The Shioda laboratory investigates molecular mechanisms of epigenetic reprogramming in the genomes of vertebrate gonads, germ cells, and stem cells. We give special emphasis to the environmental epigenomics approach, which attempts to examine how toxic substances found in the environment—such as the endocrine disruptor chemicals—affect the epigenetic regulation of gene function. Epigenetic abnormalities caused by the environmental chemicals during the early stages of fetal development may later cause adult-onset diseases including obesity and cancer. If epigenetic anomalies are introduced into the genome of germ cells, transgenerationally transmittable disorders may emerge without involving mutations in the nucleotide base sequences of genomic DNA. Taking advantage of the latest progress in stem cell biology for generation of cell culture models of germ cells and the cutting-edge massively parallel DNA sequencing technology, we have set as our present goals to characterize genome-wide epigenetic changes during germ cell development and to examine how such changes are affected by environmental pollutant chemicals.

Environmental epigenomics

Exposure of genetically male chicken embryos to exogenous chemicals that have estrogen-like hormonal activities causes phenotypic sex conversion. We have developed an improved experimental protocol of the chicken embryo feminization by injecting ethinylestradiol (the active ingredient of birth control pills) emulsified with artificial yolk-like oil mixture into three-days-incubated eggs to achieve practically 100% incidence of phenotypic sex conversion. Gonads of the feminized, genetically male chicken embryos immediately before hatching were indistinguishable from ovaries of natural, genetic females for gloss morphology, histological tissue structures, and expression of the mRNA transcripts for testes- and ovary-specific marker genes. Pyrosequencing analyses of DNA methylation at promoters of sexually dimorphic marker genes of gonads revealed that three CpG dinucleotides in the CYP19A1/Aromatase promoter are strongly hypermethylated in genetic male gonads, whereas they are strongly demethylated in genetic female gonads associated with the protranscription histone H3 trimethylation at lysine 4. These observations were consistent with the female gonad-specific strong expression of the mRNA transcripts for aromatase, which is required for normal and exogenous hormone-induced ovarian differentiation of chicken embryonic gonads. Despite the complete morphological and transcriptomal feminization of the genetic male embryonic gonads by ethinylestradiol, the three sexually dimorphic CpG epigenetic marks in the CYP19A1/Aromatase promoter were hypomethylated only partly by the chemically induced sex conversion. This study is the first published demonstration that epigenetic marks in the vertebrate genome can be more resistant against the environmental
Selected Publications:


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*Factors than transcriptional and morphological phenotypic characteristics, suggesting the existence of the persistent epigenetic memory in the transcriptionally disturbed genome.*

**Epigenetic reprogramming in mammalian pluripotent stem cells and primordial germ cells**

We have developed novel protocols for in vitro production of mouse primordial germ cell-like cells (PGC-LCs) from embryonic stem cells and induced pluripotent stem cells. Genome-wide determination of DNA methylation, DNA hydroxymethylation, and transcriptomal profiling of the PGC-LCs and their precursor pluripotent stem cells by deep sequencing demonstrates the epigenetic reprogramming in the genome of the PGC-LCs. The epigenetic reprogramming in the PGC-LCs was similar to the reprogramming observed in the natural PGCs isolated from mouse embryos, supporting the potential usefulness of the PGC-LC as a surrogate model of the hard-to-obtain natural PGCs for epigenetic research. Our present goal is to establish the similarities and differences of the epigenetic mechanisms of gene expression regulation between the PGC-LCs and natural mouse PGCs. We will then attempt to examine effects of the environmental toxic pollutants on the epigenome of the PGC-LCs. Our PGC-LC model may be useful for detecting the environmental epimutagens as well as for investigating mechanisms of actions of such a novel class of the environmental toxicants.

Fluorescence image of mouse embryoid bodies developed from iPS cells in microwells. The red squares are boundaries of each microwell, and the embryoid bodies are GFP-positive reflecting activation of the Oct-4 promoter. Our lab is generating in vitro models of germline cells from mouse embryoid bodies and analyzing epigenomic events associating the genomic reprogramming events that occur during the germline differentiation from somatic precursor cells.