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Short Report

Engineering recombinant growth factors: a tool for *in vitro* mesenchymal cell commitment

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ABSTRACT Growth factors (GFs) are part of a large number of polypeptides that transmit signals affecting cellular activities. Cells may communicate with each other through direct molecular interactions involving their cell membranes, as a result of the movement of certain molecules as GFs. They are produced by the cells and can act within the cell or in vicinal or remote cells to modulate their activities by reacting with specific receptors through either autocrine, paracrine, endocrine, juxtacrine, intracrine or extracellular matrix mediated. In the last recent years, our group have been working on genetically engineering GFs which incorporate a collagen-binding domain derived from the von Willebrand Factor (vWF). These fused proteins exhibit functional properties that do not exist in nature and have allow us to investigate their potential important implications in terms of developing strategic biomatrices for tissue repair.

Tissue regeneration is a complex process that consolidates the efforts of a variety of cells which are already present or recruited at the site of injury (Nimni, 1997). The sequence of events is guided by a series of GFs, cytokines, cell-cell and cell-matrix interactions. Therefore, inductive signals, responding stem cells and the extracellular matrix are the three key ingredientes for tissue repair (Reddi, 2000). Some carriers are effective in releasing GFs at the required rate and do not interfere with the normal process of tissue repair (Hecht *et al.,* 1999) whereas others do. In general, only a few approaches provide practical modalities for delivery in a clinical setting (Groeneveld and Burger, 2000). We will focus on collagen and hydroxyapatite matrices, which have been used in our laboratory, and have received high acceptance both in experimental animal models and in ongoing clinical trials.

Since fibroblast growth factor (FGF) is a pleotropic agent which can stimulate, inhibit, and modulate cellular events in a time- and concentration-dependent manner, it is important to control its delivery and bioavailability to advance its use as a potential therapeutic agent. Basic FGF (FGF-2) is a potent *in vitro* mitogen for capillary endothelial cells, stimulates angiogenesis *in vivo*, and participates in tissue repair. We have performed the genetic engineering of a recombinant human FGF-2 (rhFGF-2) fusion protein which, by virtue of an auxiliary vWF-derived collagen-binding domain (rhFGF-2-F2), exhibits a high affinity for collagen matrices (Fig. 1). rhFGF-2-F2 protein can be expressed at high levels in bacteria transformed with plasmids bearing the T7 polymerase system, BL21 (DE3) strain. In the presence of 1 mM IPTG, the levels of protein expression show a representative induction of the 16 kDa rhFGF-2-F1 (commercial) polypeptide (Fig. 2, lane 3) and 17 kDa for the rhFGF-2-F2 construct bearing the auxiliary vWF-derived decapeptide (Fig. 2, lane 6). After stringent washing with urea the purified proteins were finally eluted (Fig. 2, lanes 4 and 7). We also demonstrated the practical utility of rhFGF-2-F2 in the management of wound healing, since a local application of rhFGF-2-F2 mixed with type I collagen sponge into cutaneous wounds was sufficient to accelerate wound closure in normal and diabetic rats (Andrades *et al.,* 2001).

Homeostasis of bone tissue results from the right balance among it cell components. Tight metabolic regulation of osteoblasts, osteocytes and osteoclasts activity gives rise to a synthesis/resorption equilibrium of bone matrix, resulting in the bony architecture maintenance. Specific GFs have the ability to stimulate stem cells along the chondrogenic and osteogenic pathways. The differentiation and maturation of osteoprogenitor cells into osteoblasts and these into osteocytes are thought to be modulated by transforming growth factors-beta (TGF-ß1 and TGF-ß2), and TGF-ß-related bone morphogenetic proteins (BMPs). In order to capture and expand a population of cells with chondro-osteogenic potential, we have used type I collagen to develop an *in vitro* culture system for bone marrow (BM) mesenchymal progenitor cells, which includes a mesenchymal cell compatible collagen-gel impregnated with a rhTGF-ß1-collagen binding domain (rhTGF-ß1-F2). As mentioned before, such a structure bestows on the factor the ability to bind to type I collagen specifically. The results suggest that rhTGF-ß1-F2 applied to a bovine collagen matrix as vehicle and delivery system could be of advantage in promoting the *in vitro* survival, proliferation, differentiation, and colony mineralization of the osteogenic precursor cell population. Differences between rhTGF-ß1-F2 and the commercial rhTGF-ß1 may be due to the slow release of the collagen binding GF from the collagen fibrillar network to which it is bound, resulting in a longer half-life and its better availability to the target cells (Andrades *et al.,* 1999). When the *in vitro* treated cells where placed into inactivated demineralized bone matrix (iDBM) implants or diffusion chambers and implanted subdermically on the back of old rats for 28 days, they were able to form large amount of cartilage and osteoid matrix (diffusion chambers) and bone tissue (iDBM implants) (Figs. 3, 4).

