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Commentary

75TH ANNIVERSARY COMMENTARY

MCF-7 Cells—Changing the Course of Breast Cancer Research and Care for 45 Years

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Abstract

It is 45 years since a pleural effusion from a patient with metastatic breast cancer led to the generation of the MCF-7 breast cancer cell line. MCF-7 is the most studied human breast cancer cell line in the world, and results from this cell line have had a fundamental impact upon breast cancer research and patient outcomes. But of the authors for the nearly 25 000 scientific publications that used this cell line, how many know the unique story of its isolation and development? In this commentary we will review the past, present, and future of research using MCF-7 breast cancer cells.

Sister Catherine Frances (Helen Marion) Mallon was born in 1901 and attended the Immaculate Heart of Mary Convent in Monroe, Michigan (1). In 1963 she had a mastectomy for a benign tumor in her right breast, and in 1967 she underwent a radical mastectomy for an adenocarcinoma in her left breast (2). That she developed breast cancer is perhaps not surprising given the early report by Bernardino Ramazzini, the father of industrial medicine, that "tumors of the breast are found more often in nuns than any other women," and the subsequent epidemiologic literature indicating that nulliparous women are at higher risk for breast cancer. Just after she completed postoperative chest wall radiotherapy, a local recurrence in her left chest area was noted, but the recurrence was reportedly adequately controlled by radiation and hormone therapy of unknown type—perhaps diethylstilbesterol—for three years. In 1970 Helen Marion developed metastatic disease to the pleura and chest wall, and researcher Herbert D. Soule at the Michigan Cancer Foundation attempted to develop a cell line from an excision of a chest wall nodule and from a pleural effusion (2). At this time, many laboratories had documented technical difficulties in generating continuous stable cultures of cancer cell lines, including the overgrowth of fibroblasts, and several had tried to isolate cell lines using different substrates and nutrients. The process for cell line development that Soule used was relatively standard, and the cell cultures derived from the chest wall nodules were soon overgrown by fibroblasts and discarded (2). However, the cells from the pleural effusion grew initially in suspension and then ultimately formed a monolayer on plastic that grew as a continuous culture. The resulting cell line was called MCF-7, named after the Michigan Cancer Foundation, and represented Soule's seventh attempt at generating a cancer cell line. To date there have been nearly 25 000 published reports on this cell line, rivaled only by the nearly 80 000 publications using the HeLa cell line. The popularity of the MCF-7 cell lines for breast cancer research reflects its fidelity to many aspects of breast cancer in the clinical setting, particularly in the management of postmenopausal women with hormone receptor–positive breast cancer.

MCF-7 and the Estrogen Receptor

One of the most important contributions of the MCF-7 cell line to breast cancer research has been its utility for the study of the estrogen receptor (ER) alpha, as this cell line is one of a very few to express substantial levels of ER mimicking the majority of invasive human breast cancers that express ER. It is noteworthy that maintaining expression of estrogen receptor

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alpha in cultured cell lines is especially difficult, and this has resulted in generation of far more ER-negative than ER-positive human breast cancer cell lines. Similarly, development of patient-derived xenografts (PDX) has revealed the difficulty in generating ER-positive PDX. While evidence points to a role of extracellular matrix and inappropriate growth substrate (ie, two-dimensional plastic) in loss of ER in cultured cell lines (3), it does not appear that Dr. Soule performed any modifications to the standard isolation techniques of the time to specifically isolate an ER-positive cell line. Rather, it appears that the isolation of the ER-positive MCF-7 cell line was essentially fortuitous.

The original description of MCF-7 cells in 1973 did not make a reference to the fact that they are ER-positive. This finding was relegated to another publication in the same year where Soule and his colleagues reported the finding of "specific estrogen receptor in MCF-7…" Indeed, Soule ended the discussion in the pivotal *JNCI* publication by noting that, "The biologic properties of MCF-7 suggest this line to be an excellent substrate for attempting to isolate human breast cancer viruses" (2). The study of oncogenic viruses was at its high point during this time, but the publication by Soule and his colleagues, which described the presence of ER, was to have perhaps the biggest impact upon breast cancer research (4). Interestingly, the authors recognized the importance that this cell line would have to the basic understanding of ER action by providing "a stable cell line will permit experiments to be carried out which will add to the present knowledge regarding intracellular binding constants, transport mechanisms, and the mode of nuclear uptake," but they did not mention the potential of this cell line for research into hormone-driven breast cancer. In retrospect, this is surprising given that the donor, Helen Marion, clearly had hormone-responsive cancer, which was held in check by hormone therapy. In 1997, the year that Dr. Soule passed away, Levenson and Jordan wrote a comprehensive review celebrating the 25th anniversary of MCF-7 cells (5).

