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Implementation Hurdles to Closing the Gap in Breast Cancer Survivorship Care

BY SARAH DIGIULIO

A new analysis published in the *Journal of Clinical Oncology* puts the spotlight on just how much evidence there is about how to improve outcomes for breast cancer survivors. Researchers included 323 systematic reviews (yes, reviews, not single studies) for the analysis (2022; doi: 10.1200/JCO.21.02015).

The purpose of the new analysis was to identify gaps in knowledge and priorities for future research. In the paper, the authors noted that a similar analysis had been done on the evidence for survivorship interventions for prostate and colorectal cancers, and then systematically reviewed against survivorship care guidelines. But no such work had been done for breast cancer.

So, that's what the new work set out to do—mapping the evidence that currently exists against the Cancer Survivorship Care

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Novel Model Developed for Genetic Modeling of Human Testicular Cancers

BY DIBASH KUMAR DAS, PHD

Human primordial germ cells (PGCs) are the most upstream precursors of gametes (eggs and sperm). Transformed male PGCs lead to testicular cancer, which is the most common malignancy in juvenile and young-adult males ages 15-40 years old.

Because of the ethical and technical barriers to obtain PGCs from human embryos, human primordial germ cell-like cells (hPGCLCs) are a pluripotent stem cell-derived PGC model of these early precursors widely used in medical research to study their function and development. However, in vitro expansion of hPGCLCs remains a major challenge due to quick loss of PGC-like identity, being usually short-lived and difficult to maintain. Thus, their application for experiments requires a large number of homogeneous cells maintaining their PGC-like characteristic.

In research published in *Stem Cell Reports,* Toshi Shioda, MD, Associate Professor of Medicine at Harvard Medical School, and Director of the Molecular Profiling Laboratory at Massachusetts

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Potential Precision Therapies in Diffuse Large B-Cell Lymphoma

BY DIBASH KUMAR DAS, PHD

Diffuse large B-cell lymphomas (DLBCL) are aggressive and heterogeneous tumors that account for 25-30 percent of all the non-Hodgkin lymphomas. DLBCL develops from B cells transiting the germinal center (GC) humoral immune response. GCs are transient structures that form in lymphoid organs following T-cell–dependent antigen activation of mature B cells.

There are several treatment options available; however, the malignancy frequently develops resistance and/or relapse after treatment with standard therapy. Thus, there is an urgent need to determine the molecular mechanisms of DLBCL to develop effective treatment strategies.

Typically, DNA mutations are critical to the quick development of a range of antibody-producing B cells that together can identify a considerable number of specific targets. However, this process can go amiss in individuals with a mutation in a specific histone-modifying protein.

In a study published in *Cancer Discovery*, researchers at Weill Cornell Medicine described a mutation in one of the two copies of SETD2 in B cells, which can lead to the development of B-cell lymphomas with increased mutations, and they've have identified potential targets for the treatment of the disease (2022; https://doi.org/10.1158/2159-8290.CD-21-1514).

In the study, Leung and colleagues set out to elucidate the role of SETD2 mutations in the humoral immune response and how reduced dosage

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Novel Model Developed for Genetic Modeling of Human Testicular Cancers

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General Hospital Center for Cancer Research, and his colleagues describe the establishment of long-term culture hPGCLCs (LTC-hPGCLCs), which can be expanded in cell culture long term in a serum-free and feeder-free condition while retaining the transcriptomic and epigenomic characteristics of hPGC (2022; https://doi.org/10.1016/j.stemcr.2022.01.012).

In the current study, fluorescence-activated cell sorting (FACS) analysis revealed that the LTC-hPGCLC cultures maintained the hPGCLC markers TFAP2C or PRDM1 after in vitro expansion for 70-153 days. RNA-seq data showed very robust transcriptomal similarities between LTC-hPGCLCs kept in culture for up to 84 days.

Furthermore, there was strong and constant expression of several hPGC marker genes. Single-cell RNA-seq methodology was utilized to show the highly homogeneous characteristics of LTC-hPGCLCs after 120-day expansion in cell culture, while heterogeneous expression of cell cycle genes was apparent in the homogeneous clusters of cells. The data also revealed that LTC-hPGCLCs were telomerase-positive, senescence-free cells that were readily passaged with minimal apoptotic cell loss.

Overall, two male LTC-hPGCLC lines have been established in the current study. These immortal PGCLCs are being used to safely investigate how exposure to toxins might affect reproductive health and the various genetic mutations linked to testicular cancers.

Oncology Times reached out to Shioda, corresponding author of the research, for additional insights into their study. The Shioda laboratory is interested in the biology and diseases of human germline cells, which are committed to producing gametes.

Oncology Times: *What was the rationale for studying the use of primordial germ cell-like cells for toxicological research?*
Shioda: "Among all types of cells in the human body, germ cells are exclusively responsible for conveying heritable information to the next generation. While strong damages caused by exposure to toxic substances may deplete germ cells to cause infertility, modest harm that permits survival of impaired germ cells may cause familial diseases that affect not only the exposed individuals, but also their offspring.

