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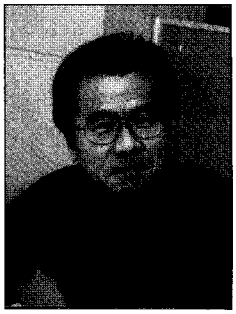

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Epigenetics of Reproduction and Development

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"Epigenetics" means the study of heritable changes in gene-activity without changes in DNA sequences. In vertebrates, methylation of DNA mainly occurs at the 5'-position of cytosine in a CpG dinucleotide forming 5-methylcytosine and DNA methylation pattern is heritable to the next generations. In general, DNA methylation associates with condensed chromatin structure and plays a profound role in transcriptional repression of gene expression through several mechanisms. CpG islands, which has higher GC contents and higher CpG frequencies compared to the entire genome, are generally believed to be unmethylated regions in normal tissues except for those under X chromosome inactivation and genomic imprinting. Recent studies, however, have shown that promoters of many genes contain tissue-dependent differentially methylated regions (T-DMRs). In general, T-DMRs are restricted to a fraction of CpG islands. Recently, we characterized a unique CpG island-associated gene, adenine nucleotide translocator 4 (Ant4), which is expressed in germ cells. Using promoter assay, we demonstrated that expression of Ant4 gene is controlled by DNA methylation at the promoter region, where a CpG island is associated. Certain CpGs of the CpG island were unmethylated in germ cells, while they were fully methylated in somatic tissues, where Ant4 is suppressed. Ant4 locus represents a new class of CpG islands that become methylated at the whole region. T-DMRs at CpG islands are important gene regulatory elements that may now be categorized into two classes: T-DMRs consisting of a fraction of the CpG island and those that occupy the whole CpG island, as demonstrated in Ant4 promoter.

Trophoblast cell lineage is established through the first cellular differentiation in mammalian embryogenesis, and its developmental potential is restricted to the extraembryonic tissues contributing solely to the placenta. Several lines of evidence suggest a relative lack of importance of DNA methylation in gene regulation in the

extraembryonic tissues when compared with embryonic ones. We analyzed the dynamics of epigenetic status in the upstream region of mouse Ddah2 gene, which was found to be specifically repressed in a stem cell population of trophoblast cell lineage. We found a T-DMR in the regulatory region of the Ddah2 gene. This region was hypermethylated in trophoblast stem cells and was hypomethylated in differentiated cells both *in vivo* and *in vitro*. This change was well correlated with Ddah2 expression. In addition, *in vitro* methylation confined to the differentially methylated region was sufficient to repress promoter activity in the reporter assay. Furthermore, a repressive pattern of

histone modifications was formed around the differentially methylated region in undifferentiated trophoblast stem cells with repressed Ddah2. Our data suggest that DNA methylation- mediated chromatin remodeling is involved in the regulation of the Ddah2 gene expression and thus is important even in trophoblast cell lineage.

A single fertilized egg gives rise to a complex multi-cellular organism consisting of, at least, 200 differentiated cell types in mammals including germ cells and trophoblast. Most cells differentiate without changes in DNA sequence through activation of a particular set of genes and inactivation of others. Epigeentics, therefore, underlies the mammalian reproduction and development. Abnormal DNA methylation profiles associate with various abnormal phenotypes. For example, cloned offspring develop a variety of abnormal phenotypes and each cloned animal has a different DNA methylation abnormality and the extent of hyper- or hypo-methylation varies among the individuals. Among the symptoms, overgrowth of the placenta is one of the commonly observed symptoms in all cloned mice regardless of the sex and strain of animal and the type of donor cell. Thus, epigenetic is a new paradigm for reproduction and development.

References

Epigenetic marks by DNA methylation specific to stem, germ and somatic cells in mice. Genes to Cells, 7, 961-969 (2002).

Epigenetics by DNA methylation for development of normal and cloned animals. Differentiation 69, 162-166 (2002).

The Sall3 locus is an epigenetic hotspot of aberrant DNA methylation associated with placentamegaly of cloned mice. Genes to Cells 9: 253-260 (2004).

DNA methylation profiles of CpG islands for cellular differentiation and development in mammals. Cytogenet. Genome Res. (Review) 105:325-334 (2004).

Preference of DNA Methyltransferases for CpG Islands in mouse embryonic stem cells. Genome Res. 14:1733-1740 (2004).

Epigenetic control of mouse Oct-4 gene expression in ES cells and TS cells. J. Biol. Chem. 279: 17063-17069 (2004).

DNA methylation mediated control of Sry gene expression in the mouse gonadal development. J. Biol. Chem. 279: 22306-22313 (2004).

Stage-by-stage change in DNA methyaltion status of DNA methyltyransferase 1 (Dnmt1) locus during mouse early development. J. Biol. Chem. 280:9627-9634 (2005).

DNA methylation-dependent epigenetic regulation of Dimethylarginine dimethylaminohydrolase 2 gene in trophoblast cell lineage. J. Biol Chem. In pres (2006).