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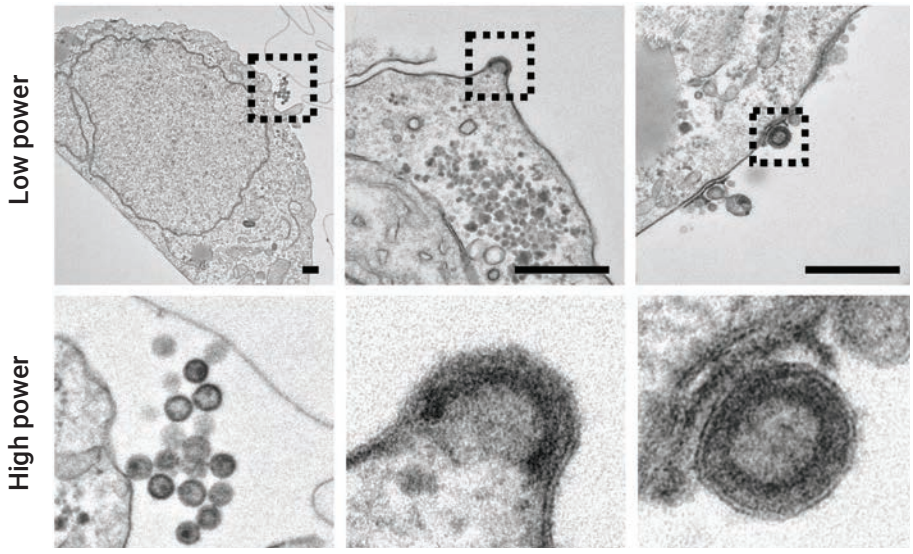
The Shioda laboratory is interested in Primordial Germ Cells (PGCs), the common precursor of gametes. Since access to PGCs in human embryos is limited, iPS cell-derived PGC-Like Cells (PGCLCs) play important roles in studying PGCs, but their lifespan is very short. Our breakthrough Long Term Culture (LTC) protocol supports perpetual expansion of PGCLCs. We found that LTC-PGCLCs produce virus-like particles resembling human-infectious retroviruses and that the responsible retrovirus (the HML-2 endogenous retrovirus) is also active in PGCs in human embryos. Testicular cancers are malignancies of PGCs, and these are the most frequent cancers among young men. About 50% of testicular cancer is seminoma, but only one seminoma cell line has ever been established due to technical difficulties. We found that the LTC protocol of PGCLC culture also efficiently supports growth of seminoma cells, and we have successfully established multiple new human seminoma cell lines and associated normal iPS cells from patient-derived tumor tissues. These cell culture resources provide unprecedented opportunities to understand mechanisms of testicular carcinogenesis and vulnerability of PGCs to toxic substances.

Long-term maintenance of human PGCLCs *in vitro*

Several labs, including ours, have established the usefulness of PGCLCs as a cell culture model faithfully resembling human embryonic PGCs. However, PGCLCs are short-lived and lost in cell culture in 10-14 days. This major technical barrier has prevented application of PGCLCs to various studies such as chemical or genetic screenings. To overcome this hurdle, we have performed a systemic evaluation of cell culture conditions and successfully established a novel protocol that supports expansion of human PGCLCs over 100 days without losing their PGC-like characteristics. Under this Long-Term Culture (LTC) protocol, PGCLCs actively migrate and rapidly proliferate without any limit by senescence as telomerase-positive cells while strictly maintaining their PGC-like transcriptomal profile and marker protein expression. The LTC-PGCLC provided us with a unique opportunity to perform proteomics analysis (with Dr. Wilhelm Haas, CCR), which detected retrovirus-like proteins expressed in this cell culture model of normal human PGCs. To our surprise, it turned out that LTC-PGCLCs robustly produce even retrovirus-like particles from their surface. The HML-2 human endogenous retrovirus is responsible for formation of the virus-like particles in PGCLCs, and analysis of previously published single cell RNA-seq data of human embryos revealed HML-2 activation in PGCs *in vivo*. Thus, the LTC-PGCLC model provides the relevant fields of research with unprecedented opportunities to access unlimited amounts of PGCLCs, facilitating studies of normal development and disease formation of human germ cells.

Genetic modeling of human testicular cancers

Testicular cancer is the most common malignancy that affects juvenile and young-adult males at 15-35 years old. The vast majority of testicular cancer is the Type II germ cell tumor, which arise from PGCs, and about 50% of them are seminomas



HML-2 human-specific endogenous retroviruses form virus-like particles at the surface of human primordial germ cell-like cells (hPGCLCs). hPGCLC is a pluripotent stem cell-derived cell culture model of human primordial germ cells, which are the earliest precursor of all germline cells. The viral capsid is assembled beneath the cell surface (center) and eventually pinched out of the cells with plasma membrane surrounding it as viral envelope (right). The virus-like particles are often released from hPGCLCs as aggregates (left).

and the others are non-seminomas such as embryonal carcinomas. Most cases of invasive testicular cancers harbor chromosome (chr) 12p amplification and are associated with Germ Cell Neoplasia In Situ (GCNIS), which consist of cells resembling PGCs and lacking chr12p amplification. Testicular cancer is known for its very strong familial predisposition. Whereas testicular cancers lack genetic mutations commonly found in many other types of adult cancers, they often harbor gain-of-function c-KIT mutations or focal amplification of the gDNA region including the wild type *c-KIT* gene. Genome-wide association studies have repeatedly suggested the involvement of the pro-apoptotic gene *BAK1* in testicular carcinogenesis. However, the mechanisms by which *c-KIT*, *BAK1*, and/or chr12p amplification contribute to testicular carcinogenesis and progression still remain largely unknown due to the lack of adequate experimental models. The genetic basis of the familial predisposition of testicular cancer is also poorly understood. In collaboration with members in Mass General Urology (Keyan Salari, Philip Saylor, Richard Lee) and Urological Pathology (Chin-Lee Wu), we are attempting to make a breakthrough by establishing novel cell lines of human testicular cancers associated with normal iPS cells, from which PGCLCs can be produced. It turned out that out LTC protocol developed for PGCLCs also efficiently support growth of seminomas, which has been represented by only a single cell line (TCam2). We are currently expanding and characterizing multiple cell lines of seminomas, non-seminomas, iPSCs derived from the same testicular cancer patients, and PGCLCs derived from these iPSCs. For example, our T548 embryonal carcinoma cells harbor four extra copies of chr12 and strongly amplified wild type c-KIT, and in direct comparison with the associating normal iPSCs by whole genome sequencing, our T548 embryonal carcinoma cells revealed LOH of loss-of-function CHEK2. Our T836 seminoma cells harbor a gain-of-function c-KIT mutation and amplified ch12p. We are currently working to introduce these prospective driver mutations into the associating normal PGCLCs – which supposedly harbor the unidentified genetic predisposition – to recapitulate the carcinogenic procedure *in vitro*.

Selected Publications:

Lee H, Blumberg B, Lawrence M, **Shioda T**. Revisiting the use of structural similarity index in Hi-C. *Nature Genetics*. 2023. – accepted.

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