Cloning, expression, purification and the study of immunotherapy status of TGFαL3-SEB chimeric protein in breast cancer treatment

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Abstract

Background & Aim: Bacterial superantigens, stimulate polyclonal T cells irrespective of their antigen specificity, resulting in a massive release of cytokines from T cells and monocytes, and suggest that they could be candidates of new antitumor agents. Recent attempts have been done to specifically target superantigens towards tumors. Here, we evaluate TGFαL3-SEB fusion protein as a new antitumor candidate by genetically fusing the third loop of transforming growth factor alpha (TGFαL3) to staphylococcal enterotoxin type B.

Methods: Recombinant TGFαL3-SEB sequence was constructed by fusing the N-terminal of tgfαL3 and C-terminal of seb using hydrophobic GGSGSGGG amino acid linker. In this study, gene coding for the SEB superantigen was amplified. The PCR product containing the seb gene was digested by EcoRI and HindIII and cloned in pET28a expression vector. Then the synthetic tgfα-Linker sequence was digested by BamHI and EcoRI and cloned in pET28::seb vector. The recombinant pET28::tgfaL3-seb transformed into E. coli BL21(DE3). Expression of recombinant protein was examined by SDS-PAGE and western blotting. In vitro antitumor activity against MDA-MB-468, human breast cancer cells expressing EGFR, was evaluated.

Results: Cloning of tgfαL3-seb was confirmed by colony-PCR, enzymatic digestion and sequencing. The recombinant TGFαL3-SEB fusion protein with molecular weights of 31kDa was expressed and confirmed by anti-his western-blot analysis. The TGFαL3-SEB chimeric protein exhibited potent in vitro antitumor activity.

Conclusion: Our findings indicated that TGFαL3-SEB fusion protein can be successfully constructed expressed and purified and may serve as a useful antitumor candidate for breast cancer immunotherapy.

Keywords: SEB, Staphylococcus aureus, Cloning, Immunotherapy, Transforming Growth Factor alpha, Breast Neoplasms

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