Original Article

Cloning and Expression of EBI3 and p28 Subunits of Human Interleukin 27 in *E. coli*

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Abstract

Background: Interleukin (IL)-27 is a heterodimeric cytokine belonging to IL-12 and IL-23 families, secreted by antigen presenting cells(APCs). The IL-27 is composed of 2 subunits: Epstein-Barr virus induced gene 3 (EBI3) and p28. IL-27 is an anti-inflammatory cytokine which has an inhibitory effect on Th17 population and suppress the IL-17 expression. It is suggested that IL-27 could be a potent drug candidate for treating auto immune diseases.

Materials and Methods: The EBI3 and p28 subunits of human interleukin 27 were constructed into plasmid vectors; they sub-cloned into pETDuet-1 expression vector in restriction sites of *BamHI*, *SacI* and *NotI*. Subsequently the induction was carried out by 1mM IPTG and the recombinant proteins were confirmed by SDS-PAGE and Western Blot analysis using anti His-tag antibody.

Results: The EBI3 and p28 subunits of human interleukin 27were cloned into plasmid vectors. The 28 and 25kDa protein bands were observed on SDS-PAGE and finally confirmed by Western blot technique.

Conclusion: In this research p28 and EBI3 proteins were produced successfully.

Keywords: Cytokine, Cloning, Recombinant protein

Introduction

IL-27 is a heterodimer protein containing Epstein-Barr-induced gene 3 (EB13) protein and also is a member of interleukin 12 and 23 families. This cytokine is a very important factor in association with the differentiation of Th1 lymphocytes and has a close relation with enhancers of cellular immune responses and also inhibitors of Th2 humoral immunity[1] and may affect other cells such as Th17 and B lymphocytesor NK cells[2]. In comparison with other cytokines, the subunits of IL-12 and IL-27 families are non-covalently bonded; therefore, the two subunits are produced by different cells and then they bind together in the extra cellular areas[3].IL-27 is an anti-tumor cytokine and inhibits angiogenesis.There has been seen a relation between IL-27 and induction of MHC class I in TBJ cells (neurocytic tumor). Furthermore there are some researches that have showed a decrease in the number of CD8+ lymphocytes that has led to IL-27 anti-tumoral effect in colon cancer cells and glial cells[4].

Low expression of MHC I and reduced function of IL-21 receptors have substantially decreased anti-tumoral effect of IL-27 in B10F16 melanoma. Transferring of IL-27 into tumoral cells has resulted in tumoral cells with lower density and slower growth rate compared to the primary tumors[5]. IL-27 has a role in decreasing metastasis possibility with about 98 percent beside its inhibitory capacity in tumor angiogenesis[1].

IL-27 is a choice to treat autoimmune diseases. Since this is an anti-inflammatory agent and inhibits the increase of IL-17, it can be used as a therapeutic potential to treat autoimmune diseases such as Multiple Sclerosis, Rheumatoid Arthritisand Psoriasis Vulgaris. It can also inhibit and reduce the severity of EAE[2] in the mice infected with MS [6,7]. Animal based studies on CIA Arthritis model showed that IL-27 inhibits the expression of transcription essential factors[8].

IL-27 is an anti-viral agent and reduces replication of HIV-1 in CD4+ T cells, peripheral blood mononuclear cells (PBMCs) and macrophages[9]. IL-27 induces the expression of interferon type 1 and IFN regulatory genes[10]. Based on mentioned researches, recombinant IL-27 is appropriate for medical care and treatment of viruses especially AIDS[11].

Interleukin 27 like other Cytokines has the main role in inducing and limiting of inflammations[12]. It has the potential to empower early phase of immune response, inhibits Th2 and plays a role in the differentiation of Th17. The main immunosuppressive role of this interleukin, which is associated with inhibition of IL-2 and opposition to IL-6, is to suppress the progression of Th17 and to induce the production of IL-10, and to activate the expression of suppressors-of-cytokine-signaling (SOCS)[13,14].

The role of IL-27, especially in cancers, Multiple Sclerosis, and Rheumatoid Arthritisfurther the activity of IL-27 may facilitate toprove IL-27 recombinant form as a drug for these diseases.

Methods

EP repairing P28 and EBI3 Segments

Nucleotide sequence of the genes encoding *P28* (NM_145659.3), and *EBI3* (NM_005755.2) subunits

Table 1: EBI3 and P28 specific primers.

EBI3 primers	F: 5'AGATCTCAGAGCTCATGACCCCGCAGCTTCTC 3'
	R: 5'CGTGGCCGCGGCCGCCTACTTGCCCAGGCTC3'
p28 primers	F: 5'AGATCTCAGAGCTCATGACCCCGCAGCTTCTC 3'
	R: 5'ACCGGTGGAGCTCATCAGGGCTGGGGGCTC 3'

were taken from NCBI. These sequences were changed based on *E. coli* (host) amino acid codon usage and synthesized by Gene ray Biotechnology. The primers were designed (Table 1) and amplification of *EBI3* (690bp) and *p28* (759bp) genes were carried out and final fragments contain *SacI*/*NotIandSacI*/*BamHI* restriction sites in the two ends respectively.

PCR was conducted using the following ingredients
1μg plasmid, 0.2mM dNTPs, 10 mM MgCl2, 1× PCR buffer, 2 pmol of each reverse and forward primer, 1U Taq DNA polymerase (Cinagen, Iran) and water was added up to the final volume of 30μL.

