Overexpression of SERPINB1 in Chinese Hamster Ovary Cells Increases Recombinant IgG Productivity

Nan Lin, Jeanne Brooks, Natalie Sealover, Christopher Limmix, Henry J. George and Kevin J. Kayser
Cell Sciences and Development, SAFC/Sigma-Aldrich, 2909 Laclede Ave., Saint Louis, MO 63103 USA

Abstract
We report the discovery and validation of a novel CHO cell engineering target (Serpinb1) that has significant effects in enhancing recombinant IgG productivity. We performed transfection studies using ODN microarrays and compared cultures with IgG heavy and light chain transcription transiently repressed versus cultures with non-targeting siRNA. The transcription of serpin peptidase inhibitor, clade B, member 1 (Serpinb1), a member of the serine protease inhibitor (Serpin) superfamily, was up-regulated 2 fold post HC and LC siRNA transfection. A lentiviral vector was used to over-express the Chinese Hamster Serpinb1 in a CHOZN® Glutamine Synthetase (-/-) recombinant IgG producing CHO cell line. The transduction led to a stable pool with 4.2-fold over-expressed SERPINB1 compared with the non-transduced control. The peak viable cell density and peak IgG volumetric productivity in fed-batch increased 1.3 and 2.0 fold, respectively, as a result of the over-expression. As verification, a plasmid expressing SERPINB1 was transfected to the CHOZN® GS (-/-) host cell line to create several stable pools. Over sixty single-cell clones were isolated from these stable pools and characterized for their SERPINB1 expression levels and exponential phase growth rate in fed-batch cultures. Parasitically, the clone (SERPINB1 Clone 27) with the highest SERPINB1 expression isolated from the plasmid stable pools had decreased exponential phase growth rate. Selected clones with varied SERPINB1 over-expression levels were subsequently evaluated for their IgG expression capabilities using GS selection. Clone 27 has much reduced outgrowth using the GS expression system, but the “minipool” transfectants demonstrated higher expression. Two intermediate level SERPINB1 OE clones (#42 and 47) demonstrated similar “minipool” productivity but reduced outgrowth under (-) glutamine selection, implying increased selection stringency. Clone #42 demonstrated increased productivity in “bulk” pool selection. We conclude that manipulating Serpinb1 expression level can lead to increased recombinant IgG productivity. We performed transcriptomic studies using cDNA microarrays and compared results

Background
Serpinb1 belongs to a superfamily of “suicide inhibitors”
Serpinb1 is up-regulated responding to hydrolysate feeds and may play a role in igG production

Methods

Overexpression Cell Engineering Target Validation

Candidate genes from Biomarker Discovery

SERPINB1

Overexpression construct design and validation (plasmid)

Cell line engineering for host CHO cell lines

Cell line for IgG producing CHO cell line

Plasmid overexpression and cell line characterization

Plasmid vector transfected in CHOZN® GS host cell line

Two levels of selection -> stable pools

Confirm OE in stable pools

Results

Serpinb1 is over-expressed in the lentiviral stable pool

Serpinb1 Lentiviral Stable Pool Demonstrated Increased IgG Volumetric Productivity

Characterization of selected clonal host cell lines derived from SERPINB1 plasmid stable pools

Growth profiles of selected clonal host cell lines derived from SERPINB1 plasmid stable pools

Conclusions

• Serpinb1 overexpression may lead to increase in both growth and productivity in IgG producing CHO cells as observed in the lentiviral stable pools derived
• Confirmed OE in single-cell clones derived from plasmid stable pools generated using CHOZN® GS host cell line
• Clones-associated biological effects were observed in the five SERPINB1 OE clones studied
  • Highest SERPINB1 OE (Clone #27) led to reduced growth rate
  • Clone #27 has much reduced outgrowth using the GS expression system, but the “minipool” transfectants demonstrated higher expression
• Two intermediate level SERPINB1 OE clones (#42 and 47) appear to have similar productivity but reduced minipool outgrowth under (-) glutamine selection, implying increased selection stringency. SERPINB1 OE Clone #42 demonstrated much increased productivity in “bulk” pool selection
• Further investigation of more clones is necessary to clarify the clone-associated effect