Biochemical and Biophysical Research Communications 427 (2012) 737–742

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Fibroblast growth factor 2 inhibits up-regulation of bone morphogenic proteins and their receptors during osteoblastic differentiation of human mesenchymal stem cells

Emmanuel Biver [a,b,c,1,*], Anne-Sophie Soubrier [a,b,1], Cyril Thouverey [c], Bernard Cortet [a,b], Odile Broux [a], Joseph Caverzasio [c], Pierre Hardouin [a]

[a] Physiopathology of Inflammatory Bone Diseases, EA 4490, University Lille North of France, Quai Masset, Bassin Napoléon, BP120, 62327 Boulogne sur Mer, France
[b] Department of Rheumatology, Lille University Hospital, Roger Salengro Hospital, 59037 Lille cedex, France
[c] Service of Bone Diseases, Department of Internal Medicine Specialties, University Hospital of Geneva, CH-1211 Geneva 14, Switzerland

ARTICLE INFO

Article history:
Received 23 September 2012
Available online 5 October 2012

Keyword:
Human mesenchymal stem cells
Bone morphogenic proteins
Fibroblast growth factor 2

ABSTRACT

Understanding the interactions between growth factors and bone morphogenic proteins (BMPs) signaling remains a crucial issue to optimize the use of human mesenchymal stem cells (HMSCs) and BMPs in therapeutic perspectives and bone tissue engineering. BMPs are potent inducers of osteoblastic differentiation. They exert their actions via BMP receptors (BMPR), including BMPR1A, BMPR1B and BMPR2. Fibroblast growth factor 2 (FGF2) is expressed by cells of the osteoblastic lineage, increases their proliferation and is secreted during the healing process of fractures or in surgery bone sites. We hypothesized that FGF2 might influence HMSC osteoblastic differentiation by modulating expressions of BMPs and their receptors. BMP2, BMP4, BMPR1A and mainly BMPR1B expressions were up-regulated during this differentiation. FGF2 inhibited HMSCs osteoblastic differentiation and the up-regulation of BMPs and BMPR. This effect was prevented by inhibiting the ERK or JNK mitogen-activated protein kinases which are known to be activated by FGF2. These data provide a mechanism explaining the inhibitory effect of FGF2 on osteoblastic differentiation of HMSCs. These crosstalks between growth and osteogenic factors should be considered in the use of recombinant BMPs in therapeutic purpose of fracture repair or skeletal bioengineering.

1. Introduction

Human mesenchymal stem cells (HMSCs) derived from bone marrow are multipotent cells [1]. They can proliferate and differentiate into osteoblasts, chondrocytes, adipocytes, or myocytes under different conditions or in response to various physiological and pathological stimuli, including growth factors, bone morphogenetic proteins (BMPs), cytokines, and hormones [2]. They are involved in the process of bone repair, especially fractures, whose effectiveness depends both on their capacity to proliferate and then to differentiate, in a time-dependent and coordinated manner. They are subjects of great interest in the field of bone research, in particular in skeletal tissue engineering [3].

BMPs belong to the transforming growth factor beta (TGFβ) superfamily and are potent inducers of osteoblastic differentiation and bone formation [4]. They are synthesized by osteoprogenitors and play a crucial role in embryogenesis, skeleton formation [5] and also in mature skeletal homeostasis [6]. They are involved in the differentiation of mesenchymal stem cells into osteoblasts [7], adipocytes [8] and chondrocytes [9]. Recombinant BMP2 and BMP7 are currently used in orthopedic and spinal surgery to improve bone healing, with variable efficacy profiles [10]. BMPs exert their actions via transmembrane receptors, the BMP receptors (BMPRs), including type 1 (BMPR1A and 1B) and type 2 receptors (BMPR2) [11], to target genes involved in osteoblastic differentiation [12].

HMSCs express BMP2, 4, 6 and 7 [13,14] and also BMPRs [15] to respond to these osteogenic signals, thus allowing their differentiation toward the osteoblastic lineage. Expressions of BMPs and BMPR by HMSCs vary during their osteogenic differentiation [16]. For instance, BMPR1B expression increases in parallel with the increase in osteoblastic markers such as Runx2 or alkaline phosphatase during osteoblast differentiation [17].