BMPs (BMP-2 and BMP-7) have been also tested under that experimental system. Because of it sensitivity to endogenous proteases, BMPs have to be either protected or delivered at a continuous rate at the site of action, if they are going to initiate and sustain the cascade of events that lead endochondral or intramembranous bone formation (Bax *et al.,* 1999). Although BMPs can induce bone formation when added as a solution, the dose needed to induce endochondral bone formation can be greatly reduced when BMPs are combined with an appropriate carrier (Andrades and

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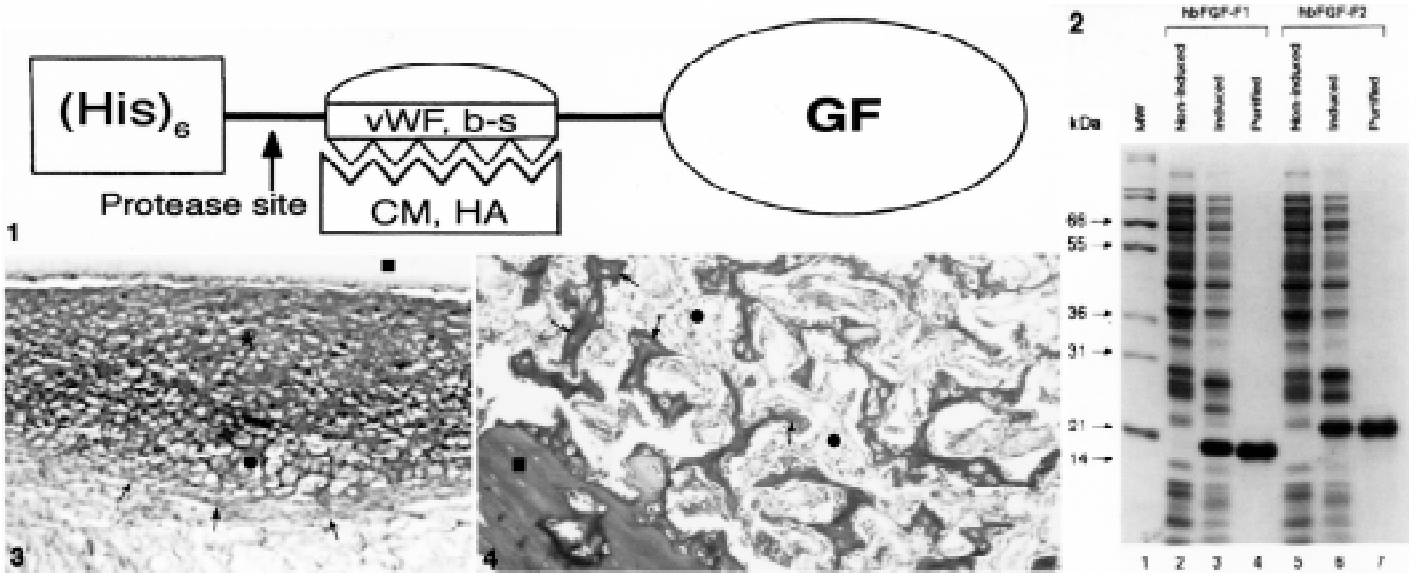

Fig. 1. A GF fusion protein *containing a collagen binding domain from von Willebrand Factor (vWF), or bone-sialoprotein (b-s) sequence, with high-affinity for collagen matrices (CM) or hydroxyapatite (HA), respectively.*

Fig. 2. Expresion and purification of the rhFGFs. *See text for explanation.*

Fig. 3. Paraffin sagittal section of a diffusion chamber containing an osteoid tissue (asterisk) close to the wall of the implant. *A more immature area appears at the opposite site (dot) surrounded by a fibrous, perichondrium-like layer (arrows). Chamber wall, upper side. Picrosirius-hematoxylin staining.*

Fig. 4. *Paraffin sagittal section of an iDBM implant containing areas of bone-like tissue showing trabecular structures (arrows), surrounded by a soft tissue with the appearance of bone marrow (dots). Implant wall, bottom left corner. Picrosirius-hematoxylin staining.*

Becerra, 2001). This study evaluates the efficiency of rhBMP-2 or rhBMP-7 to select, amplify and induce a skeletogenic cell population from rat BM cultured in a collagen gel trap device. Cell number and the expression of alkaline phosphatase (ALP), osteocalcin (OC) content and deposition of calcium, all well-known markers of osteoblastic differentiation, were measured. The results show that both BMPs are mitogenic in this system. The formation of cell colonies and their mineralization is a characteristics of a certain differentiated state. The increase in expression of ALP and OC in cultures treated with the rhBMP indicates that osteogenic induction has occurred, as has been claimed in other systems (Andrades *et al.,* 2001). Therefore, on the basis of the differentiation criteria, it can be assumed that both BMPs selects *in vitro* a progenitor cell population from BM, which can be directed along the osteogenic lineage under these experimental conditions. Our laboratory is currently working in the addition of collagen and hydroxyapatite domains to some members of the BMP family as BMP-2, -4, -6, and -7, implied in osteogenesis and bone repair.

Targeted delivery of GFs fusion proteins, with mitogenic and cell inductive potentials, combined with bioactive histoinductive-conductive matrix, as well as enhanced matrix binding properties, can be efficiently used in the clinical management of wound healing at sites of tissue repair or remodelling.

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