It was Lippman (6) and Horwitz (7) who first reported on ER status and biological function in MCF-7, with both clearly realizing and stating the importance this cell line would have in breast cancer research. While early studies in MCF-7 from Osborne (8) and Sutherland (9) showed that anti-estrogens caused a G0/G1 block and inhibited growth of MCF-7 cells, the demonstration of simple stimulation of growth with estradiol was more challenging and not as reproducible. Solving this quandary was absolutely critical for future research into ER action using MCF-7 cells. Over a decade would pass before the Katzenellenbogens discovered that phenol red, used in tissue culture media as an indicator of pH, was a weak estrogen that was sufficient to activate the ER at the high concentration used in medium (10). Removal of phenol red from medium eliminated this confounding variable and was a critical step forward in allowing complete removal of estrogen and studies to examine how estrogen activated ER and stimulated growth.

MCF-7 cells were central to the development of antibodies to ER, as Greene and colleagues developed the first monoclonal antibody to human ER using ER purified from this cell line (11). While early reports had suggested that ER was cytoplasmic and translocated to the nucleus upon binding estrogen, the availability of ER antibodies helped clarify the predominant nuclear localization of ER (12). These antibodies also aided in the identification of cDNA clones that express ER mRNA and led to the cloning and sequencing of the ESR1 gene (13). Most recently, antibodies have been used in chromatin immunoprecipitation (ChIP) to define DNA binding sites of ER in target genes (reviewed in [14]). Finally, the use of antibodies for measuring ER levels in human breast tumors has helped guide the use of hormone therapy in ER-positive tumors.

Though the MCF-7 cell is viewed as the "work horse" for studies of estrogen action in breast cancer, it is important to note that these cells also express androgen, progesterone, and glucocorticoid receptors (7). As agents targeted against all of these steroid signaling pathways are also active for treatment of some patients with metastatic breast cancer, MCF-7 cells have served as a valuable model system to elucidate other pathways of hormone response and resistance.

MCF-7 as a Model of Response and Resistance to ER-Targeted Therapy

MCF-7 cells have served as a model for the study of estrogen response both in vitro and in vivo. The exact mechanism whereby estrogen stimulates MCF-7 cells to grow remains an active area of study. Early reports focused on estrogen regulation of growth factor signaling and action, and this is certainly a key component of how estrogen regulates the cell cycle (15). But more recent studies have shown that estrogen simultaneously induces and represses a large number of genes, indicating a complex network of changes that coordinate to alter growth (16).

Studies of hormone resistance have also been fundamentally instructed by the use of MCF-7 cells. In vitro studies using estrogen withdrawal or chronic exposure to anti-estrogens led to isolation of hormone resistant variants of MCF-7 cells, which can be either ER-positive or ER-negative. Removal of estrogen initially slows cell growth, but eventually growth resumes and long-term estrogen-deprived (LTED) cells have been generated by several laboratories. The Santen laboratory showed that these cells express high levels of ER and become hypersensitive to estrogen stimulation (17). These studies, combined with those of other groups who developed their own MCF-7 LTED derivatives, have identified epigenetic and transcriptomic changes that lead to alterations in growth factor signaling. Importantly, while MCF-7 LTED cells that are obtained from bulk outgrowth of estrogen-deprived MCF-7 cells are ER-positive, single cell cloning of cells that can grow in the absence of estrogen identified both ER-positive and ER-negative clones, highlighting the tremendous heterogeneity that characterizes clinical breast cancers as a whole as well as this cell line (18).

Many groups have also developed MCF-7 cells that are resistant to the antiestrogen tamoxifen. Studies of these lines have shown alterations in growth factor signaling, epithelial to mesenchymal transition, autophagy, and other critical pathways (19). Osborne and colleagues showed that MCF-7 xenografts responded to tamoxifen in vivo and that continued exposure to tamoxifen (in the absence of estrogen) results in tamoxifen-stimulated growth (20), and this was also noted by Jordan and colleagues (21). A similar result was realized in a clinical trial of an aromatase inhibitor (anastrozole) vs tamoxifen vs the combination for treatment of postmenopausal women with early breast cancer (ATAC), where the combination of anastrozole plus tamoxifen was only equivalent to tamoxifen alone and actually worse than anastrozole alone, suggesting that in an estrogen-deprived environment, tamoxifen was actually seen as an agonist for ER. Tamoxifen-stimulated growth is now realized to be a result of the mixed partial antagonist/agonist activity of tamoxifen and has been studied in detail (19).

Finally MCF-7 cells have also provided the platform for the study of aromatase inhibitors in preclinical models. After transfection with the human placental aromatase gene, the resulting MCF-7Ca cells have been utilized as an in vivo model of postmenopausal breast cancer to evaluate the effect of aromatase inhibitors and antiestrogens (22).

