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論　文　内　容　要　旨

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学位論文題目	Genomic analysis of Chinese hamster ovary (CHO) cells (CHO細胞における染色体配列に関する研究)		

内容要旨

【Background and objective】

Chinese hamster ovary (CHO) cell lines are widely used host cells for production of protein-based biopharmaceuticals. Production of biopharmaceuticals using CHO cells has well developed, but the scientific background for CHO cells were not investigated well. Also, recently-reported CHO genomic sequences did not referred to CHO chromosome information because of the aneuploidy of chromosome number and instability of chromosomal structure of CHO. Previously, I constructed bacterial artificial chromosome library used for CHO DR-1000L-4N cell line and constructed a chromosomal physical map of the CHO DG44 cell line using these constructed 303 bacterial artificial chromosome clones. In this study, I determined the bacterial artificial chromosome end sequences of the 303 clones and analyzed it for investigation of scientific background of biological information in CHO cells for the production of biopharmaceuticals.

【Results and discussion】

Using bacterial artificial chromosome based fluorescence *in situ* hybridization and bacterial artificial chromosome end sequences, it was confirmed that the genomic sequences of CHO DG44 cells have regions which are highly homologous to the mouse genome. Among 465 bacterial artificial chromosome end sequences showed high homology to mouse genomic sequences, 23 specific narrow regions in 13 chromosomes of the CHO DG44 cell line showed high homology to mouse chromosomes. This result was similar to previous comparative genomic hybridization results between mouse and Chinese hamster genome except for sex chromosome which are conserved in rodent species. The wide area of chromosome E and P have homology to mouse chromosome X, however, there is no correlation between CHO DG44 chromosome and mouse chromosome X. These results were not able to be revealed by previous comparative genomic hybridization analysis. I compared the sequences between the bacterial artificial chromosome end sequences and genomic sequences of CHO K1 cell line. CHO K1 is well-known host cell line but the karyotype is different from CHO DG44 cell line, even though CHO DG44 and CHO K1 cells were derived from Chinese hamster. These bacterial artificial chromosome end sequences and CHO K1 cell line genomic sequences were compared and 13 bacterial artificial chromosome end sequences did not show the high homology, and most of them were low GC content. Among 13 bacterial artificial chromosome end sequences, bacterial artificial chromosome end sequence of Cg0180E19 had repeat sequences and was located near centromere and telomere region. Moreover, we determined SNPs and indels between bacterial artificial chromosome end sequences and CHO K1 genomic sequences. SNPs are approximately 0.60% of determined sequence length, and, indels are approximately 0.17% of determined sequence length.

When recombinant proteins are produced by host cell, it is essential to perform transfection and selection of suitable high-producer cell lines. In the case of CHO cell lines using as host cells, the selection process is dependent on the know-how of and selection techniques of researchers. Recently, next generation sequencing contribute to determine the whole genomic sequences of Chinese hamster and Chinese hamster derived CHO cells. Besides, in these days, targeting techniques has developed evolutionally and these techniques require specific genome sequences. Nowadays, it is very important and useful to refer their genomic sequences to their chromosome. Moreover, very recently, the 3rd generation PAC-BIO next generation sequencing analysis was developed. If using these PAC-BIO next generation sequencing data, it may be possible to compare Chinese hamster genome and CHO DG44 genome or genome sequence of other cell line, exactly. However, genomic sequences and their location in chromosomes are essential information for understanding and engineering of CHO cells, such as genome engineering technologies including genome editing. It is expected that BAC-FISH and bacterial artificial chromosome end sequences analysis could contribute to the development of scientific research in CHO cells and the construction of highly productive cell lines for protein-based pharmaceuticals.