Human growth hormone produced with recombinant DNA technology

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Abstract: Hormones affect the metabolism by stimulating the genetic apparatus of the cell, activating enzymes and changing the rate of enzymatic reactions. They increase the formation of informative ribonucleic acid, which determines the structure of the protein, and affect the biosynthesis of proteins. Somatotropin hormone (STG) is a peptide hormone consisting of 192 amino acids and is secreted from the anterior lobe of the pituitary gland. The deficiency of this hormone causes hypophysis. The demand for recombinant somatotropin is likely to increase in the near future. Because current production technologies cannot meet the demand for cheap somatotropin due to limited production capacity and high production cost. As a result, it is necessary to study the mechanisms of production of therapeutic recombinant proteins. Recombinant somatotropin is mainly synthesized using *E. coli lemo* strain and is used for therapeutic purposes.

1 Introduction

Globally, the incidence of somatotropin (STG) deficiency is increasing rapidly, and it is estimated that by 2027, the number of people with hypopituitarism will increase to approximately 10 million. Somatotropin hormone is a single-chain polypeptide hormone synthesized by acidophilic somatotrophs of the pituitary gland [1]. In humans, it is encoded by the GHN gene in the gene cluster located on chromosome 17q22–24, contains 192 amino acids and two disulfide bonds, and has a molecular mass of approximately 22 kDa [2].

The use of recombinant DNA technology to produce human growth hormone (hGH) is an emerging technology today. Our Phase I study in the treatment of short stature caused by GH deficiency is genetically engineered recombinant human GH hormone. Somatotropin is now approved for clinical use in many countries around the world, and about 4,000-5,000 children are treated with rhGH every week. [3, 4].

In order to transform the gene needed in the research process into *E. coli*, the nucleotide sequence is obtained from the NCBI database and a vector construction is designed using the PTrcHis-TOPO plasmid in the Snap Gene program. To implement the target gene expression, the GHN gene sequence is selected from the NCBI database and inserted into

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the PTrcHis-TOPO plasmid. In this process, *E.coli lemo* strain was taken as an optimal option to optimize the expression. [4, 5].

It is to develop a technology for producing recombinant somatotropin (rSTH) as a result of transferring a vector plasmid into an E. coli strain using genetic engineering.

Development of recombinant somatotropin (rSTG) technology as result of transfer of vector plasmid to *E.coli* strain using gene engineering method.

2 Experimental methods

Recombinant DNA technology is a modern way to obtain recombinant microorganisms and produce more products.

We chose the -PTrcHis-TOPO plasmid for the vector because the plasmid consists of 4390 Bp nucleotide, AmpR antibiotic marker gene, tag rrnB T1 terminator, rrnG anti terminator T7 tag, lac operator, trc promoter, ori replication parts [6, 7, 8]. (Fig.1). The somatotropin (759Bp) gene was cut using the restriction enzymes EcoR1 and Hind III to insert the other gene into the vector plasmid by taking the nucleotide sequence from the NCBI database and joining the other gene using ligase enzymes. Plasmid containing another gene was transformed into a microorganism. [9]. (Fig.2). To transform the vector construct into the E.coli Lemo strain, cells were first induced to undergo chemical transformation. Using the chemical method (CaCl2) ions, the plasmid was transformed into the microorganism cell. 5 µl of transformed GHN E.coli Lemo strain sample is grown in 495 µl of LB medium overnight culture at 37°C for 14-16 hours. 1ml of the grown culture is grown in 50ml LB medium until the OD 600 reading reaches 0.7. [10, 11].

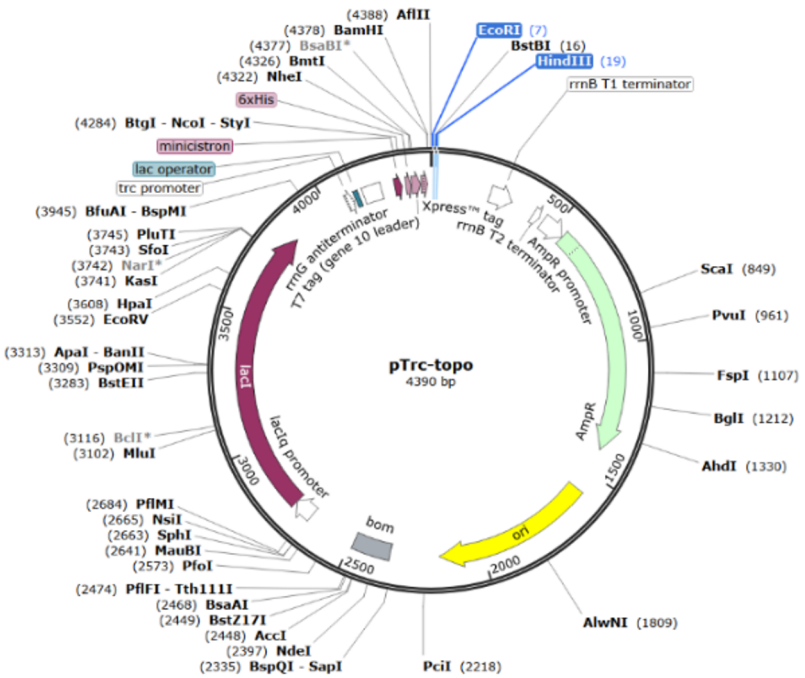

Fig. 1. Vector -PTrcHis-TOPO plasmid.