MCF-7 and HER2

MCF-7 cells do not have amplification of the HER2 (ErbB2) oncogene. But given the paucity of models for the study of HER2 and the potential cross-talk between ER and HER2, Osborne and Benz generated MCF-7 cells that overexpress HER2 (23). These cells have been an excellent model for studying how anti-HER2 inhibitors block growth and have confirmed the activity of multiple anti-HERR2 therapies, findings that have been translated and validated in clinical trials. Additionally, many laboratories have developed anti-HER2 therapy resistant MCF-7 cell lines, and interestingly one of the mechanisms is via reactivation of the ER.

Genomics and Evolution of MCF-7 Cells

Understanding the derivation and isolation of MCF-7 is critical, as fundamental concepts about breast cancer have developed from this single cell line. In the original report on the isolation of MCF-7, chromosome number was assessed in passage 2 of the pleural effusion and found to have a very wide range, from 70 to 144. However, analysis of the MCF-7 cell line at passage 39 showed that the chromosome number had narrowed to a range of 77 to 99, with a distinct stem line of 88 (2). MCF-7 cells are thus selected from the genomic heterogeneity of the cells in the initial pleural effusion. Remarkably, the current modal number of chromosomes in MCF-7 cells provided by the American Type Culture Collection (ATCC) is 82 (range 66 to 87). Despite 45 years in culture, the chromosome number has remained relatively static.

Perhaps one of the most disconcerting aspects of MCF-7 cells in the lab has been their ability to adapt and evolve over time. This facet is not too surprising given their genomic instability and the growing understanding of cancer heterogeneity and evolution; indeed, this behavior mimics clinical breast cancers that also evolve over time in the patient, both spontaneously and under the pressure of therapy. However, the inability to replicate results in MCF-7 variants across different laboratories has caused concern. The initial concern was cross-contamination, which has been well documented in other cell lines, and continues to be a major problem. Indeed a recent commentary in *Science* highlighted this general issue and recommended corrective measures, such as cell line authentication (24). Such concerns have been raised with MCF-7; it was Osborne et al. who first reported that cells being provided by ATCC were in fact cytogenetically not similar to MCF-7 (25). Furthermore, in identifying this issue, the investigators also noted that MCF-7 cells from various laboratories behaved differently in biologic assays. This led many laboratories to name their own variants of MCF-7, which have been reported in the literature and include MCF-7L (Lippman), B (Benz), KO (Kent Osborne), BK (Benita Katzenellenbogen), and many more. Some studies have now shown that these cell lines have different gene expression and genomic profiles (26). Others have noted karyotype differences between variants of MCF-7 cells (27), and our own work has shown differences in genomic rearrangements between MCF-7 cell lines from laboratories around the United States as well as between single MCF-7 cells grown out from a single culture (unpublished data). This heterogeneity of MCF-7 in culture is indeed remarkable, and it is a puzzle that a cell line that is clearly genomically unstable has grown continuously for so long. Many students who have let an MCF-7 culture "go over the weekend" will know the hardiness of this cell line, as the cultured cells rebound even after killing off 99% of the cells. It is likely the heterogeneity which allows adaptation to different growth conditions that has allowed this cell line to maintain its core properties (eg, mutations and gene rearrangements), while evolution of other less important changes has led to the emergence of different subtle variants.

MCF-7 has served as a fundamental reference cell line for many genomic studies, in part because of the ability to generate an unlimited amount of RNA/DNA to enable validation and downstream functional studies. For example, the majority of ER ChIP data come from MCF-7 (14), and the first report of genome-wide ER-directed DNA looping was performed by ChIA-PET in MCF-7 (28). RNA fusions have been analyzed in MCF-7 by numerous groups, and single molecule PACBIO whole transcriptome data is now publicly available (29). It is expected that integration of these panomic data (such as ChIP-seq and transcriptomics) will help further our understanding of the origins of this cancer cell line and that MCF-7 will continue to be a vital tool to explain how ER functions and model the genesis and progression of ER-positive breast cancer for many years to come.

Conclusion

In the 45 years since the isolation of MCF-7 cells, pivotal work using this cell line continues unabated. Despite the limitations of research on established cancer cell lines grown in tissue culture and xenografts, discoveries from the MCF-7 cell line have fundamentally altered the course of breast cancer research and have contributed to improved patient outcomes. The value of MCF-7 cells to our understanding of ER action cannot be overstated. We expect that MCF-7 and other breast cancer cell lines will remain a staple for research despite their limitations. Going forward, it is important to confirm and to validate results in other systems that incorporate more appropriate growth conditions, including growth in normoxia and hypoxia, three dimensions, tension, and coculture with other cell types. Together these results will further inform in vivo studies and continue to lead to improved outcomes in breast cancer patients. All of us owe special thanks to Sister Catherine Frances and Dr. Soule, whose legacy continues.

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