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研究成果報告書

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生活習慣病による生殖細胞のエピジェネティック変化およびゲノム変異の発生機序

Mechanisms for Epigenetic and genetic alterations in male germ cells by lifestyle diseases

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Abstract

Epigenetics is a phenomenon and mechanism, in which and by which altered phenotypes are maintained without a change in the DNA sequence. Epigenetics, involving a long-term regulation of gene expression, are regulated by DNA methylation, histone modifications and small RNAs. For example, when DNA in promoter regions becomes methylated, the gene expression becomes shut off. *Epigenome* is a term to explain the entire epigenetic information in a cell.

In the case of mammals, epigenetic phenomena are observed within an individual but not between parents and their offspring. Recently, however, studies in humans and mice have reported that lifestyle-related diseases, such as diabetes, of a parent can increases the risk of the disease in the offspring. A possible explanation for this inter-generational transmission is that lifestyle-related diseases change the methylation pattern of gametes' DNA and those changes are inherited. However, it is known that the methylation state of sperm DNA is largely erased after fertilization. Therefore, the "epigenetic inheritance hypothesis of lifestyle-related diseases" remains under hot debate.

In this study, using deep sequencing, we studied epigenomic abnormalities in sperm due to diabetes and their heritability in a mouse model. By taking advantage of our established method to purify different stages of male germ cells by FACS, we aimed to clarify at what stage of development epigenetic abnormalities emerge. On the other hand, we also analyzed the offspring of diabetic fathers to elucidate the molecular mechanisms of epigenetic inheritance. To this end, a group of mice were treated with a beta-cell-killing agent serving as the diabetic model, while the other group of mice were untreated.

Our results showed that, in the parental germ cells, no major changes were observed at the level of gene expression, but DNA methylation was altered in thousands of genomic regions. Inspection of these sequences revealed the enrichment of several transcription-factor-binding motifs, including that of ZFP57. Next, we studied their offspring. We found that offspring of diabetic fathers showed an abnormal response to insulin. Then, we determined the transcriptomes and DNA methylomes of their islets and liver. We found decreased expression of the insulin genes in the islets and increased expression of gluconeogenesis-related genes in the liver of the offspring of diabetic fathers. In addition, DNA methylation differences were again observed in thousands of genomic regions in the islets and liver, respectively. Although many of these are not promoters of differentially expressed genes, many DNA methylation changes occurred in known enhancer regions, which may therefore alter the expression of genes. Importantly, some of the DNA methylation differences seen in the parental sperm were also seen in the offspring organs. The sequences in these regions were enriched with the ZFP57 binding motif. These results suggest that the abnormal DNA methylation that occurred in sperm is protected by ZFP57 from extensive reprogramming, and thus transmitted to the offspring. Future studies, including DNA methylation analysis in early embryos and histone modification analysis, will hopefully reveal the nature of epigenetic inheritance in mammals.