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Short Communication

Cystic fibrosis transmembrane conductance regulator (CFTR) regulates the production of osteoprotegerin (OPG) and prostaglandin (PG) E_2 in human bone☆

Louis Le Heron [a], Christine Guillaume [a], Frédéric Velard [a], Julien Braux [a], Lhousseine Touqui [b], Sandra Moriceau [c], Isabelle Sermet-Gaudelus [c], Dominique Laurent-Maquin [a], Jacky Jacquot [a,*]

[a] *Inserm UMR-S 926, IFR 53, Faculté de Médecine, Reims, France*
[b] *Inserm U874, Institut Pasteur, Paris, France*
[c] *CRCM, Inserm U845, Université René Descartes, Hôpital Necker-Enfants Malades, Paris, France*

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Abstract

Bone loss is an important clinical issue in patients with cystic fibrosis (CF). Whether the cystic fibrosis transmembrane conductance regulator (CFTR) plays a direct role in bone cell function is yet unknown. In this study, we provide evidence that inhibition of CFTR-Cl^- channel function results in a significant decrease of osteoprotegerin (OPG) secretion accompanied with a concomitant increase of prostaglandin (PG) E_2 secretion of primary human osteoblast cultures ($n=5$). Our data therefore suggest that in bone cells of CF patients, the loss of CFTR activity may result in an increased inflammation-driven bone resorption (through both the reduced OPG and increased PGE_2 production), and thus might contribute to the early bone loss reported in young children with CF.

Keywords: Cystic fibrosis; CFTR function; Osteoprotegerin; Prostaglandin E_2; Osteoblasts; Bone mass

1. Introduction

Low bone mass is common in patients with cystic fibrosis (CF) and has been termed *CF-related bone disease* [1]. The clinical manifestations of CF-related bone disease include an increased risk of fracture and kyphosis with the potential consequence of an accelerated decline in lung function [2–4]. The loss of cystic fibrosis transmembrane conductance regulator (CFTR) functional activity in lung tissue is associated with dysregulated NF-$_k$B-driven pro-inflammatory genes [5–7] and progressive lung destruction leading to patient's death [8]. An association between systemic inflammation during lung infective exacerbations and increased bone resorption has been demonstrated in CF patients [9]. The mechanisms for early bone loss and fractures in CF patients are multifactorial and are likely due to several CF-related factors that also influence bone metabolism [1,3,10]. These include the malabsorption of vitamin D, poor nutritional status, hypogonadism, delayed pubertal maturation, inactivity, and the frequent use of glucocorticoid therapy. Another potential mechanism is that the chronic pulmonary inflammation associated with CF leads to elevated levels of circulating cytokines, which in turn promote bone resorption and suppress bone formation [9,11].

The maintenance of bone mass requires an exquisite balance between the bone-forming osteoblasts and the bone-resorbing osteoclasts that is regulated by osteotropic factors, inflammatory cytokines, and prostaglandins [3,12]. Whether CFTR functional activity plays a direct role in bone mineralization is unknown, but this has been hypothesised on the basis of animal studies with the CFTR-null mouse [13,14]. A recent work has demonstrated

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* Corresponding author. Inserm UMR-S 926, Faculty of Medicine, IFR 53, 1, rue du Maréchal Juin, 51100, Reims, France. Tel.: +33 3 26 91 82 91; fax: +33 3 26 91 86 43.
E-mail address: jacky.jacquot@inserm.fr (J. Jacquot).

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osteopenia and sex-related differences in bone formation in CF mouse model [15]. Smaller bones with decreased density was found in CF mice, and more particularly in females, despite the absence of difference in osteoblast and osteoclast surfaces, suggesting that CFTR influences bone cell activity rather than number. In human bone cells the expression of CFTR protein has been reported by immunohistological observations [16]. Thus, a dysfunction of CFTR-Cl^- channel activity in bone cells might result in a low bone mineral density that we found in young children with CF, independently of their nutritional status and the severity of lung disease [17].

