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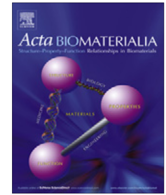

Osteostatin improves the osteogenic activity of fibroblast growth factor-2 immobilized in Si-doped hydroxyapatite in osteoblastic cells

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ABSTRACT

Si-doped hydroxyapatite (Si-HA) is a suitable ceramic for the controlled release of agents to improve bone repair. We recently showed that parathyroid hormone-related protein (PTHrP) (107–111) (osteostatin) has remarkable osteogenic features in various in vitro and in vivo systems. Fibroblast growth factor (FGF)-2 modulates osteoblastic function and induces angiogenesis, and can promote osteoblast adhesion and proliferation after immobilization on Si-HA. In the present study we examined whether osteostatin might improve the biological efficacy of FGF-2-coated Si-HA in osteoblastic MC3T3-E1 cells in vitro. We found that Si-HA/FGF-2 in the presence or absence of osteostatin (100 nM) similarly increased cell growth (by about 50%). However, addition of the latter peptide to Si-HA/FGF-2 significantly enhanced gene expression of Runx2, osteocalcin, vascular endothelial growth factor (VEGF) and the VEGF receptors 1 and 2, without significantly affecting that of FGF receptors in these cells. Moreover, secreted VEGF in the MC3T3-E1 cell conditioned medium, which induced the proliferation of pig endothelial-like cells, was also enhanced by these combined factors. The synergistic action of osteostatin and Si-HA/FGF-2 on the VEGF system was abrogated by a mitogen-activated protein kinase inhibitor (U0126) and by the calcium antagonist verapamil. This action was related to an enhancement of alkaline phosphatase activity and matrix mineralization in MC3T3-E1 cells, and also in primary human osteoblastic cells. These in vitro data show that osteostatin increases the osteogenic efficacy of a Si-HA/FGF-2 biomaterial by a mechanism involving mitogen-activated protein kinases and intracellular Ca^{2+}. These findings provide an attractive strategy for bone tissue engineering.

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1. Introduction

Si-doped hydroxyapatite (Si-HA) has biocompatibility, bioactivity and osteoconductivity properties, thus it has been proposed as a suitable matrix for the controlled release of biological agents to improve bone repair following fracture and other skeletal injuries [1]. The observed beneficial effects of Si substitution in this ceramic involve several passive and active mechanisms which have been critically reviewed by Bohner [2]. In addition, osteointegration of this type of material can be further improved by incorporation of growth factors which stimulate the repair mechanisms and thus functional restoration of the damaged tissue [3].

Recent findings indicate that parathyroid hormone-related protein (PTHrP) may affect bone formation and bone remodeling through distinct structural domains [4]. In this regard, the native C-terminal PTHrP (107–139) fragment has been shown to be a strong bone resorption inhibitor [5], but can also stimulate osteoblastic function both in vitro and in vivo [6–10]. The bioactivity of this fragment in bone appears to reside into its N-terminal domain, namely the pentapeptide known as osteostatin [6,11–14]. Of interest, osteostatin itself might be generated from PTHrP (107–139) upon proteolytic processing by the product of the PHEX gene (phosphate-regulating gene with homologies to endopeptidases on the X chromosome), which is abundant in osteoblasts [15]. Supporting the osteogenic action of this pentapeptide, we recently showed that its loading onto Si-based ceramics (including Si-HA) made these materials efficient in inducing cell growth and cell differentiation in

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osteoblastic MC3T3-E1 cell cultures [13,16]. Interestingly, this bioactivity in vitro was similarly observed with osteostatin coated onto Si-HA scaffolds by either adsorption, and thus deliverable, or covalent immobilization, leaving free its N-terminus [16]. Moreover, osteostatin coating conferred osteoinduction properties to Si-based ordered SBA-15 mesoporous materials when implanted in a cavitary defect in the femoral epiphysis of healthy rabbits [17].

Fibroblast growth factors (FGFs) are a group of proteins which control the proliferation and differentiation of various cell types, including osteoblasts [18]. FGF-2 increases osteoblast proliferation and can also modulate osteoblast differentiation [19–22]. In this regard, we recently demonstrated that low doses ($\leqslant 1$ ng ml^{-1}) of FGF-2 immobilized on Si-HA improved adhesion and proliferation of osteoblastic SaoS-2 osteosarcoma cells [23]. In addition, FGFs are strong inducers of angiogenesis [24]. This is of particular interest, since neovascularization is crucial to meet the oxygen and nutrient demands of cells involved in bone regeneration [25]. Hence, enhancement of bone growth by FGF-2 might be a result of increased osteoblastic growth and improved vascularization. Thus immobilization of FGF-2 on biomaterials would represent a promising approach for bone tissue engineering [26,27].

