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EGF-IL-18 fusion protein as a potential anti-tumor reagent by induction of immune response and apoptosis in cancer cells

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Abstract

We report here the generation and characterization of EGF-IL-18 fusion protein as an anti-tumor reagent. The epidermal growth factor (EGF) and interleukin-18 (IL-18) fusion protein was shown to induce interferon-γ (IFNγ) expression and secretion in KG-1 cells, and to promote PBMNC proliferation. It also stimulated activation of $CD4^+$ T cells, and increased other immune responses. Moreover, EGF-IL-18 could induce significant tumor regression in SMMC-7721-xenografted Balb/c nude mice when administered together with peritumoral injection of X-ray-irradiated NK-92 cells, and this regression is associated with arresting of the tumor cells in G1 phase and induction of apoptosis.

Keywords: Epidermal growth factor (EGF); Interleukin-18 (IL-18); Fusion protein; Anti-cancer; Apoptosis

1. Introduction

Interleukin-18 (IL-18), initially known as interferon-γ inducing factor (IGIF), is a member of interleukin (IL)-1 cytokine superfamily which plays an important role in regulating immune response [1]. IL-18 is mainly produced in macrophages, and its expression has been shown to be activated by lipopolysaccharide (LPS), interferon consensus sequence-binding protein (ICSBP) and PU.1 [2]. IL-18 enhances cytotoxic activity of NK cells and proliferation of T cells, and stimulates production of IFNγ, IL-2, granulocytic–monocytic colony-stimulating factor (GM-CSF). In addition, it induces IL-8, MIP-1β, MCP-1 from peripheral blood mononucleated cells (PBMC). It is reported that IL-18 induce expression of FasL in several types of cell [3]. Due to its immune-stimulating

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effects, IL-18 has been proposed as a therapy vehicle against cancer. However, in some cases, cancers cells could escape immune recognition [4]. Thus the usage of IL-18 in cancer treatment is still under debate.

Epidermal growth factor receptor (EGFR) is a member of the tyrosine kinase family of growth factor receptors. Binding of ligands such as the EGF with EGFR could activate cell proliferation or differentiation. EGFR is highly expressed in many types of human tumor [5–8]. EGFR overexpression has been reported to be involved in carcinogenic processes, such as cell proliferation, angiogenesis and metastasis. Vaccinia virus growth factor (VGF) is a virus encoded EGF-homologous polypeptide. The third loop of VGF exhibited high affinity binding to the EGFR but low levels of mitogenic and colonogenic activities *in vitro* [9].

In the present study, a fusion protein, consisting of EGF-homologous polypeptide fused to human IL-18 mature peptide was constructed and the anti-tumor activities were analyzed. It was proposed that the targeted delivery directed by EGFR-binding capability of the fusion protein would enhance the specific anti-tumor activities of IL-18.

2. Materials and methods

2.1. Construction of the EGF-IL-18 expression vector (Fig. 1)

Human IL-18 cDNA was linked with the encoding sequence of the third loop of vaccinia virus growth factor (VGF) via a linker sequence encoding 15 amino acid residues $(G\text{-}G\text{-}G\text{-}G\text{-}S)_3$ (GenBank AF454397), as described previously [10]. Based on the gene sequence of EGF-IL-18, we designed upstream primer P1, 5′-ATGGTACCGACGACGACGACAAGCGCTGCTCCCATG-3′ (KpnI site is underlined and enterokinase recognition site is framed) and downstream primer P2, 5′-CGCCTCGAGCTAGTCTTCGTTTTGAACAGTGA-3′ (XhoI site is underlined), respectively. The EGF-IL-18 coding sequence was amplified by standard polymerase chain reaction (PCR) using primers P1, P2 and pFUS plasmid as the templates. The PCR products were purified and cloned into the KpnI/XhoI site of pET32a(+) to produce the pET32a(+)-EGF-IL-18 expression plasmid. The pET32a(+)-EGF-IL-18 vector was designed for expression of the recombinant TrxA-EGF-IL-18 protein that contained a thioredoxin of 109 amino acids (11.7 kDa), a His-tag of 6 amino acids, and a S-tag sequences of 15 amino acids upstream of target protein. The fusion tags together had a molecular mass of 13.9 kDa, and could be removed from the TrxA-EGF-IL-18 protein by protease cleavage using enterokinase.

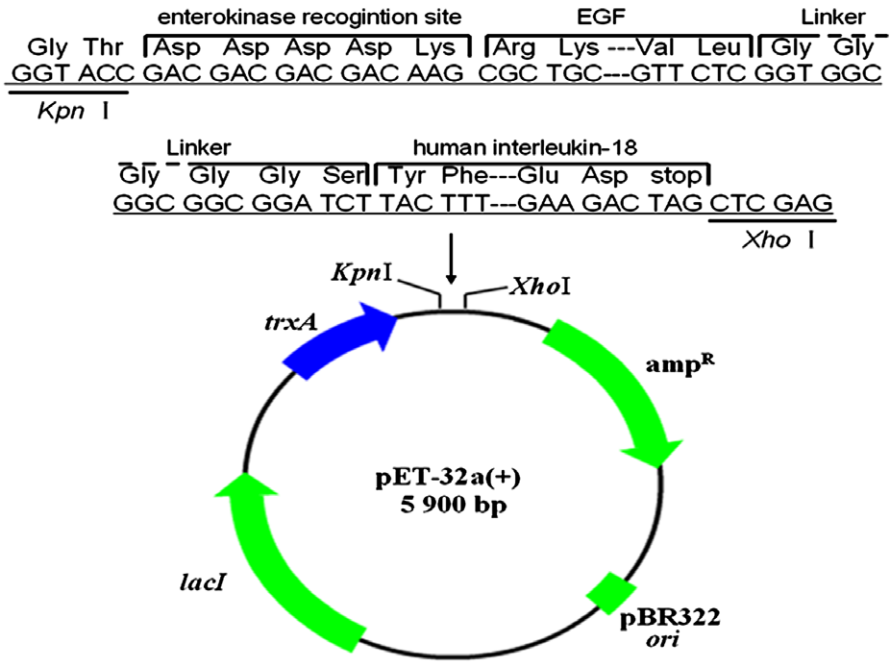

Fig. 1. Schematic representation of the recombinant expression vector for EGF-IL-18. The coding sequence of EGF-IL-18 with enterokinase recognition site was amplified by polymerase chain reaction (PCR), and the PCR products were digested with KpnI and XhoI, and then ligated to pET32a(+) to construct pET32a(+)-EGF-IL-18 expression plasmid.