The following PCR program was used
Denaturation at 94°C for 30 seconds, annealing at 68°C for 30 seconds, and extension at 72°C for 40 seconds; cycles were repeated for 30 times. At the beginning and end of the reaction, the adjusted temperatures (94 and 72°C) were fixed for 5 minutes. amplicons were detected by electrophoresis on 1.5% agarose gel and results were observed under UV light by SYBR Green.

Gene Segments Cloning into Expression Vector pET Duet-1
The PCR products, digested by the restriction enzymes. Final products were electrophoresed on agarose gel, and were recovered using a specific kit (Bioneer, Korea). Expression plasmid pET Duet-1 was digested by restriction enzymes on both sided of the inserts. The ligation of inserts into vectors was carried out by using T4 DNA Ligase (Fermentas, Lithuania)15.

The cloning of these two genes were confirmed by universal PCR and sequencing of PCR product.

Expression of Recombinant Proteins EBI3 and p28

Recombinant pET Duet-1 plasmids containing EBI3 and p28 were transformed into *E. coli* BL21 on LB agar media with ampicillin. Cells with recombinant plasmids were cultured in LB broth at 37°C and incubate overnight in 200 rpm. 1mM IPTG has been used for induction of it. After different hourssediment samples were collected. Proteins of each sample were prepared for electrophoresis on 15% SDS-PAGE gel, the non-recombinant bacteria were used as negative control. To approve the expression of proteins in the bacteria, Western Blot test was conducted using specific his-tag conjugated antibody.

Results

Amplification of EBI3 and p28 Fragments

PCR was performed by the specific primers for both EBI3 and P28 genes
(Figure 1a and b).

Cloning confirmation of genes

After sub-cloning of EBI3 and p28 genes, PCR was performed by pET-Duet1 universal primers. The PCR products were electrophoresed on agarose gel 1.5% (Figure 2).

EBI3 and P28 Expression and Approved by Western-Blotting

The expressions of proteins were induced using 1mM IPTG. The samples were electrophoresed on 12% SDS-PAGE gel and transferred to nitrocellulose membrane. Western-blot analysis by alkaline phosphatase conjugated His-Tag antibody revealed the protein bands (Figure 3).

Discussion

Studies conducted on IL-27 showed that it can affect the growth and density of tumors and also can inhibit angiogenesis[5] of early tumors and metastasis[1]. So scientists believe that IL-27 can help the treatment of tumors[8].Since IL-27 is an anti-inflammatory agent and inhibits IL-17, it can be introduced as a therapeutic agent against autoimmune diseases[7]. It can also be considered as a strong obstacle for HIV-1 replication[9]. Based on previous reports recombinant IL-27 can be used in the treatment of viral infections,
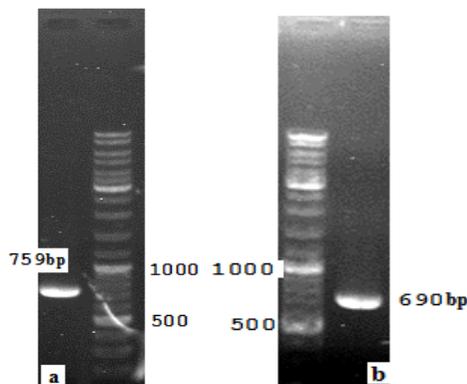
Figure 1. P28 (a) and EBI3 (b) PCR Products against 100bp DNA Ladder

Figure 2. The Confirmation of EBI3 and P28 cloning by Universal PCR

Figure 3. Western Blot analysis of EBI3 and P28

especially AIDS[11]. Considering the extensive biological properties of this valuable cytokine, and the importance of this factor in the treatment and its pharmaceutical role, and considering the results of other researches, the authors of the current study decided to produce two subunits of IL-27 in vitro using one of the recombinant protein production techniques[13]. At the present study, we used pre-synthesized genes for the experiment.

Pflanz et al. evaluate the early identification of IL-27[3]. Hu et al. also performed pig P28 subunits cloning[16].

The applied method is common for cloning and has been used in different studies[17]. Lack of disulfide bond in IL-27 allows these two subunits to be produced by different cells and be conjugated together in the extracellular space. Evaluation of pure IL-27 p28 or EBI3 biological activity in vitro showed that any one of them individually is active and does not need another one[13]. The aforementioned results confirm the separate expression of EBI3 and P28 in different vectors of the current study, since evaluating the performance and role of these subunits by separate proteins leads more useful results. To express IL-27 subunits in the *E. coli* expression system, the pET Duet-1 expression vector was used. There are different plasmid vectors to express genes in *E. coli*, including pGEX, pQE30 and pET vectors. Phage T7 promoter was used in pET system, it is a specific strong promoter and RNA polymerase of *E. coli* cannot recognize it, while promoters of pGEX and pQE are transcribed by *E. coli* RNA polymerase[18]. Expression of IL-27 subunits by pET Duet-1 vector resulted in the production of IL-27 subunits which any one of them is observed as a separate band in the competent bacteria. The results of the current study showed that this system is a suitable host to produce the recombinant IL-27 and using bacterial system (BL21DE3) is a quick and less costly method. The current study is the first report for human IL-27 gene cloning and expression in Iran.

Conclusion

In this research p28 and EBI3 recombinant proteins were produced in prokaryotic system successfully.

Acknowledgements

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