The extent of bone remodeling is mainly driven by the cellular interactions of osteoblasts and osteoclasts. These interactions are mediated by receptor activator of nuclear factor-$_k$B ligand (RANK) on the osteoclast surface and by a balance in RANK ligand (RANKL) and osteoprotegerin (OPG) production by osteoblasts, i.e., the RANKL/OPG/RANK system [18]. OPG, a member of the tumor necrosis factor receptor superfamily, acts as a soluble receptor inhibiting osteoclast differentiation and resorption by binding to and neutralizing RANKL. Prostaglandin (PG) E_2, a major eicosanoid product of the COX-2-catalyzed reaction in osteoblasts, is reported to induce osteoclast differentiation and bone erosion at sites of inflammation [19,20]. Interestingly, reports show that the suppression of OPG expression by PGE_2 is crucially involved in lipopolysaccharide-induced osteoclast formation [20,21]. In murine bone models, OPG is produced by osteoblasts when PGE_2 synthesis is inhibited [22,23]. To date, whether CFTR activity in human osteoblasts is a determinant factor in bone metabolism is yet unknown, and more particularly in the context of CF bone disease. The aim of this study was to assess the impact of CFTR-Cl^- channel conductance on the basal and TNF-α-stimulated production of OPG, RANKL and PGE_2 by human normal osteoblast in primary cultures.

2. Methods

We used a model of primary human osteoblast culture previously established in our laboratory from freshly trabecular bone fragments obtained from 45 to 75-year-old female donors undergoing orthopedic surgery (Professor Dehoux, CHU, Reims). Bone samples ($n=5$) were obtained with informed patients consent after approval by the local research ethics committee. Primary human osteoblast cell cultures (used at the second–third passage and after confluence within 6–8 weeks) were validated according to the main specific markers of osteoblastic phenotype: osteocalcin, alkaline phosphatase, bone sialoprotein and type I collagen [24]. The CFTR mRNA expression of human osteoblasts in primary culture compared to positive control human bronchial cells (16HBE14- cell line) was analyzed by RT-PCR; PCR products were separated by 1% agarose gel electrophoresis and visualized with ethidium bromide with an ImageMaster® VDS (Pharmacia Biotech).

3. Results

By RT-PCR analysis, we confirmed the expression of CFTR mRNA in primary human osteoblasts (Fig. 1A, lane 2) compared to positive control normal human bronchial cells (16HBE14-cell line, Fig. 1A, lane 1) in culture. Immunofluorescence of human osteoblasts in culture (Fig. 1B) revealed the location of CFTR protein in the cytoplasm using the mouse IgG2A anti-human CFTR (C-terminus specific) monoclonal antibody (clone 24-1 from R&D Systems, Abington, UK).

To provide now evidence of the CFTR functional activity in the changes of bone cell physiology, we first incubated human osteoblast cultures with 10 μM of CFTR-$_{inh}$172 (a selective inhibitor of CFTR-Cl^- channel conductance) added every 24 h for 72 h. Then, for the following 5-h period, we analyzed the levels of basal and stimulated (TNF-α, 20 ng/ml) release of OPG, RANKL, PGE_2 and chemokine IL-8 (by using specific ELISAs) in human osteoblast cultures compared to control human osteoblasts (cell cultures treated with vehicle alone).

