cancer treatments.

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