HYBRID CELL LINE DEVELOPMENT SYSTEM UTILIZING SITE-SPECIFIC INTEGRATION AND METHOTREXATE-MEDIATED GENE AMPLIFICATION IN CHINESE HAMSTER OVARY CELLS

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Site-specific integration has emerged as a promising strategy for streamlined and predictable Chinese hamster ovary (CHO) cell line development (CLD). However, the low specific productivity of the targeted integrants limits their practical applications. In this study, we developed a hybrid CLD platform combining site-specific integration of a transgene and dihydrofolate reductase/methotrexate (DHFR/MTX)-mediated gene amplification to generate high-yield recombinant CHO cell lines. We used the CRISPR/Cas9-based recombinase-mediated cassette exchange landing pad platform to integrate the *DHFR* expression cassette and transgene landing pad into a CHO genomic hot spot, the *C12orf35* locus, of *DHFR*-knockout CHO-K1 host cell lines. When subjected to various MTX concentrations up to 1 μM, EGFP-expressing targeted integrants showed a 3.6-fold increase in EGFP expression in the presence of 200 nM MTX, accompanied by an increase in *DHFR* and *EGFP* copy number. A single-step 200 nM MTX amplification increased the specific monoclonal antibody (mAb) productivity (q_{mAb}) of recombinant mAb-producing targeted integrants by 2.8-fold, reaching a q_{mAb} of 9.1–11.0 pg/cell/day. Fluorescence in situ hybridization analysis showed colocalization of the *DHFR* and mAb sequences at the intended chromosomal locations without clear amplified arrays of signals. Most MTX-amplified targeted integrants sustained recombinant mAb production during long-term culture in the absence of MTX, supporting stable gene expression in amplified cell lines. Our results suggest that the present CLD platform can increase the productivity of targeted integrants by amplifying transgene copies.

Given the wide use of the CHO cells and potentials of site-specific integration-based CLD, accelerated generation of highly producing targeted integrants is promising. Increasing the transgene copy number from established and well-characterized targeted integrants can minimize clonal variation and increase productivity without the need for multicopy targeted integration. The present method could provide a new CLD platform for the efficient production of biopharmaceuticals and will be of broad interests to both medical and biotechnological research communities. In this presentation, I will discuss pros and cons of the present method by comparing it with the classical CLD method. Furthermore, I will discuss the perspectives of this new platform for product-specific CHO CLD and cell engineering.