Bone bio-engineering strategies face two related challenging issues: on one hand, to stimulate the proliferation of stem cells

Abbreviations: BMPs, bone morphogenic proteins; TGF-β, transforming growth factor beta; BMPR-IA/IB/II, bone morphogenic proteins receptor-IA/IB/II; ERK, extracellular signal-regulated kinases; JNK, c-Jun amino-terminal kinases.

* Corresponding author at: Department of Rheumatology, Lille University Hospital, Roger Salengro Hospital, 59037 Lille cedex, France. Fax: +33 320446120.
E-mail address: ebiver@yahoo.fr (E. Biver).

[1] Equal work participation.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.09.129

and osteoprogenitors with growth factors to obtain an adequate tissue volume; on the other hand, to promote their differentiation into osteoblasts by osteogenic factors such as BMPs to obtain a bone tissue. Understanding the crosstalk between signaling by growth factors and BMPs remains an important issue to optimize the use of BMPs and HMSCs in therapeutic perspectives and bone bioengineering.

Growth factors such as platelet-derived growth factors (PDGFs) or fibroblast growth factors (FGFs) are secreted during the healing process of fractures or in surgery bone sites [18]. They were identified as regulators of HMSC differentiation. FGFs belong to a family of 22 members identified in humans and regulate cells proliferation, differentiation and migration [19]. FGF1 and FGF2 are the most abundant in adult tissues. FGFs bind tyrosine-kinase receptors, FGF receptors 1–4, activating various signaling pathways, in particular mitogen-activated protein kinase (MAPK) ERK and JNK. FGF2 is expressed by osteoblasts, osteocytes and osteoprogenitors, activates their proliferation [20] and is an important regulator of bone and cartilage cells [21]. Disruption of FGF2 or its receptors is associated with severe skeletal defects [22,23]. The role of FGF2 in osteoblastic differentiation depends on cells type and maturation stage, and its concentration: a stimulating effect on osteoprogenitors commitment and early differentiation; an inhibitory effect on terminal osteoblastic differentiation and mineralization [24]. Some data suggest that FGF2 might modulate the response to BMPs [25]. Signaling pathways activated by tyrosine-kinase receptors, especially MAPKs, are likely to interact with BMP response of bone cells [26].

Our working hypothesis was that FGF2 modulates the expression of BMPs and their receptors during HMSC osteoblastic differentiation. Having documented that BMPs and BMPRs expression is up-regulated during osteoblastic differentiation, and that FGF2 inhibits this effect in HMSCs, we aimed to study the molecular mechanism by which FGF2 inhibits BMP and BMPR expressions. We found that FGF2 prevents this cellular response via activation of MAPK ERK and JNK.

2. Materials and methods

2.1. Cell cultures

HMSC were obtained from two healthy donors (HMSC1 and HMSC2, Lonza, Verviers, Belgium). Cells were seeded at 10000 cells/cm^2 in expansion medium composed of Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS), 1% penicillin/streptomycin and 1% glutamine (Dutscher, Brumath, France). Cell cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO2, and the media were changed every 2–3 days. Osteoblastic differentiation experiments started when HMSCs reached confluence (day 0). To induce osteogenesis, cells were grown in DMEM medium supplemented with 50 μM ascorbic acid, 10 mM β-glycerophosphate, 10 nM dexamethasone and 10^{-8} M vitamin D3 (Sigma–Aldrich, France). To test the effect of FGF2 (human basic Fibroblast Growth Factor, New England BioLabs, Cell Signaling Technology, MA, USA) on osteoblastic differentiation, FCS concentration was decreased to 5% in the osteogenic medium. To test the effect of MAPK inhibitors (U0126 and SP600125, Sigma–Aldrich, France) or their vehicles, cells were pre-incubated for 30 min prior to adding FGF2. Cell proliferation was determined by cell counting (Coulter counter).

2.2. Alkaline phosphatase activity assay

Alkaline phosphatase (ALP) activity was assessed using *P*-nitrophenyl phosphate as a chromogenic substrate by the method of Lowry, as previously reported [27].

