Soluble Expression and Purification of Bioactive Recombinant Human Bone Morphogenetic Protein-2 from *Escherichia coli*

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https//:doi.org/10.12982/CMUJNS.2020.0048

Received: *September2, 2019*
Revised: *November 28, 2019*
Accepted: *December 17, 2019*

ABSTRACT

Human bone morphogenetic protein-2 (hBMP-2) is a potent growth and differentiation factor for bone induction and regeneration. Recombinant hBMP-2 (rhBMP-2) was cloned and expressed as a soluble protein using E. coli-based expression system. A full-length gene encoding mature hBMP-2 was amplified by RT-PCR, cloned into an expression vector and expressed using SHuffle E. coli cells. The rhBMP-2 was successfully expressed as a soluble protein under the control of the lacUV5 and protein A promoters by IPTG induction. The rhBMP-2 fused with ZZ domain at its N-terminus was successively purified with a single step by using IgG Sepharose 6 fast flow affinity chromatography. Analysis of the purified protein on SDS-PAGE, Western blot analysis and LC-MS/MS, verified that the purified protein was rhBMP-2. The biological activity testing on hFOB 1.19 showed that rhBMP-2 had the ability to significantly induce cell proliferation in a dose dependent manner. ALP staining and activity assay also increased after rhBMP-2

treatment. The mRNA expression of the osteogenic genes by quantitative real-time PCR (qRT-PCR) showed that rhBMP-2 was able to up-regulate the gene expression of ALP, COL1, BMP-2, Runx2, and OPN. This data indicates that rhBMP-2 is functionally active to induce human osteoblast proliferation and differentiation. The production of rhBMP-2 by this developed method could be useful for bone regeneration and repair applications.

Keywords: Human bone morphogenetic protein-2, Recombinant protein, Soluble protein, Osteoblast differentiation, *Escherichia coli*

INTRODUCTION

Bone morphogenetic proteins (BMPs) are the secreted growth factors which belong to the transforming growth factor-β (TGF-β) superfamily of multifunctional cytokines. BMPs have been involved in the regulation of cell proliferation, survival, differentiation, regulation of cell-matrix interactions and the stem cell development in a wide variety of tissues including bone (Hogan, 1996; Lind et al., 1996; Massague, 1996; Nissinen et al., 1997; Balemans and Van Hul, 2002; Xiao et al., 2007; Du and Yip, 2010). BMPs are key regulatory factors in the growth and regeneration of bone and cartilage (Tuan et al., 2003; Ishikawa et al., 2007; Poon et al., 2016), and also function in the repair and remodeling of the adult skeletal system (Sellers et al., 2000). Of the BMP family, BMP-2 is the best characterized protein which has the strongest bone-inducing activity (Gao et al., 2006), that also plays an important role during bone regeneration, repairs and the induction of mesenchymal stem cells into osteocytes (Wang et al., 1990).

Native human BMP-2 is a homodimeric protein of identical monomer with a dominant beta-sheet structure and forms the cystine-knot assembly. Each monomer connected together with three intramolecular disulfide linkages and one interchain disulfide bridge to form an active dimer (Scheufler et al., 1999). The interface between the two monomers is stabilized by hydrophobic interactions and an intermolecular disulfide bond. For BMP-2 processing, a 396 amino acid precursor is proteolytically cleaved to yield the mature form of 114 amino acids (Hillger et al., 2005), and demonstrates the biological activity only in a dimeric form. The stabilization of disulfide bonds involves the protein interaction with transmembrane serine/threonine kinase receptors on osteogenic cells, leading to activate cell proliferation and differentiation of osteoblasts (Reddi, 2000). Owing to its osteoinductive capacity, BMP-2 renders a therapeutic protein for *de novo* bone formation in clinical use, which is utilized as an alternative to bone autografting during the healing of critical fractures, for spinal fusions or the treatment of bone and periodontal defects including dental implants (Gautschi et al., 2007; McKay et al., 2007; Bessa et al., 2008; Tang et al., 2009; Kimura et al., 2010; Shimono et al., 2010; Luo et al., 2012; Marques et al., 2015; Gomes-Ferreira et al., 2016; Poon et al., 2016; Herford, 2017; Gonzaga et al., 2019).