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Oral Presentation

A novel engineered insect cell line for pro-protein processing and activation

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Background

A large number of therapeutically relevant proteins, including secreted enzymes, hormones and neuropeptides, need post-translational proteolytic processing to become mature and functional. A problem in their heterologous expression is often represented by the limited ability of the host cell to efficiently and accurately mature the recombinantly expressed zymogens and precursors. This hurdle often impairs their expression level and solubility, and an *in vitro* maturation step is always required to recover functionally active products. However, the latter procedure cannot be universally applied, since in many cases functional enzymes can be obtained only when the post-translational processing is accomplished by the physiological peptidase in the proper cell compartment.

Results

To tackle this bottleneck in recombinant protein production, we have developed an insect cell line able to process inactive zymogens. Stably transfected cells have been isolated through serial limiting dilutions and clone pool analysis has been performed to ensure the expression of the recombinant gene. Functional screening has been applied to characterize the final clones, based on the ability of the cells infected with recombinant baculovirus to secrete properly processed human enzymes. The selected clone was demonstrated to be competent for processing of different enzyme classes, including lipase, serine endopeptidase and metalloprotease. The processing extent of the secreted enzymes strictly correlated with an increase in their catalytic activity, confirming that cleavage occurred at the proper activation site. The final validation was performed applying a high-throughput approach, whereby media of insect cells grown in 24-deep-well blocks were processed using a 96-well plate fully-automated robotic procedure to purify the recombinant proteins, whose catalytic activity was assayed in 384-well plate format with a homogeneous fluorescence-based readout. Pro-protein processing stably transfected cells were easily adapted to grow in miniaturized format (2 ml in 24-deep-well block) and were used for mid-scale production of a recombinant human peptidase, demonstrating good compliance with different cultivation conditions.

Conclusion

We have developed a novel stably transfected insect cell line able to effectively process recombinant pro-containing proteins.