2.3. Matrix mineralization detection

Calcium deposition was analyzed by Alizarin Red-S staining (Sigma–Aldrich) and quantified by extracting the alizarin red stain with 100 mM cetylpyridinium chloride (Sigma–Aldrich).

2.4. RNA extraction, RT-PCR and real-time PCR

Total RNA was extracted using Extract-All reagent (Eurobio, Les Ulis, France), according to manufacturer's instructions. Reverse transcription and polymerase chain reaction (PCR) were performed as previously described [28]. Samples were loaded and run through agarose gels. Those last ones were scanned and analyzed by densitometry using ImageJ software (National Institutes of Health) to calculate relative expressions. Obtained expression level of a gene of interest was normalized with (Glyceraldehyde 3-phosphate dehydrogenase) GAPDH expression level in the same sample. Real-time PCR was performed using a Light Cycler system (Roche Diagnostics), according to manufacturer's instructions as previously described [28]. Primers were obtained from Tib MolBiol (Berlin, Germany), the sequences and PCR conditions for each cDNA are listed in Table 1. The house keeping gene GAPDH was used to normalize transcription levels. Relative quantification analyses were performed using RelQuant 1.01 Software (Roche Diagnostics).

2.5. Statistical analyses

All statistical analyzes were performed using STATVIEW® software (SAS Institute, NC, USA). Results were expressed as the mean ± SEM. Comparative studies of means were performed using one-way analysis of variance with a significance value of $p < 0.05$. Gene expressions were compared using the Mann–Whitney test.

3. Results

3.1. FGF2 inhibits osteoblastic differentiation of HMSCs

We first investigated the effect of FGF2 on HMSCs proliferation and osteoblastic differentiation. FGF-2 increased cells proliferation (Fig. 1A) while alkaline phosphatase activity was dramatically decreased by FGF2 (Fig. 1B). Mineralization at 18 days of differentiation (Fig. 1C) was evaluated after various FGF2 treatment duration from baseline (0 to 14 first days of osteogenic medium). As the amount of mineral results from both cell number and the degree of osteogenic differentiation, we analyzed in parallel the number of HMSC at day 18 and the amount of mineral accumulated in the extracellular matrix to correct alizarin red staining by the proliferation ratio compared with non treated HMSC (0 day) (Fig. 1D). We found a treatment-duration dependant inhibitory effect of FGF2 on matrix mineralization, indicating that promoting cell proliferation by FGF2 at the early time of the osteoblastic differentiation did not result in higher absolute mineralization, probably because of a counterbalancing effect on osteogenic differentiation. Taken together, these data indicate that FGF2 has an inhibitory effect on HMSC osteoblastic differentiation.

3.2. FGF2 prevents up-regulation of BMPs and BMPRs during HMSC osteogenic differentiation

As BMPs are among the most potent osteogenic factors, we wondered whether BMPs and BMPRs are modulated in differentiating HMSCs and if FGF2 interferes with this process. We hypothesized that the inhibitory effect of FGF2 on osteoblastic differentiation might be due in part to a down regulation of BMPs

Table 1
Primer sequences of human genes used for PCR analysis. F: forward; R: reverse.

Gene	Forward and reverse primers	Annealing temperature T_a (°C)	Product length (bp)	GenBank
BMP2	F 5′-3′GTGATGCGGTGGACTGCACAG R 5′- 3′AAGGGCATTCTCCGTGGCAG	58	355	NM_001200
BMP4	F 5′- 3′CAGCATCCCTGAGAACGAGG R 5′- 3′TGGGTCCGAGTCTGATGGAG	58	306	NM_001202
BMP7	F 5′-3′TCATGAGCTTCGTCAACCTCG R 5′-3′GTTGCTGGTGGCTGTGATGTC	58	302	NM_001719
BMPR-1A	F 5′-3′TGGTCCGGCAAGTTGGTAAAGG R 5′- 3′ACGACGAGCCATCTCCCAAATG	58	623	NM_004329
BMPR-1B	F5′-3′AAGTGGATCAGGCCTCCCTCTG R 5′-3′CCAGGCCCAGGTCAGCAATA	57	500	NM_001203
BMPR-2	F 5′-3′GACTTTGGACTGTCCATGAG R 5′-3′CTTTCCTCAGCACACTGTGCAG	57	449	NM_001204
Noggin	F5′-3′GGCCAGCACTATCTCCACAT R5′-3′ ATGAAGCCTGGGTCGTAGTG	58	167	NM_00540
GAPDH	F 5′TACGTCGTGGAGTCCACTGG3′ R 5′AGAGGCAGGGATGATGTTCTG3′	58	327	NM_0020463

