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Functional Expression of Recombinant Tumstatin in Stably Transformed *Drosophila melanogaster* S2 Cells

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Objectives

The purpose of this experiment is to confirm whether the recombinant tumstatin revealed from the stably transformed *Drosophila melanogaster* S2 cells has in vitro capacity.

Materials and Methods

Materials - Cell line : *Drosophila melanogaster* Schneider 2 (S2) cells

- vector : pMT/BiP/V5-His and pCoHygro (Invitrogen)

Methods - Construction of expression plasmids, Stable transformation,

Cell culture and analysis of gene expression, Purification of recombinant tumstatin,

Bovine capillary endothelial cell proliferation assay, Western blot analysis

Results and Discussion

Recombinant tumstatin was expressed in stably transformed *Drosophila melanogaster* S2 cells and secreted into the medium with a molecular weight of 29 kDa. Recombinant endostatin was also purified to homogeneity using a simple one-step Ni^{2+} affinity fractionation. Purified recombinant tumstatin inhibited endothelial cell proliferation in a dose-dependent manner. The concentration at half-maximum inhibition (ED_{50}) for recombinant tumstatin was approximately 0.69 μg ml^{-1}. A maximum production level of 4.6 μg recombinant tumstatin $(10^7 \text{ cells})^{-1}$ was obtained in a T-flask culture of S2 cells, 7 days after induction with 0.5 mM $CuSO_4$.

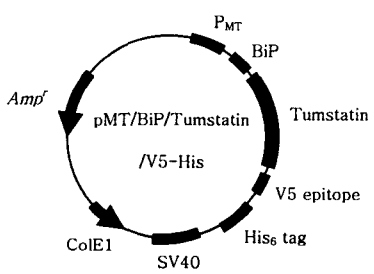

Fig.1. Schematic representation of the expression plasmid pMT/BiP/T-V5-His.

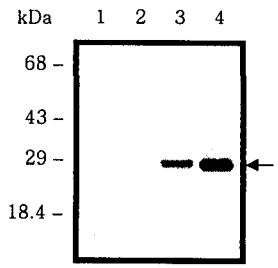

Fig.2. Western blot analysis of non-transfected and stably transformed S2 cells.

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