C205 Chinese hamster ovary (CHO)細胞の長期継代培養時における染色体数と細胞性質
Clonal Variability and Chromosome Instability in Chinese Hamster Ovary Cell Lines

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1. INTRODUCTION

Chinese hamster ovary (CHO) cells were established from ovary of an adult Chinese hamster (*Cricetulus griseus*) in 1958. The proline-requiring CHO-K1 and the dihydrofolate reductase (DHFR)-deficient CHO-DG44 are the most widely used industrial host cells for production of biopharmaceuticals [1]. Despite their industrial values, efforts to characterize the CHO genome has lagged behind the human and mouse genome projects. For further improvement of CHO cell lines, genome-wide analysis of CHO cells, including CHO genome sequencing, transcriptomics, proteomics, metabolomics and glycomics are essential. In previous study, we constructed the bacterial artificial chromosome (BAC) library for genome-wide analysis of CHO cells and selected 13 BAC- fluorescence *in situ* hybridization (FISH) markers to identify all of their 20 individual chromosomes of CHO-DG44 cells [2].

However the chromosome number heterogeneity of CHO cell populations and the contribution of chromosome variability to production variability have not been investigated to date. In this study the chromosome number distribution of CHO cell pools and the effect of different chromosome number on the cell growth and recombinant protein production have been investigated. In addition the chromosome number distribution together with growth and productivity of gene-amplified CHO DR1000L-4N (CHO-4N) clones in the presence and absence of methotrexate (MTX) were studied.

2. MATERIALS AND METHODS

-Cell line and Medium: CHO-DG44 (dhfr$^-$) and CHO-4N. IMDM medium supplemented with 10% fetal bovine albumin, hypoxanthine and thymidine for CHO-DG44 and the same medium but without hypoxanthine and thymidine with 1000 nM MTX for CHO-4N. Cells were maintained in 5% CO_2 at 37°C.

-Cell Cloning, Chromosome Count and Chromosome Identification: Limited dilution method was used to isolate individual clones using established cell lines. Identification of chromosomes was performed by FISH as described previously [2].

-Productivity: Cell counting by hemocytometer with trypan blue dye exclusion and enzyme-linked immunosorbent assay (ELISA) were carried out to determine the specific production rate of established CHO-4N clones as described previously [2].

3. RESULTS AND DISCUSSION

After two rounds of cloning and examining 156 clones, finally 8 CHO-DG44 and CHO-4N (4 each) clones were selected. Clonal analysis of isolated clones showed that these cell lines are basically heterogeneous. Our BAC-FISH and DAPI images indicate that in CHO cells mitosis is a vulnerable process. Abnormal ploidy and nucleation are quite prevalent among clones of CHO cell lines resulting in cell population heterogeneity in terms of chromosome number. Study of chromosome number stability of the representative by counting 100 metaphase spreads of DAPI-stained slides for three consecutive months shows that clone's chromosome number distribution changes over time (Fig.1). Comparing the growth rate of CHO-DG44 and CHO-4N revealed that gene-amplification slowdowns the growth rate of gene-amplified cells. Culturing the CHO-4N clones in the presence and absence of MTX indicates that removing MTX might affect the production rate negatively and growth rate fairly positively in CHO-4N cells. After correlating the chromosome number and specific production rate of CHO-4N clones it became clear that chromosome number variability is associated with productivity of clones and that polyploid CHO cells are good candidates for production of biologics, since they possess extra copies of gene-amplified region, as shown by FISH images, enabling them to produce higher mounts of target recombinant proteins.

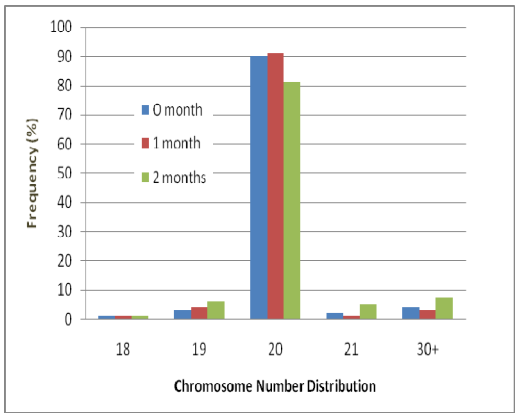

Fig. 1: Investigation of chromosome stability over time.

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