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

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INTRODUCTION

Bone tissue engineering has been an increasing field of research during the last years. The ideal approach for a regenerative application would consist in the use of cells from the patient, scaffolding materials and differentiation growth factors. **Bone morphogenetic protein-2** (BMP-2) is one such growth factors with a strong ability to induce new bone and cartilage formation and has been used as a powerful osteoinductive component of several late-stage tissue engineering products for bone grafting. In this work, we aimed at obtaining high yields of human recombinant BMP-2 in a stable, pure and biologically active form by use of a new bacteria expression system that circumvents the disadvantages of conventional recombinant protein preparation methods and to perform a study of the stability conditions and functionality of these peptides *in vitro* in human mesenchymal stem cells and C2C12 murine cell line.

MATERIALS & METHODS

rhBMP-2 was cloned in **pET-25b** vector, expressed in fermentor under controlled conditions in BL21DE3 *E. coli* strain, purified by affinity chromatography and size exclusion chromatography and tested in mesenchymal stem cells and C2C12 cell line.

Fig. 1. The sequence coding for mature rhBMP-2 was cloned in a pET-25b vector containing a periplasmatic secretion signal and a histidine tag for affinity chromatography purification.

Fig. 2. Expression of recombinant bacteria was performed in a **fermentor** allowing large yields of rhBMP-2, around 110mg/L.

RESULTS & DISCUSSION

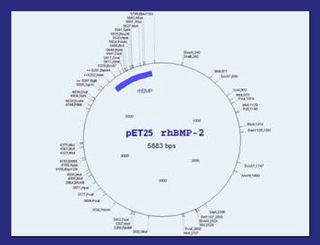

Fig. 3. pET-25b/BMP-2 vector

This novel approach allows secretion of soluble protein into periplasmatic space of *E. coli* permitting *in loco* dimer formation.

Production and purification of rhBMP-2

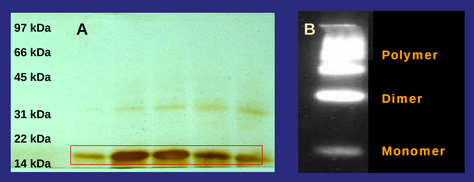

Fig. 4. A) Silver stained reduced SDS-PAGE reveals purification growth factor to up 95%. B) Non-reduced western-blot permitted observe monomer, dimer and polymer fractions.

Biological activity assays in C2C12

Morphology of C2C12

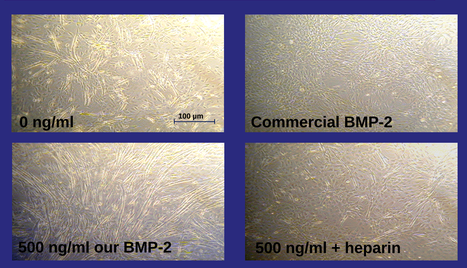

Fig. 5. Effect of rhBMP-2 added to C2C12 after 5 days of cell culture. Changes in morphology are observed but not similar to positive control.

BMP signalling pathway

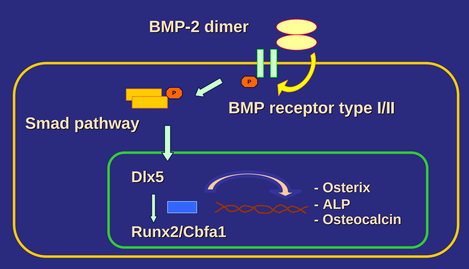

Fig. 6. BMP-2 human signalling pathway which triggers differentiation of MSCs into osteoblasts.

RT-PCR expression of osteogenic markers in C2C12

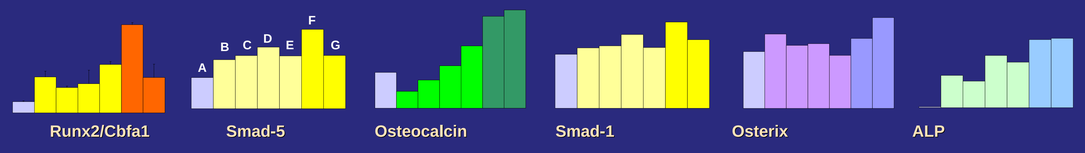

Fig. 7. RT-PCR shows increase in markers of osteogenic differentiation such as Runx2/Cbfa1, Smad-5 and Osteocalcin after 5 days of cell culture with our BMP-2. A) control, B) 500ng/ml, C) 1000ng/ml, D) 2000ng/ml, E) 5000ng/ml, F) 500ng/ml commercial BMP-2, G) 1000ng/ml commercial BMP-2

Biological activity assays in mesenchymal stem cells

ALP bioassay in human adipose MSCs

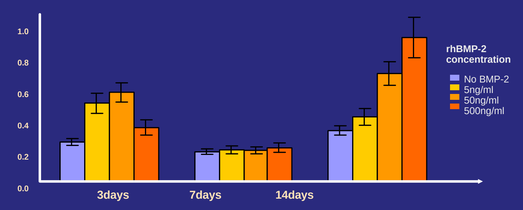

Fig. 8. ALP bioassay revealed an increase in ALP levels with continuous purified 5-500ng/ml rhBMP-2 stimulation. 3, 7 and 14 days of cell culture.

von Kossa in rat bone marrow MSCs

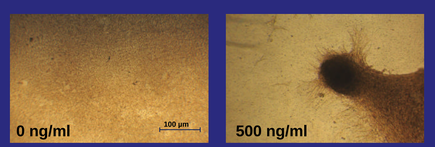

Fig. 10. von Kossa bioassay revealed evidence of bone nodule formation in bone marrow MSCs after stimulation with 500ng/ml purified rhBMP-2. 14 days of cell culture.

Morphology of human MSCs

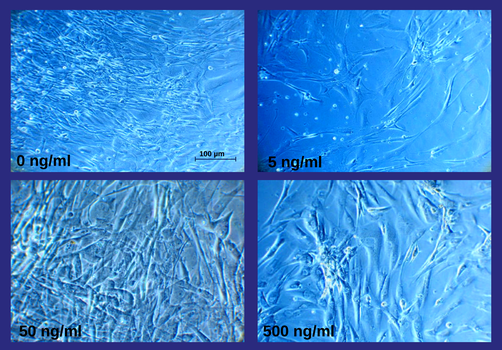

Fig. 9. Addition of 5-500ng/ml rhBMP-2 to human adipose mesenchymal stem cells resulted in changes of morphology. 10 days of cell culture.

MTS cytotoxicity bioassay

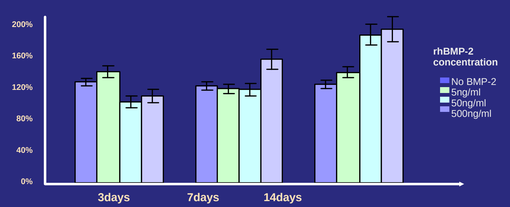

Fig. 11. MTS bioassay revealed no significant cytotoxicity of purified rhBMP-2 at 5-500ng/ml. 3, 7 and 14 days of cell culture.

CONCLUSIONS

➢ The novel approach described herein shows to be a promising way for obtaining significant amounts of partially purified rhBMP-2 for use in future bone tissue engineering applications.

ACKNOWLEDGMENTS

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