"The risk that toxicants cause infertility or heritable diseases increases when germ cell precursors are affected rather than terminally differentiated gametes. Because primordial germ cells (PGCs) are the earliest precursors of all germ cells, it is very important to understand how the environmental toxic chemicals or therapeutic drugs impact their viability or create mutations in their genome. Since PGCs uniquely experience genome-wide epigenetic imprinting even involving the imprinting genes, they may be especially vulnerable to exposure-induced epigenetic disturbance.

"Because PGCs exist in human embryos during only limited stages of development, access to PGCs for research purposes is extremely challenging for technical and ethical barriers. As recent studies on biology of PGCs have been revealing significant differences between rodents and primates, significant limitations exist in estimating toxicological characteristics of human PGCs based on data obtained using rodent models.

"For these reasons, the use of PGC-like cell culture models derived from human pluripotent stem cells for toxicological research has promise. Our previous study has shown largely homogeneous characteristics of human PGCLCs generated from various precursor pluripotent stem cells using different methods, supporting the use of this cell culture model with modest inter-laboratory deviations for evaluation of toxic substances."

Oncology Times: *Your team was able to successfully develop a method to preserve human PGCLCs. What were some of the challenges faced previously when maintaining human PGCLCs, and how was your team able to successfully develop a method of preserving human PGCLCs?*
Shioda: "Preceding studies aiming at long-term cell culture of human PGCLCs largely suffered from the lack of proliferation, loss of germ cell identity, and cell death. One of such studies reported long-term maintenance of human PGCLCs, but their protocol required FACS-enrichment of cells showing germ cell characteristics from cells that lost expression of germ cell markers. Such non-germline cells rapidly emerged and dominated the cell culture. The unstable nature of these human PGCLCs in vitro cast a doubt about the use of these cells for toxicological assessments.

"When we tackled this problem, we excluded the use of special feeder layer cells or highly complicated medium, which will limit the practical use of our cell culture for future high-throughput applications. Instead, we attempted to simplify the system by identifying factors that are not favorable for preservation of germ cell characteristics. Through this strategy, we quickly realized that the use of serum does not render positive effects and the feeder layer of the standard STO cell line is sufficient to support unlimited expansion of human PGCLCS without losing the germ cell identity. Soon we found that the conditioned medium of STO cell culture replaces the feeder layer cells, permitting us to establish a feeder-free and serum-free system for unlimited expansion of human PGCLCS. Later, we developed a chemically defined human PGCLC cell culture medium that even eliminated the requirement of any conditioned medium (unpublished)."

Oncology Times: *When it comes to testicular cancer research, what is the potential significance of generating this human PGCLC model?*
Shioda: "The vast majority of human testicular cancers are type II germ cell tumors, which are derived from PGCs. An important characteristic of this type of malignancy is that their genomic DNA does not typically harbor a lot of mutations, whereas increase in ploidy is common. The long-term culture human PGCLCs may provide us with unique opportunities to reconstitute the carcinogenic and progression events by introducing a small number of commonly found mutations into them.

"Our laboratory has been working to establish novel human testicular cancer cell lines and normal iPS cell lines from the same mass of tumor. From these iPS cells, human PGCLCs are generated. Because family history is one of the strongest risk factors of testicular cancers, the PGCLCs harboring the same (but normal) genetic background as the cancer cell line are expected to harbor the predisposition loci, possibly increasing our chance to reconstitute testicular cancers by introducing relatively limited numbers of genomic DNA mutations identified by genome-wide deep sequencing comparisons between the normal iPSCs and the cancer cell line.

"Human testicular cancers are categorized into seminomas and non-seminomas. Whereas a number of non-seminoma human testicular cancer cell lines have been established and are currently available as research models, there has been only a single trustable human seminoma cell line. This cell line (TCam-2) was established decades ago, and a large number of studies on seminomas have been solely dependent on this particular cell line. By applying the technique of long-term cell culture expansion of human PGCLCs, we have successfully established a novel human seminoma cell line, as well as iPSCs derived from the same patient (unpublished). This pair of novel cell cultures may permit detailed genomic analyses of seminoma cells in unprecedented depth."

Oncology Times: *Were there any markers identified that indicate a possibility that long-term culture human PGCLCs alone can undergo a certain degree of maturation in vitro?*
Shioda: "Our published study (Kobayashi, et al) has shown two signs of in vitro maturation of human PGCLCs. The first is the absence of GATA3/4 mRNA, which are expressed in freshly isolated PGCLCs. These pioneer factors play important roles in the lineage determination of human PGCs from their precursors. It appears that the freshly isolated PGCLCs resemble the earliest stage of PGCs, whereas cell culture-expanded PGCs reflect more advanced stage of PGCs.

"The second sign is the gradual decrease in the global methylation of genomic DNA during long-term expansion of PGCLCs. Because conversion of PGCLCs to the pluripotent embryonic germ cells restores genomic DNA methylation to the level of the precursor iPSCs, we are speculating possible biological importance of the small decrease in DNA methylation during the extended culture. When human PGCLCs are co-cultured with mouse embryonic gonadal somatic cells, their genomic DNA is dramatically de-methylated and they start to express markers of more advanced germline differentiation such as DAZL." OT

Dibash Kumar Das is a contributing writer.