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KARYOTYPE-BASED ANALYSIS OF CELL LINE INSTABILITY AND CLONALITY IN CHO CELLS

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Chinese hamster ovary (CHO) cell line instability that can result in unexpected changes in phenotypes such as cell growth, productivity, or product quality is challenging for the biomanufacturing of therapeutic proteins. In addition, understanding cell line instability and its relationship to clonality is critical. We hypothesize that chromosomal rearrangements resulting from genomic instability are associated with cell line instability.
We developed cell line instability models using two cell lines: secreted alkaline phosphatase (SEAP)-expressing CHO cells (CHO-SEAP) and their host cells (CHO-DUK). We also developed a karyotype-based framework to quantify chromosomal rearrangements. In the absence of methotrexate (MTX), long-term cultured CHO-SEAP cells exhibited a slightly increased growth rate, a significantly decreased specific productivity, and changes in the chromosomal rearrangement ratio of seven chromosomes when compared to the CHO-SEAP cells grown with MTX, demonstrating production instability. Fluorescence in situ hybridization and karyotyping analyses indicated a chromosomal loss of the SEAP gene-containing region, leading to the emergence of a non-producing subpopulation. These results support a mutation-and-selection mechanism of production instability wherein random chromosomal rearrangements can give rise to faster growing cells with low or non-producing phenotypes.
Long-term cultured CHO-DUK cells exhibited an increased growth rate and an increase in the population ratio containing a particular chromosome. Growth rate and karyotyping analyses of limiting dilution CHO-DUK clones showed a correlation between the faster growing clones and the presence of that particular chromosome. Finally, karyotyping analysis indicated that CHO-DUK cells, as well as limiting dilution clones, were karyotypically heterogeneous, suggesting that chromosomal rearrangements occur spontaneously and can compromise the clonality of a cell line that has been developed from a single cell.