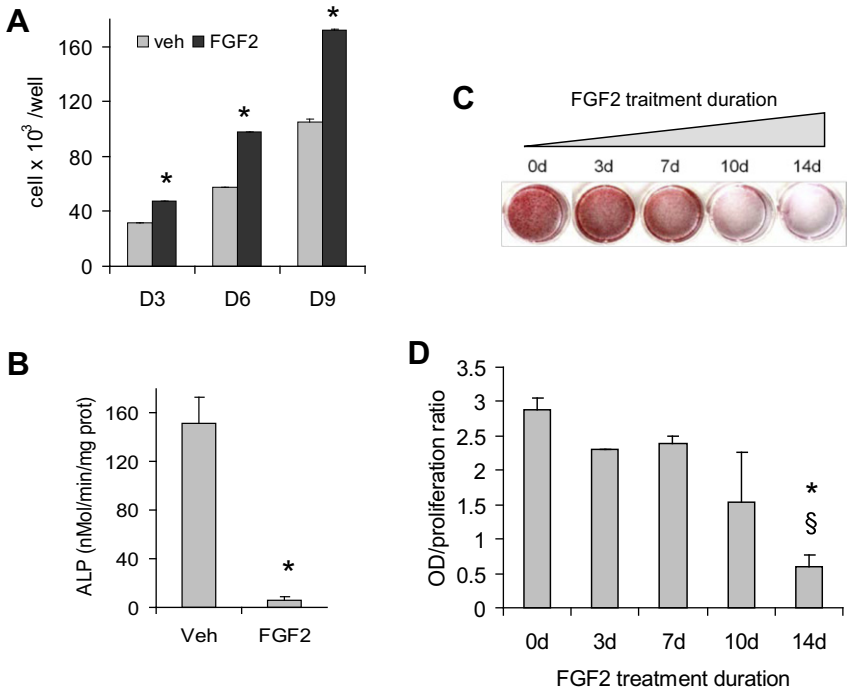

Fig. 1. FGF-2 increases HMSC proliferation and inhibits osteoblastic differentiation. (A) HMSC1 proliferation in osteogenic medium (FCS 5%) after 3, 6 and 9 days of culture with and without FGF2 (10 ng/mL). $^{*}p < 0.01$ Compared with respective vehicle. (B) Alkaline phosphatase activity after 9 days of culture in osteogenic medium with and without FGF2 (10 ng/mL). (C) Matrix mineralization after 18 days of differentiation after 0, 3, 7, 10 and 14 days of FGF2 treatment (10 ng/mL) from baseline. (D) Mineralization quantification (mean ± SEM) by extracting alizarin red staining (OD 595), corrected by the proliferation ratio compared to HMSC1 not treated with FGF2 (0 days). $^{*}p < 0.01$ Compared with 3d, $^{§}p < 0.05$ compared with 0d and 7d.

and BMPRs. We analyzed expression of the three main BMPRs, BMPR1A, BMPR1B, and BMPR2 during the course of HMSC osteoblastic differentiation. BMPR1A and BMPR2 were expressed at baseline in HMSCs whereas BMPR1B was not or poorly expressed (Fig. 2A). We therefore analyzed BMPR1B expression by conventional PCR on agarose gel. We found that BMPR1B expression was induced and increased importantly during early osteoblastic differentiation (Fig. 2A). BMPR1A expression was slightly up-regulated during early differentiation stage, whereas no significant change was noticed for BMPR2 (Fig. 2A).

Regarding BMPs, expressions of BMP2, BMP4 and BMP7 were investigated (Fig. 2B). We did not find any expression of BMP7 in our cells. Interestingly, expressions of BMP2 and BMP4 increased during HMSC osteoblastic differentiation, in the early 24 h for BMP4, and later for BMP2. In addition, Noggin, a BMP antagonist, was not expressed at baseline but up-regulated during osteoblastic differentiation (Fig. 2C).

