ASSESSMENT OF KARYOTYPE VARIATION OF CHINESE HAMSTER OVARY (CHO) CELLS

USING TWO CYTOGENETIC METHODS, SCIENTISTS AT ACIB REVEALED HIGH KARYOTYPIC VARIATION OF CHO CELL POPULATIONS (CELL POOLS AND SUBCLONES).

Chinese Hamster Ovary (CHO) cells are the most important expression systems for the production of complex therapeutic proteins. This has many reasons, including their flexibility to grow under different culture conditions. This flexibility is in part due to, but at the same time set off by their inherent genomic heterogeneity. Chromosomal rearrangements are a common phenomenon in CHO cells as well as in other immortalized, rapidly growing cell lines. In the context of an industrial producer cell line, this may lead to cell line- and product instability, resulting in prolonged screening phases in order to isolate cells with sufficiently stable properties. Genomic rearrangements may occur during each cell division and include small variations of one or a few nucleotides, or larger scale aberrations, mostly translocations of genomic regions. These structural variations are further complemented by numerical variations, such as the loss or gain of whole chromosomes.

Only few methods exist to assess genome-wide instability. Scientists at acib evaluated two cell-based (cytogenetic) methods to reveal karyotypic variations of individual CHO cells. With chromosome counting, the number of chromosomes per cell can be determined, while with chromosome painting structural changes of chromosomes can be quantified.
Assessment of the distribution of chromosome numbers per cell and the karyotypic variation via chromosome painting showed a clear discrimination between different CHO host cell lines. Each host cell line analysed has a characteristic modal chromosome number and a predominant karyotype, which, however, can change over time in culture with new karyotype variants appearing. Furthermore, the genomic heterogeneity between a CHO cell pool and a subclone thereof (thus a clonal population origination from a single cell) was compared. Cytogenetic analyses indicated that the subclone has a comparable chromosomal instability as the originating cell pool, concluding that the process of subcloning does not contribute to an improved genomic homogeneity of a cell population.

**Impact and effects**

A prerequisite for a cell line to be used for industrial production of biopharmaceuticals is to be clonal, thus originating from a single cell in order to be genotypically and phenotypically identical. The presented study investigated the assumption that subcloning has a positive influence on the genomic homogeneity of CHO cells via two cytogenetic methods. Assessment of karyotypic changes revealed that genomic variance is high in all populations of CHO cells, be it a clonal cell line or a cell pool, as it occurs randomly with each division, making subcloning an unsuitable tool to enhance population homogeneity. Subclones had the same wide spread in chromosome number distribution and structural variants as a cell pool. Based on these findings the main focus of quality control and product safety should be on the quality attributes of the product itself, which is finally administered to the patient and not on demonstration of clonality of the cell line used for production, as currently required by the regulatory authorities.

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**Project coordination (Story)**

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