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Molecular Profiling Laboratory

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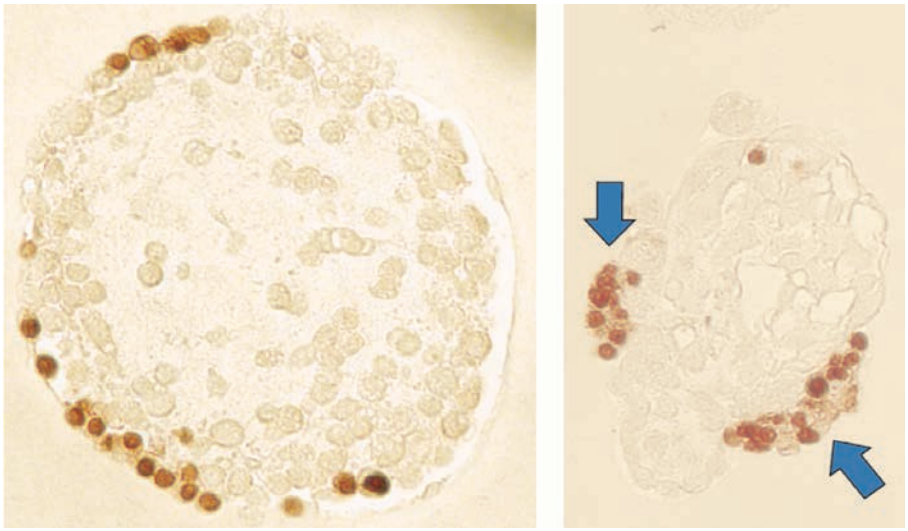
The Shioda laboratory is interested in the biology and diseases of human germline cells, which are committed to producing gametes (sperm or eggs). Primordial germ cells (PGCs) are the first germline cells emerging in human embryos during the third week of gestation. Malignant transformation of male PGCs results in testicular cancers, the most common cancers in young US men between the ages of 15 and 35. Whereas the DNA of PGCs loses most of its gene-silencing machinery to reset its gene expression program, DNA regions encoding the Human Endogenous Retroviruses (HERVs), which are remnants of ancient retroviral infection, selectively remain silent. Activation of HERVs may cause various disorders such as autoimmune diseases and cancers. Although mechanisms of HERV activation in diseases are largely unknown, we hypothesize that relaxed HERV silencing in PGCs under stresses may predispose HERVs to be activated beyond fertilization. Because access to human embryonic PGCs is extremely challenging, our laboratory takes advantage of human iPSC-derived PGC-like cell culture models to study normal biology and mechanisms of diseases involving PGCs.

Epigenetically Provoked Multi-generational Disease Predispositions Involving Aberrant Germline Epigenetic Reprogramming in Primordial Germ Cells

The germline is a series of specialized cell population destined for gametogenesis – i.e., production of sperm and eggs. Thus, germline cells are solely responsible for conveying genetic and epigenetic information to the subsequent generation. All heritable genetic aberrations, including mutations causing familial cancer predispositions, occur exclusively in the germline. Recent studies, including ours, showed that *in utero* exposure of mammalian germline cells to various types of stresses such as therapeutic drugs, toxic environmental chemicals, or malnutrition may create trans-generationally heritable epigenetic aberrations that could cause adult-onset diseases such as cancers or metabolic disorders. In the third week of gestation, human primordial germ cells (PGCs), the earliest-stage germline cells, are observed in the embryonic yolk sac as a cluster of only 40 cells. While rapidly proliferating, PGCs migrate towards genital ridges, where they differentiate into sex-specific germline stem cells. Genomic DNA of PGCs lose cytosine methylation globally and almost completely except for a few specific elements such as regions encoding the Human Endogenous Retroviruses (HERVs). We presume that this robust epigenetic reprogramming occurring uniquely in PGCs may make PGCs especially vulnerable to epigenetic aberrations that cause disease predispositions. Since activation of HERVs are linked to various human diseases such as cancers, autoimmune diseases, and resistance to cancer immunotherapy, our current hypothesis is that stress-induced relaxation of epigenetic machineries silencing HERVs in PGCs may predispose a subset of HERVs to accidental activation and thus increase risks of diseases observed in the



Emergence of human PGC-LCs on the surface of embryoid bodies. Human PGC-LCs are visualized by anti-OCT4 immunohistochemistry of FFPE slides. Most PGC-LCs are localized in the outermost surface layer of embryoid bodies (left). PGC-LCs often form dense clusters (arrows; right), which may mimic the embryonic niche involved in germline commitment of precursor cells.

subsequent generations.