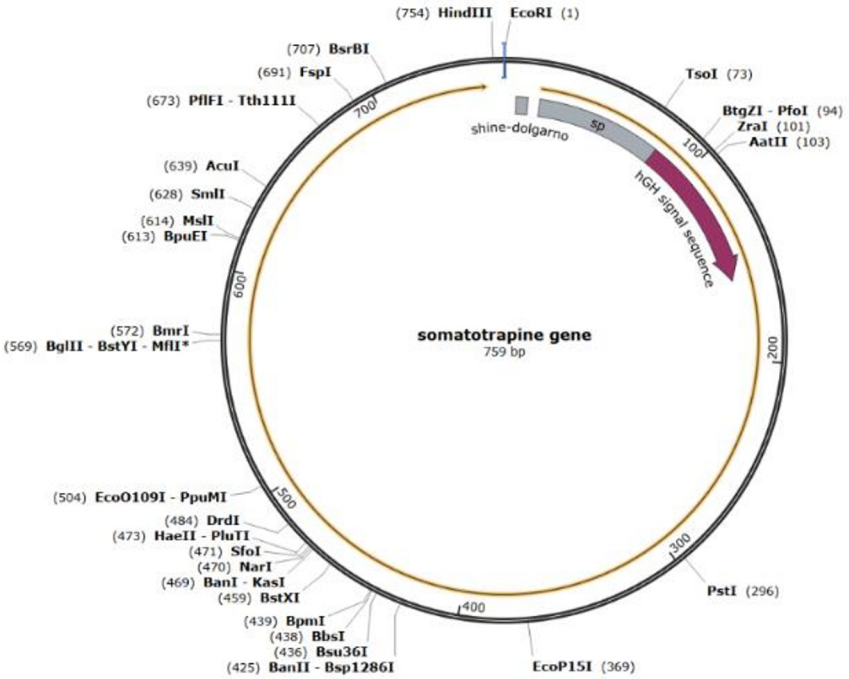

Fig. 2. An overview of the 759 base pair human somatotropin gene in vector construction.

Culture reaching an OD600 of 0.7 was induced with IPTG at a concentration of 0.5 mM. It is expressed in a thermal shaker at 220 rpm and 370C for 4 hours. To remove the protein expressed inside the cell, it is treated with 5 ml of buffer A and 4 mg/ml lysozyme, placed at room temperature for 30 minutes, then 5 ml of buffer B is added, and the cell wall is disintegrated in an ultrasonic device. After the cell wall is broken, it is centrifuged at a speed of 20,000 rpm, and the supernatant separated from the biomass is collected. Affinity chromatography method was used due to the addition of His tag (histidine protein) to PTrcHis-TOPO plasmid as a vector. In this case, a Ni (nickel) column is selected. For chromatography, the column is prepared with 50 mM Tris-HCl, 300 mM NaCl pH-8 buffer. After the sample is passed through the column, unnecessary proteins are washed out again in this buffer. 250 mM imidazole is used to extract the necessary proteins from the column. Pure recombinant somatotropin was obtained by affinity chromatography.

3 Results and discussion

Somatotropin obtained by genetic engineering (rSTG) by transferring the gene responsible for the production of human somatotropin to the *E.coli lemo* strain, a strain that synthesizes the rSTG hormone was created.

Fig.3. Fermentation and hervesting of E.coli lemo to produce hGH.

First, *E.coli lemo* strain was grown in 1 ml of LB nutrient medium with antibiotics for 14-16 hours to isolate transformed strains. The strain grown in nutrient medium supplemented with antibiotics was transferred to another 10 ml flask and grown in a thermomixer for 14 hours [12-15]. (Fig.3).

Second, to produce biomass, the microorganism was multiplied for 5 hours in a 10-liter fermenter by periodically feeding the nutrient medium, and homogenization was performed to extract biomass from the resulting culture liquid, and the cell wall was broken down with lysis buffer and centrifuged at a speed of 2000 rpm, separated from the biomass. the supernatant is collected. Affinity chromatography method was used due to the addition of His tag (histidine protein) to PTrcHis-TOPO plasmid obtained for the vector. In this case, a Ni (nickel) column is selected. For chromatography, the column is prepared with 50 mM Tris-HCl, 300 mM NaCl pH-8 buffer. After the sample is passed through the column, unnecessary proteins are washed out again in this buffer. 250 mM imidazole is used to extract the necessary proteins from the column. Pure recombinant somatotropin was obtained by affinity chromatography. [11, 12].

The recombinant hormone obtained during the research was injected into mice 0.5-3.5 µl 3 times a week for 3 months. In this case, in the 5th week, the amount of somatotropin in the blood is normalized, and the weight of the mouse is from 12 to 53 g, and the length is from 6 to 10.2 cm. The production of somatotropin obtained from animals on the basis of biosynthetic, modified, genetic engineering methods has been established, and the demand for somatotropin obtained on the basis of modification is very high. Somatotropin hormone produced by the method of genetic engineering is highly effective when used in STG deficiency. Somatotropin hormone is recommended to be given to STG in humans after the test. In this case, MRI of the bone age is performed, and if the growth cone is open, that is, STG deficiency is detected, the average age of starting treatment is in children (12-13 years old). For 3-6 months, pituitary hGH was used, the growth rate of patients was observed, and during the study period, bone age developed in parallel with chronological age. maximum in the 1st year can grow height from -4 to -6 SDS). The somatotropin hormone obtained by this technology is of high quality and relatively cheap compared to other analogues, which allows not only the domestic demand, but also the expertise of other countries.

4 Conclusion

Research results have shown that, production of biosynthetic, modified, genetically engineered somatotropin from animals has been launched, and the demand for modified somatotropin hormone is very high. Somatotropin hormone produced using the method of genetic engineering was highly effective when administered to the body in case of deficiency. It is recommended to give somatotropin hormone to STG in humans in a small amount based on the test regimen, based on the amount of hormone in the blood plasma and whether the hand growth zone is open or closed.

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