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Expression of RBMY1A1 and CDY1 Genes in Korean Infertile Men

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Azoospermia factor (AZF) has been reported to disrupt spermatogenesis and caused male infertility. Deletions involving AZF factor seem to be associated with a severe testicular phenotype containing almost complete absence of germ cells. It is subjected to the influence of many genes (DFFRY, DBY, RBMY, CDY, VCY2,and DAZ). Among these genes, RBMY and CDY genes are strongly associated with male germinal cell development and expressed specifically in testis. These genes were expressed in different stages during spermatogenesis process. RBMY1A1 gene has been reportedto be expressed in stage of spermatogonia, spermatocyte, and round spermatid, while the CDY1 gene was expressed in the following stage of elongate spermatid. In this study, RT-PCR approach was used to investigate the expression pattern of RBMY1A1 and CDY1genes (AZFc region) in testicular tissues from 42 Korean infertile men. The result indicated that negatively expressed RBMY1A1 was present in 33% of the patients. In case of CDY1 gene, no expression of 64% of the patients was appeared. Patients who have no expression of the RBMY1A1 gene also showed no expression of the CDY1 gene in their testis tissues. Taken together, these genes on human Y chromosome could be important for male infertility.

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Comparative Analyses of Genomic Sequences in Human Chromosome 21 and Chimpanzee Chromosome 22

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The identifying the types and extent of DNA of DNA sequence variation existing between human chromosome 21 and chimpanzee chromosome 22 will be important for understanding the genetic basis of recently evolved human and chimpanzee specific traits. To obtain chimpanzee sequences derived from the BAC clones, assembly were generated by using the megablast program. The resulting alignments were checked by eye to remove poorly aligned regions. This set was used to analyze the degree of conservation of noncoding and coding sequences between chimpanzee and human. Orthologous chimpanzee sequences were obtained by performing pipmaker server against the human genome. Human and chimpanzee specific repetitive sequences were identified by Repeatmasker. We compared human chromosome 21 to chimpanzee chromosome 22 sequences, and analysed rearrangement event between two species. The result showed that a variety retroelements are almost present at the deletion boundaries in the human and chimpanzee genome, respectively, suggesting that duplication, translocation. insertion, and deletion events could be actively associated with retroelements.

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Regulation of Xist Expression by Binding of CTCF and MeCP2 to Tsix Promoter

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Tsix inhibits the expression of Xist gene responsible for the initiation of X inactivation. Recently, the control process of Xist expression by Tsix is under massive investigation. The promoter of Tsix contains DMR (differently methylated region), hyper-methylated on Xa (active X chromosome) and hypo-methylated on Xi (inactive X chromosome). Binding of various trans-acting factors to the DMR of Tsix promoter determines the level of Tsix expression. Although it has been known that CTCF (CCCTC-binding factor) binds to the unmethylated promoter of Tsix on Xi, it is unclear whether binding of CTCF activates the expression of Tsix or inhibits. We investigated the actual function of CTCF on the unmethylated promoter of Tsix on Xi using ChIP (chromatin immunoprecipitation) assays. Also, it is assumed that MeCP2 (Methyl-CpG binding protein 2), reported to bind to the methylated promoter on X-linked genes, would be a factor regulating the transcription of Tsix, which binds to the methylated promoter of Tsix on Xa. Binding of MeCP2 to Tsix promoter was investigated using ChIP assays and transcriptional activity of the MeCP2-bound promoter was estimated using RT-PCR. These results imply that CTCF and MeCP2 could regulate Tsix expression through the binding to Tsix promoters on Xi and Xa, respectively.

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The γ-H2AX Affects the Binding of XIST RNA and BRCA1 to Inactive X Chromosome

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Histone proteins, major components of chromosomes, affect the chromosome structure and gene expression and often function in combination with other proteins. Their functions are modulated by protein modifications such as the methylation, phosphorylation, acetylation, etc. In inactive X chromosome (Xi), histone proteins play important roles in maintaining the inactive state of the genes on Xi. Especially, γ-H2AX, a phosphorylated form of H2AX, has been known to specifically bind with XY body during male meiosis, which mimics Xi of somatic female cells in transcriptional activity and methylation status. Recently, it is reported that BRCA1 contributes to binding of the XIST RNA to Xi. Here, we focused whether H2AX is phosphorylated in Xi of somatic female cells as well as XY body in male meiotic cell and affects the binding of BRCA1 and XIST RNA to Xi. We performed the immuno-fluorescence assay (IFA) with γ-H2AX specific antibody in male and female cells. And we exposed the wortmannin to cultured female cells for blocking the phosphorylation of H2AX and observed the localization of BRCA1 and XIST RNA on Xi using IFA and RNA FISH. The results demonstrate that γ-H2AX may affect the binding of XIST RNA and BRCA1 to Xi and, thereby, function in Xi maintenance in conjunction with each other.