第 266 回つくば分子生命科学セミナー

TSUKUBA MOLECULAR LIFE SCIENCE SEMINAR

演題: Intra-clonal variation in CHO cells producing a recombinant therapeutic monoclonal antibody: Implications for the Biopharmaceutical Industry.

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会場: 筑波大学総合研究棟 D 311 室

要旨: When establishing stable production cell lines by non-targeted methods it is well known that specific productivity in transfectant pools can vary considerably from cell to cell due to differences in vector copy number and site of integration in the host genome. There can also be variation in other cell characteristics due to insertional mutagenesis. On subsequent passaging, gene amplification or gene silencing can further increase heterogeneity. Less well understood is why, after isolating single cells from amplified or non-amplified pools, the resulting clonal populations still show marked phenotypic heterogeneity, even over relatively short timescales.

In this work, we studied intra-clonal variation in antibody-expressing CHO-K1 transfectants using a pair of novel bicistronic transcriptional reporter vectors separately encoding IgG light and heavy chains. Transcription of each immunoglobulin chain is independently coupled to translation of enhanced green or yellow fluorescent protein by an attenuated internal ribosomal entry site. We monitored heterogeneity in fluorescence levels of these reporter proteins in a metal-amplified high- producing clone using fluorescence-activated cell sorting (FACS) and fluorescence microscopy at the single cell level and Genetix ClonePixFL analysis at the colony level. An imaging flow cytometer (Amnis Imagestream) was used to acquire additional subcellular and morphological information to enhance our interpretation of the conventional flow cytometry data. Simultaneous measurements of secreted IgG in the same cells were made using red fluorescently tagged secondary antibodies, either on the surface of individual cells or as fluorescent halos around secreting colonies.

We found several possible sources of variation contributing to observed cell heterogeneity in clonal populations. Firstly, measurement error can play a role in observed variation. Secondly, cell cycle position and cell size can affect fluorescence measurements at the single cell level. Thirdly, by studying the dynamic response of sorted subpopulations from the parental clone we found evidence of temporal phenotypic variation in which cells and their descendants drift between high and low expression levels, and vice versa, over a period of several cell cycles, eventually returning to a common steady state population distribution. We also observed that compensated EGFP and EYFP fluorescence remained highly correlated in the presence of variation, despite each reporter being driven from a separate promoter. This suggests coordinated regulation of light and heavy chain cassette expression in this clone. Lastly, as is the commonly invoked explanation for clonal variation, genetic and epigenetic changes may accumulate randomly in different cell lineages within the population, contributing to heterogeneity and instability over longer timescales.

Overall, our findings exemplify the power of newly emerging techniques for single cell analysis. We were able to separate clonal variation into several components acting over different timescales. In particular, by focusing on shorter timescales this study highlights the importance of accounting for the contribution of non- or partially-heritable phenotypic variation when performing cell selection and screening, and when assessing the stability of freshly-isolated clones.

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