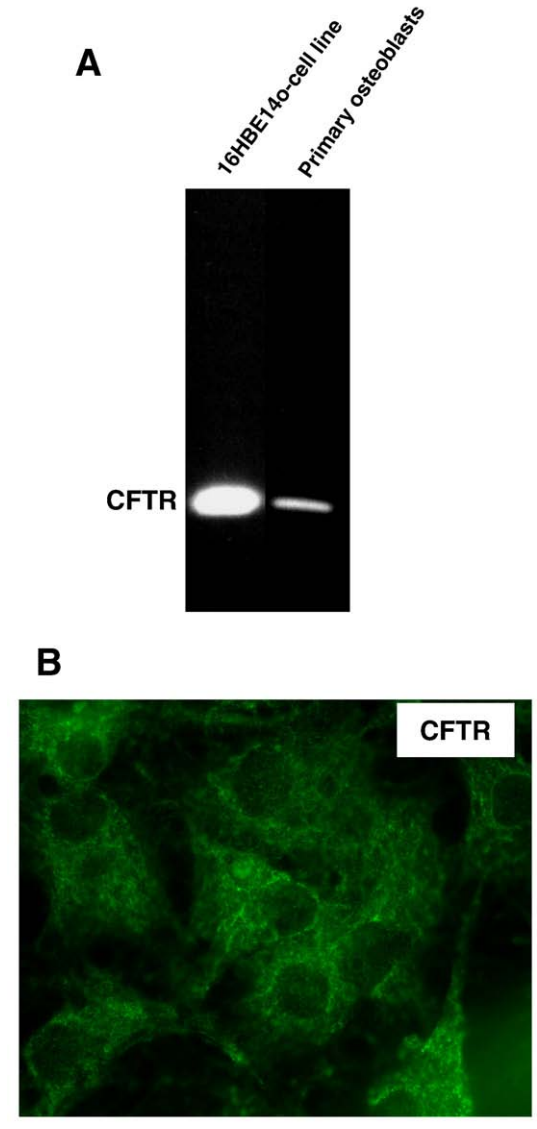

Fig. 1. Panel A: CFTR mRNA expression of human osteoblasts in primary culture (lane 2) compared to positive control human bronchial cells (16HBE14-cell line, lane 1) analyzed by RT-PCR; PCR products were separated by 1% agarose gel electrophoresis and visualized with ethidium bromide with an ImageMaster® VDS (Pharmacia Biotech). Panel B: CFTR protein immunofluorescence in normal human osteoblasts (passage 3, 7 week-culture).

Supernatants of resting and TNF-α-stimulated osteoblast cultures ($n=5$) treated with CFTR-$_{inh}$172 significantly released less OPG (up to a 3.5-fold decrease) than untreated control osteoblast cultures (Fig. 2A). Under TNF-α stimulation we observed that treatment of osteoblasts with CFTR-$_{inh}$172 resulted in a lower OPG secretion (124.1 +/− 32.8 pg/ml/10^6 cells, $n=5$) in comparison with untreated osteoblasts (434.6 +/− 58.1 pg/ml/10^6 cells, $n=5$). Interestingly, the decreased OPG release by CFTR-$_{inh}$172-treated osteoblasts was accompanied with a concomitant increase (up to a 2.6-fold increase) of PGE_2 secretion (Fig. 2B). Under TNF-α stimulation, we observed that treatment of osteoblasts with CFTR-$_{inh}$172 resulted in a strong increase of PGE_2 secretion (373.4 +/− 51.3 pg/ml/10^6 cells, $n=5$) compared to untreated osteoblasts (132.1 +/− 37.2 pg/ml/10^6 cells, $n=5$). The effect of CFTR-$_{inh}$172-mediated increase of PGE_2 release was fully reversible (after a 24-h period) upon CFTR-$_{inh}$172 removal from osteoblast cultures. The PGE_2 secretion was thus returned to a value similar to that observed in untreated osteoblasts (data not shown). Undetectable levels of RANKL (detection limit < 0.1 pg/ml) were observed in supernatants from both the CFTR-$_{inh}$172-treated and untreated osteoblast cultures (data not shown). Further, we found a large amount of IL-8 released by osteoblast cultures (171.7 +/− 19.3 and 2467.6 +/− 110.6 pg/ml/10^6 cells, $n=5$, in basal and TNF-α-stimulated production for 5 h, respectively) which was enhanced (a 20% increase) by the CFTR-$_{inh}$172 treatment, both before and after TNF-α challenge.