Experimental testing of the above hypothesis faces multiple hurdles. Access to human embryonic PGCs is extremely challenging due to technical and ethical reasons. Molecular mechanisms of PGC commitment and differentiation are significantly different between human and the conventional laboratory rodents. HERVs are unique to humans although the genome of mice harbors IAPs (Intra-cisternal A Particle), a rodent-specific group of endogenous retroviruses that are known to cause various epigenetically provoked diseases. To overcome these hurdles, my laboratory takes advantage of PGC-LCs (PGC-Like Cells), a cell culture model of PGCs generated from iPSCs. In contrast to other protocols that produce PGC-LCs inside iPSC aggregates, our protocol produces PGC-LCs exclusively on the surface of embryoid bodies. This is an important advantage to study effects of exposures to drugs or toxic chemicals on PGC-LCs. In our initial studies, we have shown robust and global DNA demethylation in the genome of mouse PGC-LCs whereas a few types of repetitive elements such as IAPs escaped the erasure, resembling late-stage mouse embryonic PGCs. We also demonstrated that aberrant DNA hypermethylation artificially introduced in mouse iPSCs was effectively repaired in PGC-LCs during the course of germline epigenetic reprogramming. Our recent studies produced PGC-LCs from human iPSCs using our own protocol for improved robustness and experimental reproducibility and showed that human PGC-LCs produced in our lab as well as other labs reflect an earlier stage of embryonic PGCs than mouse PGC-LCs. Thus, global DNA demethylation in the current version of human PGC-LCs was still in its early initiation state and weak. Nonetheless, we were able to detect activation of a specific subset of HERVs in human PGC-LCs that were strictly silenced in the precursor iPSCs, suggesting the existence of a group of HERVs that are especially prone to activation in human germline. Our current research focuses on the molecular mechanisms that silences HERVs in PGCs and their vulnerabilities to stresses. Attempts are also being made to determine whether germline activation of HERVs is involved in mechanisms of the epigenetically inherited disease predispositions to cancers and other human diseases.

Selected Publications:

Diaz-Castillo C, Chamarro-Garcia R, **Shioda T**, and Blumberg B. Transgenerational self-reconstruction of disrupted chromatin organization after exposure to an environmental stressor in mice. *Scientific Reports*. 2019, in press.

Mitsunaga S, Shioda K, Owa C, Isselbacher KJ, Hanna JH, and **Shioda T**. Generation of human primordial germ cell-like cells at the surface of embryoid bodies from primed-pluripotency induced pluripotent stem cells. *J Vis Exp*. 2019; 11(143).

Mitsunaga S, Odajima J, Yawata S, Shioda K, Owa C, Isselbacher KJ, Hanna JH, and **Shioda T**. Relevance of iPSC-derived human PGC-like cells at the surface of embryoid bodies to prechemotaxis migrating PGCs. *Proc Natl Acad Sci U S A*. 2017; 114(46):E9913-E9922.

Chamorro-Garcia R, Diaz-Castillo C, Shoucri BM, Kach H, Leavitt R, **Shioda T**, and Blumberg B. Ancestral perinatal obesogen exposure results in a transgenerational thrifty phenotype in mice. *Nature Communications*. 2017; 8(1):2012.

Miyoshi N, Stel JM, Shioda K, Qu N, Odajima J, Mitsunaga S, Zhang X, Nagano M, Hochedlinger K, Isselbacher KJ, and **Shioda T**. Erasure of DNA methylation, genomic imprints, and epimutations in a primordial germ-cell model derived from mouse pluripotent stem cells. *Proc Natl Acad Sci U S A*. 2016; 113(34):9545-5.

Janesick AS, **Shioda T**, Blumberg B. Transgenerational inheritance of prenatal obesogen exposure. *Molecular and Cellular Endocrinology*. 2014; 398(1-2):31-35.