Since expressions of BMPs and their receptors are up-regulated during HMSC osteogenic differentiation, we tested whether FGF2 exerts its negative effect on osteogenic differentiation by inhibiting BMP and BMPR expressions. FGF2 completely blocked the increase of BMP2, BMP4, BMPR1A and BMPR1B (Fig. 2B and C). Interestingly, FGF2 also inhibited the expression of Noggin, in parallel with the modulation of BMPs and BMPR expression (Fig. 2C).

3.3. ERK and JNK MAPKs mediate the inhibitory effect of FGF2 on BMP and BMPR up-regulation

It was previously reported that FGF activates ERK and JNK MAPKs. Western blot analyses confirmed these data in our HMSC

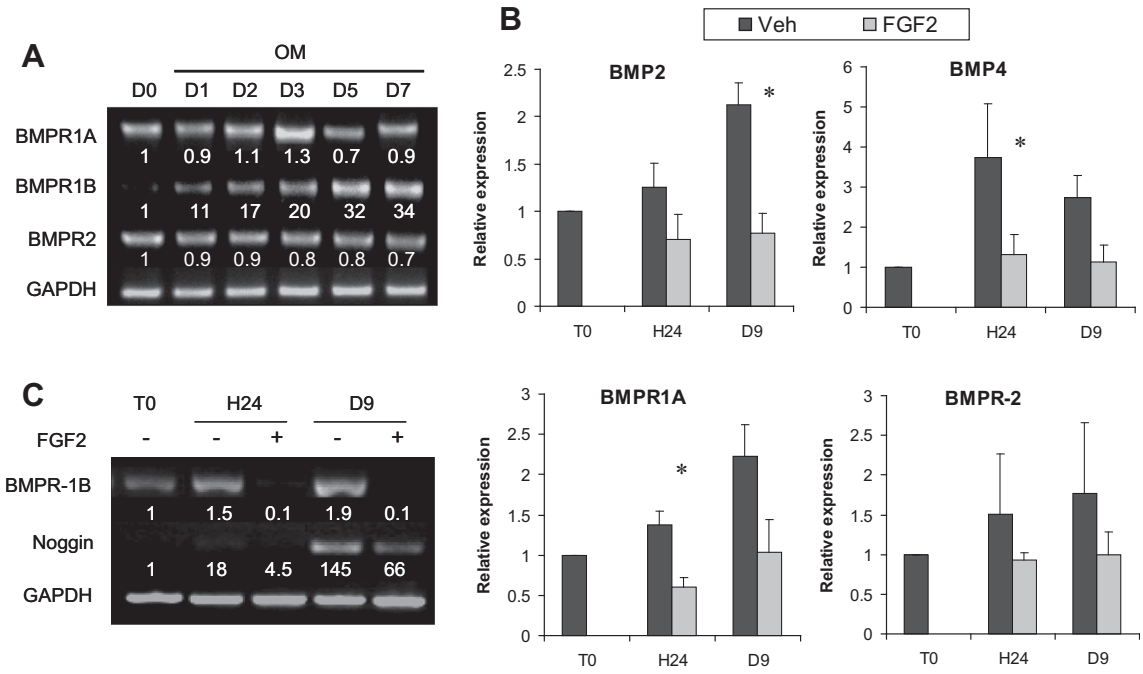

Fig. 2. Effect of FGF2 on BMP, BMPR and Noggin expression during osteoblastic differentiation of HMSCs. (A) PCR analysis of BMPR expression in confluent HMSC1 after 0, 1, 2, 3, 5 and 7 days (D1–D7) of incubation in osteogenic medium (OM). (B) Quantitative BMP and BMPR expression levels at 24 h and 9 days in response to FGF2 (10 ng/mL) in HMSC2. Data presented are mean of three independent cultures ± SEM. $^{*}p < 0.05$ versus untreated HMSCs. (C) PCR analysis of BMPR1B and Noggin expression in HMSC2 after 24 h and 9 days of treatment with or without FGF2 (10 ng/mL) in osteogenic medium. Expression of a gene of interest at an indicated time was normalized with basal expression and GAPDH expression and the resulting relative expression was indicated under each gel spot.

