Expression, purification and in vitro biological activity from human recombinant BMP-2 produced by a novel approach

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Introduction

Bone morphogenetic proteins have promoted great biomedical interest due to their ability in inducing new bone formation when used as powerful osteoinductive components of several late-stage bone grafting products. Recombinant human bone morphogenetic protein-2 (rhBMP-2) is obtained from mammalian cell expressing systems in low amounts or from bacteria inclusion bodies after time-consuming refolding methods. Thus, there is a need to establish novel approaches for producing rhBMP-2 in high yields by simple and cheap procedures.

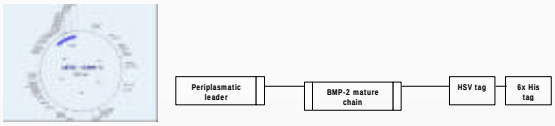


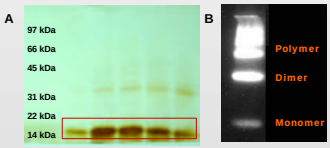
Figure 1 – pET-25b expression vector. A novel approach allows secretion of soluble protein into periplasmatic space of E. coli allowing dimer formation.

Aims of this work

- Obtaining high yields of soluble, stable and bioactive purified rhBMP-2.
- Evaluate the cytotoxicity and bioactivity of rhBMP-2 in stem cells and C2C12 cell line.

Results

Solubilization and purification of rhBMP-2



Yields of purified rhBMP-2:
110mg/ L fermentation
Fermentor conditions

Figure 2 – A) Silver stained reduced SDS-PAGE reveals purification growth factor to up 95%. B) Monomer, dimer and polymer detected by western-blot in non-reduced conditions.

Bioactivity in mesenchymal stem cells

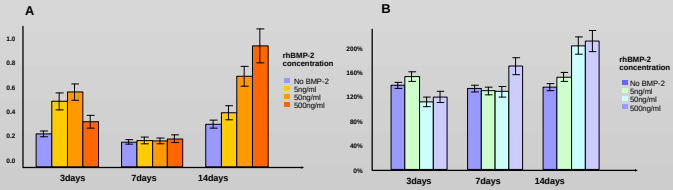


Figure 3 – A) Alkaline phosphate (ALP) bioassay revealed increased levels of this late-marker of osteoblast differentiation after addition of rhBMP-2 to fat-derived human mesenchymal stem cells.
B) Tetrazolium salt (MTS) bioassay revealed no significant cytotoxicity of purified rhBMP-2 in fat-derived human mesenchymal stem cells.

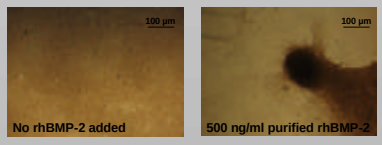


Figure 4 – von Kossa bioassay revealed evidence of bone mineralizing nodules in rat bone marrow mesenchymal stem cells after two weeks of 500 ng/ml stimulation with purified rhBMP-2. Magnif. 400x

Bioactivity in C2C12 cells

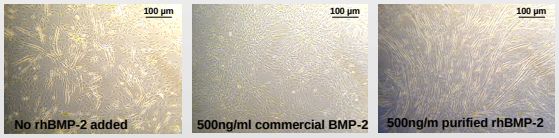


Figure 5 – Morphology of C2C12 cells after 5 days of addition of rhBMP-2 to the cell culture. A magnification of 400x was used.

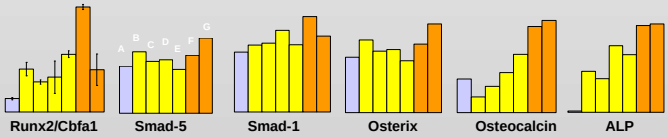


Figure 6 – Semi-quantitative RT-PCR shows an increase in early markers of osteogenic differentiation (Runx2/Cbfa1, Smad-5 and Smad-1) after 5 days of cell culture with our BMP-2. A) negative control, B) 500ng/ml, C) 1000ng/ml, D) 2000ng/ml, E) 5000ng/ml, F) 500ng/ml commercial BMP-2, G) 1000ng/ml commercial BMP-2.

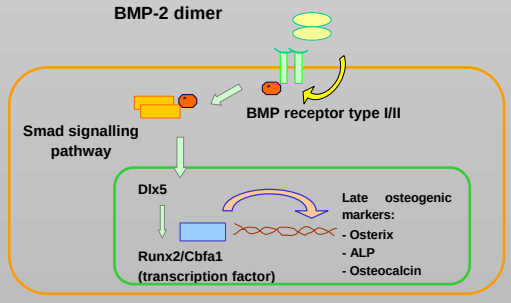


Figure 7 – BMP-2 signalling pathway which triggers differentiation of mesenchymal stem cells into osteoblasts.

Conclusions

- Recombinant human BMP-2 has been produced in monomer, dimer and polymer fractions and purified up to 95% purity.
- Absence of cytotoxicity was revealed by MTS bioassay. Evidence of bioactivity was shown in both mesenchymal stem cells and C2C12 cells by ALP bioassays and semi-quantitative RT- PCR.
- The novel approach described herein shows to be a promising way for obtaining large amounts of partially purified rhBMP-2 that may be use in several future bone biomedical applications.

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