Expression, Purification and Bioactivity of a Recombinant Human BMP-7 Expressed in 293T Cells

Halcsik, E.[1], Bassi, Ê. J.[1], Bustos-Valenzuela, J. C.[1], Granjeiro, J. M.[2], Sogayar, M. C.[1]

[1] NUCEL – Núcleo de Terapia Celular e Molecular, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil.

[2] Departamento de Biologia Celular e Molecular, Universidade Federal Fluminense, Niterói, RJ, Brazil.

Bone morphogenetic protein-7 (BMP-7) is a multifunctional secreted growth factor of the TGF-β superfamily which is predominantly known for its osteoinductive properties, though it has also been implicated as playing a role in embryonic development. The mature protein consists of a 34-38 kDa disulfide linked homodimer. Recombinant human BMP-7 (rhBMP-7) induces bone formation *in vivo* and increased osteoblasts cell proliferation and collagen synthesis *in vitro*. Moreover, clinical efficacy of rhBMP-7 has been demonstrated for treatment of orthopedic injuries through topical application. In this study, the full-length sequence of hBMP-7 was cloned, expressed and purified by heparin affinity chromatography from the conditioned medium of 293T cells transfected with a lentiviral vector (pCMV-hBMP7-IRES-GFP). The protein was recognized by mouse monoclonal and rabbit polyclonal anti-BMP7 antibodies, with a band of apparent molecular mass of 34-38 kDa being detected by Western blotting under non-reducing conditions, corresponding to the mature protein dimer form. The purified rhBMP-7 displayed osteoinductive properties at 800 ng/ml, demonstrated by alkaline phosphatase activity and morphological alterations of murine pre-myoblastic and pre-osteoblastic C2C12 cells upon osteogenic differentiation. These results, along with the good yield obtained, encourage further studies on signaling pathways related with the early steps of osteoblastogenesis stimulated by this growth factor. We are also carrying out studies on the ability of our purified rhBMP-7 to induce ectopic bone formation *in vivo* for future applications in bone Tissue Engineering.