treated with FGF2 (data non shown). To investigate the implication of these signaling pathways in the inhibitory effect of FGF2 in HMSC osteoblastic differentiation, we used the biochemical inhibitors U0126 and SP600125 that respectively inhibit ERK and JNK. As indicated in Fig. 3, FGF2-mediated inhibition of matrix mineralization was completely reversed by pre-treatment with either U0126 or SP600125 (Fig. 3A). ERK or JNK inhibition prevented FGF2-mediated inhibition on BMP2 and BMP4 expression (Fig. 3B). Interestingly, we found that JNK inhibition promoted expressions of BMPR1A and BMPR1B (Fig. 3C). Taken together, these data indicate that ERK and JNK MAPKs interfere with BMP and BMPR expressions, suggesting that they are involved in the inhibition of HMSC osteoblastic differentiation by FGF2.

4. Discussion

Understanding the interactions between growth factors and BMP signaling remains a crucial issue to optimize the use of BMPs in therapeutic perspectives and bone tissue engineering. BMPs and growth factors activating tyrosine-kinase receptors (FGF, PDGF, VEGF) are among the main factors acting on the skeleton. They are embedded within the bone matrix or secreted in bone microenvironment and contribute to the bone repair process [29]. BMPs can also be used in bone therapeutic strategies. In bioengineering strategies, BMPs are combined with various insoluble substrata in implanted carriers to be delivered in situ in clinical practice [6]. FGF2 is expressed during the bone healing process of fractures

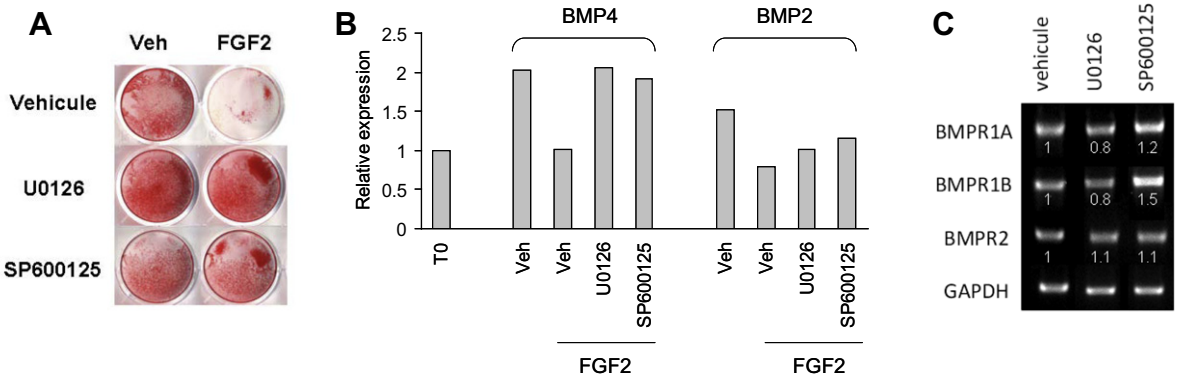

Fig. 3. ERK and JNK MAPKs activated by FGF2 are associated with the inhibitory effect of FGF2 on BMP and BMPR up-regulation in HMSCs. (A) Alizarin Red-S staining of matrix mineralization by HMSC2 co-treated with ERK or JNK inhibitors (U0126 and SP600125 respectively) and with FGF2 (10 ng/mL) or its vehicle for 15 days in osteogenic medium. (B) BMP gene expression was determined by quantitative RT-PCR in HMSC2 after 3 days of treatment with ERK or JNK inhibitors (U0126 and SP600125 respectively) and with FGF2 (10 ng/mL) or its vehicle in osteogenic medium. (C) BMPR gene expression was determined by PCR in HMSC1 after 3 days of treatment with ERK or JNK inhibitors (U0126 and SP600125 respectively) in osteogenic medium. Expression of a gene of interest at an indicated time was normalized with basal expression and GAPDH expression and the resulting relative expression was indicated under each gel spot.

and surgery bone sites. BMPs used in skeletal tissue engineering and surgery are likely to interact with FGF.

