Preparation of Recombinant Bone Morphogenetic Protein 2 at Laboratory Scale

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Introduction: Millions cases of bone fracture are reported annually worldwide. The conventional methods for the repair often fail. The complications of using routine methods include stimulation of bone repair which should be performed without infection risk and immunologic responses. The aim of this study was cloning and expression of bone morphogenenic protein 2 (BMP2), a key growth factor used in bone repair. **Materials and Methods:** mRNA was extracted from human maxillary osteosarcoma and reverse transcribed. The BMP2 gene was amplified by PCR and cloned into pETDuet vector and was expressed. **Results:** The BMP2 gene was expressed successfully and expression was confirmed by western blotting using anti- His tag antibody. **Conclusion:** Recombinant protein preparation method has been recognized to be accessible and the production is cost effective.

Keywords: BMP2; Bone regeneration; Bone fracture

Introduction

Millions cases of bone fracture are reported annually in the world. The conventional methods for repair often fail which cause cost burden and stress for the society (1-3). By the improvement in the surgical techniques, fixation, and biological sciences, healing of some fractures are delayed; nonetheless, in 4% of the cases relapse occurs after treatment. There is an increase in the usage of fast dental implant for quicker recovery and prevention of alveolar bone atrophy; however, if there is no similarity between implant shape and socket, the implant could be failed (4). These cause challenges for reconstructive surgeons due to the fracture repair complexity which leads to dysfunction movement and chronic mental state in patients (5).

Conventional treatments for bone repair include using autograft, allograft, and xenograft materials. The complications of using these routine methods are stimulation of bone repair with infection risk and immunologic responses. Moreover, the problems with application of autograft includes the need for appropriate selection of recipient and donor sites, and morbidity in donor site (5).

Application of appropriate growth factors for fast bone regeneration could be an alternative solution to overcome those obstacles (6, 7). Bone morphogenetic proteins 2 (BMP2) is a growth factor which is produced in human organs with a significant role in bone repair process. BMP2 extraction from human body is quite a problematic method and has not been identified as a desirable method for patients; in addition, it is costly. However, mass production of recombinant BMP2 is accessible and comfortable for patients (8, 9). The aim of the present study was cloning and expression of BMP2 and preparation for mass production to be used for future studies.

Materials and Methods

Gene cloning

mRNA was extracted from human maxillary osteosarcoma by RNA extraction kit (Fermentas, Waltham, Massachusetts, USA, 02451) and reverse transcribed using oligo DT. BMP2 gene was amplified by specific primers based on Accession NO M22489. PCR product was detected on 1.5% agarose gel electrophoresis and cloned into pBluescript plasmid via T/A cloning method. Ligation reaction was transformed into DH5α competent cell and spread on agar plate containing 100µg/ML ampicillin and X-gal and IPTG. White bacterial colonies containing recombinant plasmid were selected.

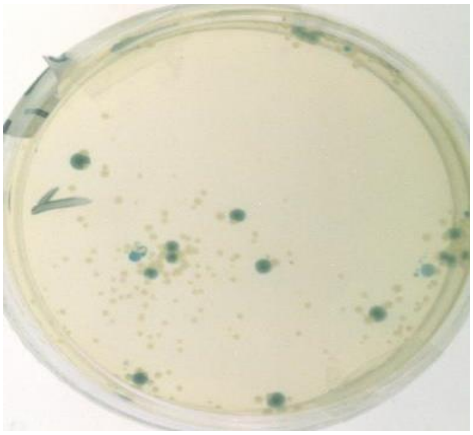

Figure 1. Bacterial plate containing white and blue colonies

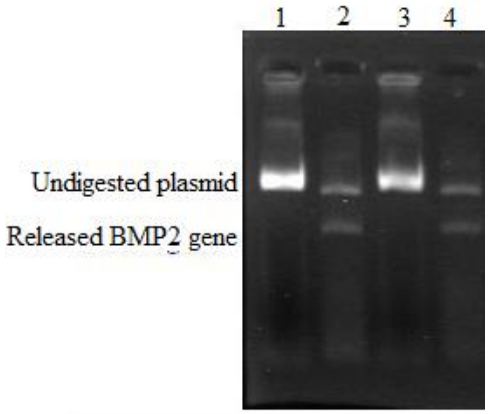

Figure 2. 0.8% agarose gel electrophoresis for digested and undigested plasmid. Lane 1 and 3 present undigested plasmids. Lane 2 and 4 present BamHI and EcoRI digested plasmid. Released BMP2 gene is shown.

