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Production of recombinant A254-GMCSF immunotoxin by a non-lytic insect cell expression and evaluation of its cytotoxicity by *in vitro* studies

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Background and Aims: One of the emerging therapeutic strategies for targeted treatment of most cancers is the use of immunotoxins which are fusion proteins consisted of a targeting and a toxic moieties. Until now, various expression systems have been utilized to express recombinant immunotoxins. Here we report the expression and purification of the A254-GMCSF immunotoxin by the non-viral insect cell expression system. Then, the purified protein was in evaluated for its specific and non-specific cytotoxicities.
Methods: The coding sequences of the A254-GMCSF fusion protein was cloned into the pMIB/V5-His plasmid. Then the recombinant plasmid was transfected into the Sf9 cells and stable cell lines were selected by treatment with Blasticidin S HCl. Producer colonies were subjected to the suspension cell culture and finally the expressed protein was purified. SDS-PAGE and Western blot analysis using anti His-tag and anti-GMCSF antibodies were used to confirm the purified proteins. Subsequently, specific cytotoxicity of the purified protein was evaluated on GMCSF receptor positive cells lines, i.e. HL-60 and U937, comparing to the GMCSF receptor negative Vero cell line.
Results: The fidelity of cloning was confirmed by DNA sequencing. Following transfection and antibiotic screening some stable colonies were obtained. SDS-PAGE and Western blot analysis authenticated the purified protein by showing a band of about 60 kD. Cytotoxicity assay showed specific activity of these proteins on HL-60 and U937 cell lines with IC50s ranging from 2-2.6 µg/ml.
Discussions: This study showed the ability of non-viral insect cell expression system to express the A254-GMCSF recombinant immunotoxin. The expressed protein showed a specific killing activity on the GMCSF receptor positive cells comparing to non-bearing GMCSF receptor cells. The obtained protein could be used as a therapeutic agent, following further in vitro and in vivo studies.

Keywords: Granulocyte-Macrophage Colony Stimulating Factor; Immunotoxin; Insect cell; Shiga toxin