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Communication

[*Comunicação*]

Expression of human *bone morphogenetic protein (BMP-2* and *BMP-4)* genes in transgenic bovine fibroblasts

[*Expressão dos genes* bone morphogenetic protein *(BMP-2 e BMP-4) em fibroblastos bovinos transgênicos*]

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Losses of bone tissues occur due to several factors, most importantly due to accidents. The bone tissue has an uncommon capacity to regenerate with the complete substitution of the harmed tissue by *de novo* formed tissue. However, there is a physiological limit in which the organism is capable to accomplish the task of self-reconstruction without surgical intervention. When the bone loss is larger than this critical limit, a surgical intervention is necessary in order to achieve tissue reparation. With the development of internal rigid fixation systems (use of metallic reconstruction plates and screws), the opened fracture reductions reached a satisfactory level. Nevertheless, the results achieved with the use of these techniques are still uncertain in cases of large tissue destruction in which the losses do not allow a correct juxtaposition of the bone fragments. In order to try to solve these limitations, surgeons have been searching and testing several types of therapeutic approaches to obtain aesthetic and functional restorations of the harmed anatomical area. Among the alternatives, the most used are autografts (bone transferred from one site to another in the same individual), alografts (bone transferred from one individual to another), xenografts (bone transferred from one specie to another) and aloplastics (synthetic materials). Although the autografts are successfully used, they have some disadvantages, such as the limited amount of bone tissue that can be obtained and necessity of a surgery in the donor area. Consequently, there is considerable interest in developing novel alternatives to *de novo* regenerate bone tissue. With the increase of the knowledge of the genetic osteogenic factors and genetic engineering, genetic therapy is becoming a viable alternative to obtain a satisfactory result in bone regeneration.

The *in vivo* repair of bone tissue is controlled by the temporary expression of growth factors genes (Bolander, 1992; Sandberg et al., 1993; Linkhart et al., 1996; Bostrom et al., 1998), trigged by the presence bone morphogenetic proteins (BMPs). BMP can be produced in animal cells in culture or in transgenic animals. Transfected bovine fibroblasts lines can be used as a source of donor nucleus to generate transgenic animals through nuclear transfer into enucleated bovine oocyte. These transgenic animals can further produce proteins with pharmaceutical relevance (Houdebine, 2000).

It was isolated the human bone morphogenetic proteins cDNAs (*BMP-2* and *BMP-4*) that were positioned under control of a mammalian constitutive promoter. The vectors were used to transfect bovine fibroblasts and express the *BMP-2* and *BMP-4* genes.

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Total RNAs were isolated from bone tissue collected from patients with facial trauma (jaw fractures between the 7th and 10th day pos-trauma). All samples were harvested to get the best fracture reduction. All surgical procedures were carried out in the Unidade de Cirurgia e Traumatologia Buco-Maxilo-Facial, Hospital de Base de Brasília in accordance with the ethical standards of the institutional committee and the Helsinki Declaration.

RNA was isolated using Trizol[1] and the cDNA synthesis was conducted with the Superscript II kit[1] according to the manufacturer's instructions.

The BMP genes were amplified by PCR using the primer pairs BMP2forw (gtgcttcttagacggactgc) / BMP2rev (gtactagcgacacccacaac) to amplify the 1,233 bp *BMP-2* gene, and BMP4forw (agccattccgtagtgccatc) / BMP4rev (aaggactgcctgatctcagc) to amplify the 1,373bp *BMP-4* gene. Each PCR reaction was carried out in a 25μl mixture containing 60mM Tris-SO_4 (pH 8.9), 18mM ammonium sulfate, 2mM $MgSO_4$, 200μM of each dNTP, 200nM of each primer, 1U of *Taq* Platinum high fidelity polymerase[1]. The mixture was overlaid with mineral oil, denatured for 2min at 94°C in a MJ Research thermal cycler[2] and amplified for 30 cycles (94°C for 15s, 55°C for 30s, 68°C for 2min). The products were run on a 1% agarose gel, stained with ethidium bromide and visualized with UV light. The amplified DNA sequences were cloned into the pGEM-T easy vector[3] and sequenced. The genes were then cloned in the *Not*I restriction site from the pCMV-β vetor, replacing the β-galactosidase gene[4], under control of the cytomegalovirus (CMV) promoter for expression in mammalian cells to generate the vectors pCMV-BMP2 and pCMV-BMP4.