Our study confirms an increase in the expression of BMPs and their receptors BMPR1A and BMPR1B during osteoblastic differentiation of HMSC. We found that FGF2 has an inhibitory effect on HMSC osteoblastic differentiation and prevents the up-regulation of BMPs and BMPRs during HMSC osteogenic differentiation. It was previously reported that endogenous BMPR-IB signaling is required for early phase of osteoblast differentiation of human bone cells [17,30]. In osteoblasts of transgenic mice which express a truncated dominant-negative BMPR1B, BMP signaling is blocked. This model highlighted in vivo the essential and specific role of BMPR1B in osteoblast commitment and differentiation [31]. Interestingly, we found that BMPR1B is the most up-regulated BMPR during HMSC osteoblastic differentiation and that FGF2 completely prevented BMPR1B expression. Our results on BMPR1B are consistent with previous data supporting an essential role of this BMPR and provide a mechanism explaining the inhibitory effect of FGF2 on HMSC osteoblastic differentiation.

We also found that FGF2 inhibited BMP2 and BMP4 expressions in HMSC. The inhibitory effect of FGF2 on BMP2 expression had been previously reported in MC3T3 pre-osteoblastic cells [32]. Autocrine BMP production is necessary to activate RUNX2, a transcription factor stimulating osteoblast-specific gene expression [33]. By inhibiting the autocrine production of BMPs, FGF2 prevents the activation of osteoblastic differentiation. Expression of Noggin, a BMP antagonist, was not increased by FGF2, suggesting that the inhibitory effect of FGF2 is not mediated by an up-regulation of BMP antagonists.

FGF2 activates the MAPK ERK and JNK [34–36]. The role of ERK and JNK MAPK in BMP-induced osteoblastic differentiation is well established [37–39]. We found that FGF2 interfere with HMSC osteoblastic differentiation and BMP/BMPR expression by activating ERK and JNK. Despite the multiplicity of signaling pathways activated in response to FGF2 and potential redundancies between pathways, we restored the level of matrix mineralization and the expression of BMP4 and BMP2, by inhibition of either ERK or JNK, supporting the implication of these MAPKs in the inhibition of BMP and BMPR expression by FGF2. We highlight one of the potential mechanisms explaining the inhibitory effect of FGF2 on HMSC osteoblastic differentiation. In addition, other signaling pathways activated by tyrosine kinase receptors, such as the PI3K/Akt or the phospholipase C, might interfere with the effect of FGF2 [36]. Moreover, FGF2 can also modulate the response to BMPs by interfering with the Smad pathways that are activated by BMPR. Indeed, previous works indicated that the stability of the Smad complex can be modulated by MAPK [40]. By activating ERK and JNK, FGF2 is likely to decrease Smad signaling in response to BMPs. Our data on the level of expression of BMPs and BMPR provide an additional mechanism supporting the inhibition by FGF of BMPs osteogenic properties.

Understanding the interactions between growth factors and BMPs is of particular interest. As mentioned in the introduction, the inhibitory effect of FGF2 on BMPs and their receptors must be integrated in approaches attempting to optimize skeletal bioengineering strategies using both growth and osteogenic factors. The effectiveness of bone tissue engineering relies on the combination of three pillars: cells, including HMSC and osteoprogenitors, a vascularized scaffold and signaling factors such as FGF to promote cells proliferation [41]. High concentrations of FGF2 have an inhibitory effect on the response to BMPs; sequential supplementation of FGF2 followed by BMP2 shows higher osteogenic properties compared to the simultaneous delivery of both factors [25,42]. In the field of orthopedic and spine surgeries that use recombinant BMPs, inhibition of BMPR by growth factors secreted at fracture and surgery sites during the healing process might decrease the capacity of HMSC and bone cells to respond to BMPs. This hypothesis partially explains why supra-physiological concentrations of recombinant BMP2 or 7 are needed to obtain osteo-inductive effects.

In conclusion, data presented in this study indicate that FGF2 can modulate BMP pathway in HMSCs by down-regulating BMP/BMPR expression, thereby inhibiting the osteoblastic differentiation of HMSCs. This effect is mediated by ERK and JNK MAPKs pathways. These data provide an additional mechanism of crosstalk between growth factors and BMPs and must be integrated in the use of HMSCs and BMPs in therapeutic perspectives or skeletal bioengineering.