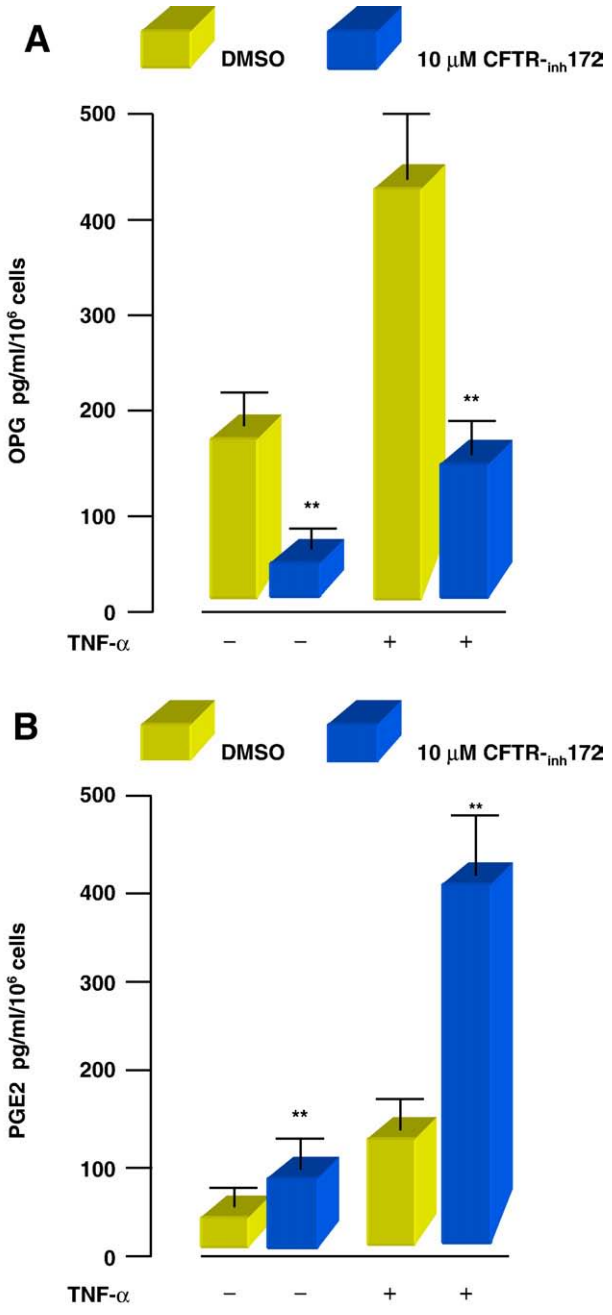

Fig. 2. Panels A and B: Osteoprotegerin (OPG) and prostaglandin E_2 (PGE_2) secretion (pg/ml/10^6 cells) by cultured human osteoblasts and maintained for a 5-h period in the absence (−) and presence (+) of TNF-α (20 ng/ml) following a 72-h pretreatment of 10 μM of CFTR-$_{inh}$172, respectively. ** Significantly different ($p<0.05$) from control (DMSO). Results are the mean and S.E. of five independent experiments (realized in triplicate).

4. Discussion

The present findings show for the first time the contribution of CFTR-Cl^- channel activity in the regulation of two key regulators of bone resorption, i.e., the OPG, which inhibits osteoclastogenesis and PGE_2, a well known inducer of inflammatory bone loss. Our data suggest that in bone cells of CF patients, the loss of CFTR activity may result in an increased inflammation-driven bone resorption (through both the reduced OPG and increased PGE_2 release), and thus might contribute to the low bone mineral density found in young children with CF, independently of their nutritional status and the severity of lung disease [10,17]. Further studies are now required to characterize the function of normal and defective CFTR in the signaling of bone mineralization and formation–resorption to develop new therapies for CF bone disease.

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