Bovine fibroblasts were transfected with the vectors pCMV-BMP2 and pCMV-BMP4 using the LipofectAmine Plus[1] according to the manufacturer's instructions. In order to detect *BMP-2* and *BMP-4* genes expression (mRNA) in the transgenic fibroblasts cells, RT-PCR was carried out. Total RNA isolation and PCR were conducted as previously described. Fig. 1 shows the RT-PCR amplification of the 1,233 bp (*BMP-2*) and 1,373 bp (*BMP-4*) DNA sequences in 3 independent fibroblasts lines. RT-PCR with total RNA without cDNA synthesis revealed no amplification attesting no contaminations with DNA (Fig. 1, lane 6). RT-PCR analyses with non-transfected fibroblasts lines (negative control) revealed no DNA amplification (Fig. 1, lane 5). Western blot analysis, carried out as described (Ramoshebi et al., 1999; Viñals et al., 2002) using polyclonal antibodies[5] revealed that both BMP-2 and BMP-4 protein is produced in the transfected fibroblasts (Fig. 2).

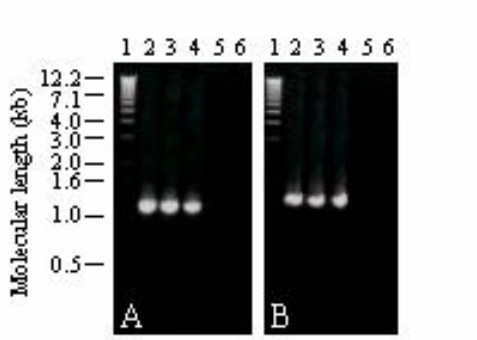

Figure 1. RT-PCR analysis of bovine fibroblasts cells transfected with the vector pCMV-BMP2 (A) and pCMV-BMP4 (B). Lane 1: 1 kb Ladder (Invitrogen); lanes 2-4: transfected fibroblasts cell lines; lane 5: non-transfected fibroblasts lines (negative control); lane 6: total RNA without cDNA synthesis.

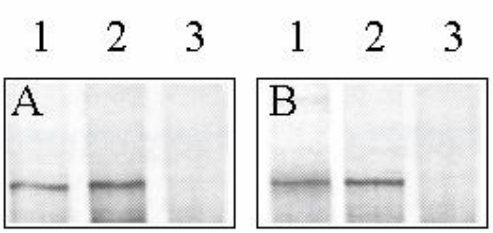

Figure 2. Western blot analysis showing the presence of BMP-2 (A) and BMP-4 (B) protein in transfected bovine fibroblasts. Lanes 1 and 2 (A and B): transfected fibroblasts; lane 3 (A and B): non-transfected fibroblast line.

[1] Invitrogen, Carlsbad, CA, USA.
[2] Waltham, MA, USA.
[3] Promega, Madison, WI, USA.
[4] Clontec, Palo Alto, CA, USA.
[5] Creative BioMolecules, Hopkinton, MA, USA.

In this report it was able to express human *BMP-2* and *BMP-4* genes in bovine transgenic fibroblasts. It suggested that the BMPs could be produced in cell in culture. In addition, the BMP genes can be introduced and expressed *in vivo* (Rech et al., 1996), in order to trigger the *de novo* osteogenesis process.

Keywords: BMP, gene expression, fibroblast, oral and maxilofacial surgery, tissue reconstruction

RESUMO

cDNAs dos genes bone morphogenetic protein-2 (BMP-2) e bone morphogenetic protein-4 (BMP-4) *foram sintetizados a partir de RNA total extraído de tecidos ósseos de pacientes que apresentavam trauma facial (fraturas do maxilar entre o 7º e o 10º dia pós-trauma) e clonados num vetor para expressão em células mamíferas, sob controle do promotor de citomegalovírus (CMV). Os vetores contendo os genes* BMP-2 *e o* BMP-4 *foram utilizados para a transfecção de fibroblastos bovinos. mRNAs foram indiretamente detectados por RT-PCR nas células transfectadas. As proteínas BMP-2 e BMP-4 foram detectadas mediante análises de Western blot. Os resultados demonstram a possibilidade de produção desses fatores de crescimento celular em fibroblastos bovinos. Essas células poderão ser utilizadas como fontes doadoras de material genético para a técnica de transferência nuclear na geração de animais transgênicos.*

Palavras-chave: BMP, expressão gênica, fibroblasto, cirurgia oral e maxilofacial, reconstrução de tecidos.

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