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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.

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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