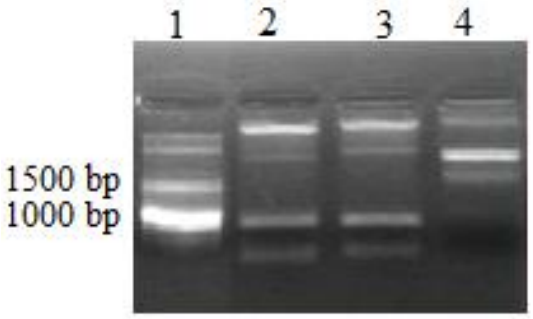

Figure 3. 2% gel electrophoresis of recombinant plasmid PCR product. Lane 1, DNA size marker. Lane 2 and 3 present PCR product for recombinant plasmid. Lane 4, PCR reaction of empty plasmid

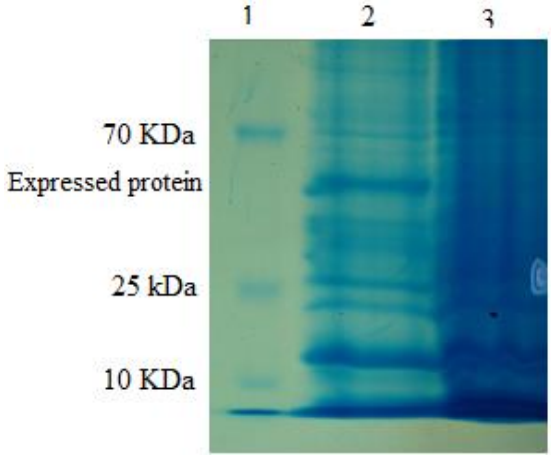

Figure 4. SDS-PAGE of recombinant plasmid. Lane 1, protein marker. Lane 2, bacterial lysate containing recombinant plasmid expressed approximately 42 KDa protein. Lane 3 bacterial lysate containing empty plasmid

Gene expression

Plasmid was extracted using alkaline lysis method (10) and it was digested by BamHI and EcoRI restriction enzymes. The released fragment (BMP2 gene) was purified, and sub-cloned into enzyme digested expression pETDuet vector. Recombinant expression plasmid was transformed into Bl21 (DE3) *E. coli* strain. Recombinant BMP2 plasmid was extracted and the results were confirmed by PCR.

E. coli which contained recombinant plasmid was cultured into YT medium (11) and incubated overnight; consequently, plasmid promoter was induced by 0.5 mM IPTG (12). Sampling was performed before induction for 4 times by one-hour interval after induction. Bacterial samples were lysed and electrophoresed on 12% SDS-PAGE along with protein marker and *E. coli* without plasmid. Expressed protein was transferred into nitrocellulose membrane and detected by anti His Tag antibody and Horse Radish Peroxidase (western blotting) (13).

Results

mRNA was extracted from human maxillary osteosarcoma and reverse transcribed by oligo dT. Then, BMP2 gene was amplified by specific primers and PCR product (BMP2 gene) was gel electrophoresed and cloned via T/A cloning method. Plasmid screening was performed by X-gal–IPTG and white colonies (Figure 1) containing recombinant plasmid were cultured; eventually,' plasmid extraction was attained.

Recombinant plasmid was digested using BamHI and EcoRI restriction enzymes, then, it gel electrophoresed (Figure 2). Released fragment (BMP2 gene) was purified and sub-cloned into expression pETDuet plasmid.

Sampling was performed before and after induction, then, it lysed, and electrophoresed. Figure 4 shows commassi brilliant blue stained expressed approximately 42 KDa protein on SDS-PAGE. Figure 5 shows detected protein by anti-His tag antibody and Horse Radish peroxidase by western blot

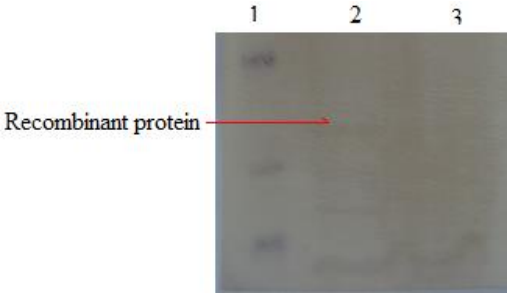

Figure 5. Western blot detected by anti His tag antibody. Lane 1, protein marker. Lane 2, bacterial lysate containing recombinant plasmid. Lane 3 bacterial lysate containing empty plasmid

Discussion

Bone morphogenetic proteins 2 (BMP2) is a growth factor produced in human organs which plays a significant role in bone repair. BMP2 has been used in bone regeneration as well (14). BMP2 protein has been demonstrated to be a stimulator for bone generation in fracture repair such as non-union fracture (15, 16).

Xiong *et al.* prepared rhBMP2 in E.coli and purified protein implanted into mesenchymal cells; in addition, they showed new cartilage and bone formation (17). Ma *et al.* expressed rhBMP2 in E. coli and proposed that rhBMP2 has biological capacity for ectopic bone formation (18). Ihm *et al.* expressed hBMP2 in soluble form in *E. coli* (19).

Conclusion

Extraction and purification of BMP2 growth factor from human blood is a complex method and its production has low efficiency, however, preparation by recombination is an easy method to be used. Human product cannot be stored for long time and has a high possibility of infection by viruses. Recombinant protein preparation method has been recognized to be accessible and the production is cost effective.

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Conflict of Interest: 'None declared'.

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