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Epigenetics by DNA Methylation for Normal and Cloned Animal Development

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"Epigenetics" means the study of heritable changes in gene-activity without changes in DNA sequences. Methylation of the cytosine residue in a CpG dinucleotide sequence is a characteristic of the vertebrate genome. In vertebrates, methylation of DNA mainly occurs at the 5'-position of cytosine in a CpG dinucleotide forming 5-methylcytosine. Methylation of DNA plays a profound role in transcriptional repression of gene expression through several mechanisms. Generally, DNA of inactive genes is more heavily methylated than that of active ones; conversely demethylation of DNA reactivates gene expression *in vivo* and *in vitro*.

CpG islands with tissue-dependent and differentially methylated region (T-DMR)

Sequences of CpGs are not evenly distributed in the mammalian genome. They appear at a 10 to 20 times higher density in selected regions than in other regions, and regions enriched with CpGs are known as CpG islands. These CpG islands are used as landmarks to find genomic regions in bulk DNA sequences, because CpG islands are generally found in transcription units. Generally, it has been recognized that CpG islands are unmethylated in normal tissues, except the CpG islands involved in X inactivation and genomic imprinting. However, most data on DNA methylation mediated gene repression concerns TATA-less and CG-rich promoters that are associated with CpG islands. The human genome project identified 30,000-40,000 protein coding genes,and there are

approximately 29,000 CpG islands. There are 30,000 genes and 15,000 CpG islands in the mouse genome. Tissue-specific promoters revealed that 50% of CpG islands are linked to tissue-specific genes. The remaining tissue-specific promoters do not associate with CpG islands.
Restriction Landmark Genomic Scanning (RLGS) can perform rapid analysis of methylation profiles of thousands of CpG islands associated with genes in parallel. Scanning of 1,500 CpG islands of genomes from 10 different cell types and tissues, including ES, EG, TS cells before and after differentiation revealed 247 T-DMR. Considering that there are 15,000 CpG islands in the mouse genome, the total number of CpG islands with T-DMR will be much greater. It is clear that CpG islands having T-DMR were numerous and widespread in the genome. A recent study by restriction enzyme-based library cloning identified normally methylated CpG islands in the human genome (Liora Z. Strichman-Almashanu Benome Reseacr 12:543-554, 2002).
The T-DMR panel clearly indicates that DNA methylation is cell type specific. Consequently, it is clear that genes associated with CpG islands should be included in the list of genes investigated for DNA methylation mediated gene-silencing.

Abnormal DNA methylation status in cloned animals

The rate of cloned animal production is generally quite low average 2-3% or less of reconstituted eggs develop into live offspring. It is most likely that incomplete DNA methylation does not allow cells of the embryo to correctly express genes required for development and survival of the embryo and fetus. Cloned animals that survived birth and beyond have nearly correct DNA methylation patterns as previously reported. In cloned mice, the DNA methylation pattern atT-DMR was 99% identical to the normal mated control. Therefore, the cloned animals that developed to full term have almost normal DNA methylation patterns and are fairly good copies of nuclear donor animals. So, the epigenetic system is more flexible than previous thought. However, cloned animals have a variety of abnormal symptoms at and after birth. It is important to note that all cloned mice have imperfect DNA methylation without exception. Each cloned animal has different DNA methylation aberrations, and the extent of abnormality and loci varies among different individuals.

Conclusions and feature directions

A single fertilized egg gives rise to a complex multi-cellular organism consisting of at least 200 differentiated cell types. Most cells differentiate without changes in DNA sequence through activation of a particular set of genes and inactivation of others. The molecular basis for the memory for activated or inactivated gene sets, which is inherited to the cells'next generations, is critical for differentiation and development of multicellular organisms. Epigenetic errors cause other diseases as suggested in cloned animals.

References

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