FGF-2 binding to specific tyrosine kinase receptors activates multiple signal transduction pathways involving mitogen-activated protein kinase (MAPK), phosphatidylinsositol-3 kinase (PI3K)-Akt and intracellular Ca^{+2}/protein kinase C in target cells, including osteoblasts [19,28]. In addition, both native PTHrP (107-139) and osteostatin have previously been shown to affect these signalling pathways in osteoblastic cells, related to various osteogenic effects [7,8,12]. Although the putative osteostatin receptor is presently unknown, these findings suggest that both FGF-2 and osteostatin appear to interact, at least in part, with common pathways in osteoblastic cells to promote bone formation.

In the present study, using the well-characterized non-transformed osteoblastic cell line MC3T3-E1, we extended our initial observations in an osteoblastic osteosarcoma cell line to confirm that FGF-2 maintains its osteogenic activity after immobilization on Si-HA. In addition, we aimed to evaluate whether addition of osteostatin would increase the activity of this biomaterial.

2. Materials and methods

2.1. FGF-2 immobilization on Si-HA

Si-HA with the nominal formula $Ca_{10}(PO_4)_{5.7}(SiO_4)_{0.3}(OH)_{1.7}\square_{0.3}$, where $\square$ represents vacancies at the hydroxyl position, was prepared by aqueous precipitation reaction of $Ca(NO_3)_2{\cdot}4H_2O$, $(NH_4)_2HPO_4$ and $Si(CH_3CH_2O)_4$ solutions, as previously described [29]. The precipitated Si-HA powder was treated at 700 °C to remove nitrates without altering the material structure, and elemental chemical analysis was carried out by fluorescence X-ray spectrometry. The particle size distribution of Si-HA powder in aqueous suspension showed a bimodal distribution centered at 10 and 50 μm [23,29].

The human full length FGF-2 (155 residues) used in this study was synthesized and purified by Dr. R.M. Lozano (Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Cientificas,

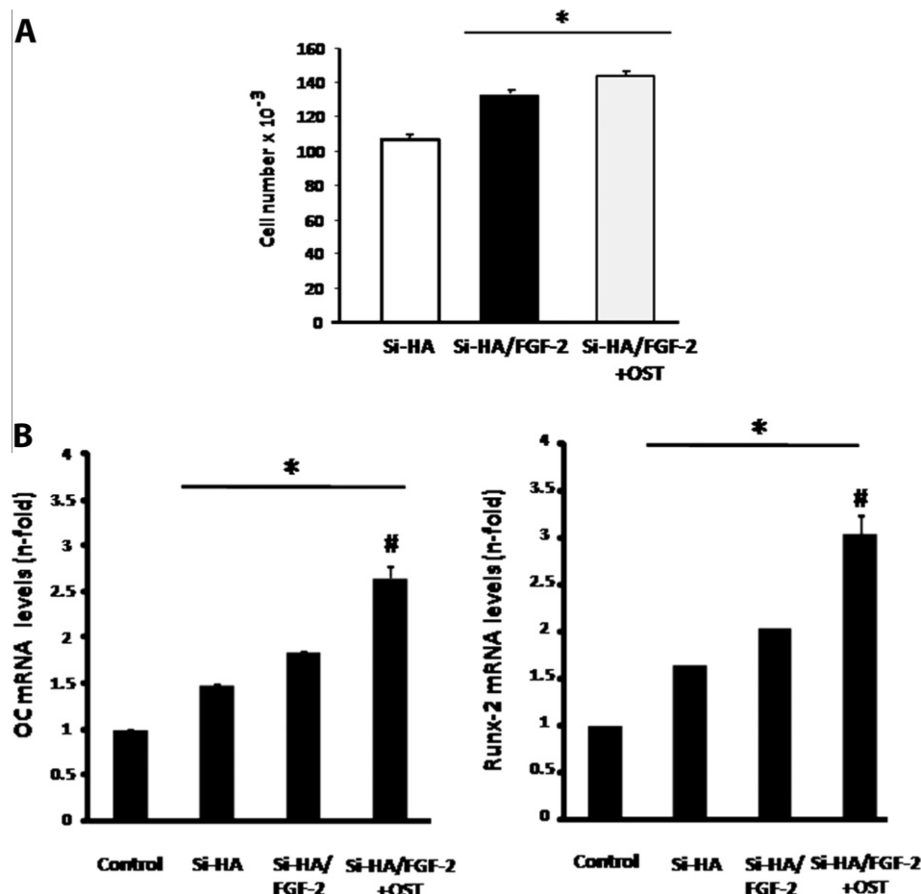

Fig. 1. (A) MC3T3-E1 cell proliferation in the presence of Si-HA, with or without immobilized FGF-2 alone or combined with 100 nM exogenous osteostatin (OST) after 4 days culture. (B) Changes in gene expression levels (assessed by real time PCR) of OC and Runx2 induced by Si-HA, with or without immobilized FGF-2 alone or combined with 100 nM exogenous osteostatin (OST) on day 4. Results are means ± SEM of at least three determinations in triplicate. $^{*}P < 0.05$ vs. Si-HA or control; $^{\#}P < 0.05$ vs. Si-HA/FGF-2.

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