Acknowledgements

The authors thank S. Delplace for her expert technical assistance. This study was supported by a mobility grant from the "Société Française de Rhumatologie SFR", from the « Collège Doctoral Européen Université Lille Nord de France », and the « Région Nord Pas de Calais ».

References

[1] P. Bianco, M. Riminucci, S. Gronthos, P.G. Robey, Bone marrow stromal stem cells: nature, biology, and potential applications, Stem Cells 19 (2001) 180–192.
[2] N. Jaiswal, S.E. Haynesworth, A.I. Caplan, S.P. Bruder, Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro, J. Cell. Biochem. 64 (1997) 295–312.
[3] J.D. Boerckel, Y.M. Kolambkar, K.M. Dupont, B.A. Uhrig, E.A. Phelps, H.Y. Stevens, A.J. Garcia, R.E. Guldberg, Effects of protein dose and delivery system on BMP-mediated bone regeneration, Biomaterials 32 (2011) 5241–5251.
[4] X. Guo, X.F. Wang, Signaling cross-talk between TGF-beta/BMP and other pathways, Cell Res. 19 (2009) 71–88.
[5] X. Li, X. Cao, BMP signaling and skeletogenesis, Ann. N.Y. Acad. Sci. 1068 (2006) 26–40.
[6] E. Biver, P. Hardouin, J. Caverzasio, The "bone morphogenic proteins" pathways in bone and joint diseases: translational perspectives from physiopathology to therapeutic targets, Cytokine Growth Factor Rev. (2012), http://dx.doi.org/10.1016/j.cytogfr.2012.06.003.
[7] Q. Kang, W.X. Song, Q. Luo, N. Tang, J. Luo, X. Luo, J. Chen, Y. Bi, B.C. He, J.K. Park, W. Jiang, Y. Tang, J. Huang, Y. Su, G.H. Zhu, Y. He, H. Yin, Z. Hu, Y. Wang, L. Chen, G.W. Zuo, X. Pan, J. Shen, T. Vokes, R.R. Reid, R.C. Haydon, H.H. Luu, T.C. He, A comprehensive analysis of the dual roles of BMPs in regulating adipogenic and osteogenic differentiation of mesenchymal progenitor cells, Stem Cells Dev. 18 (2009) 545–559.
[8] H. Huang, T.J. Song, X. Li, L. Hu, Q. He, M. Liu, M.D. Lane, Q.Q. Tang, BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage, Proc. Natl. Acad. Sci. USA 106 (2009) 12670–12675.
[9] B.S. Yoon, K.M. Lyons, Multiple functions of BMPs in chondrogenesis, J. Cell. Biochem. 93 (2004) 93–103.
[10] S.N. Lissenberg-Thunnissen, D.J. de Gorter, C.F. Sier, I.B. Schipper, Use and efficacy of bone morphogenetic proteins in fracture healing, Int. Orthop. 35 (2011) 1271–1280.
[11] B. Bragdon, O. Moseychuk, S. Saldanha, D. King, J. Julian, A. Nohe, Bone morphogenetic proteins: a critical review, Cell Signal. 23 (2011) 609–620.
[12] K. Miyazono, Signal transduction by bone morphogenetic protein receptors: functional roles of Smad proteins, Bone 25 (1999) 91–93.
[13] M.S. Friedman, M.W. Long, K.D. Hankenson, Osteogenic differentiation of human mesenchymal stem cells is regulated by bone morphogenetic protein-6, J. Cell. Biochem. 98 (2006) 538–554.
[14] F.P. Seib, M. Franke, D. Jing, C. Werner, M. Bornhauser, Endogenous bone morphogenetic proteins in human bone marrow-derived multipotent mesenchymal stromal cells, Eur. J. Cell Biol. 88 (2009) 257–271.
[15] K. Lavery, P. Swain, D. Falb, M.H. Alaoui-Ismaili, BMP-2/4 and BMP-6/7 differentially utilize cell surface receptors to induce osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells, J. Biol. Chem. 283 (2008) 20948–20958.
[16] E. Abe, M. Yamamoto, Y. Taguchi, B. Lecka-Czernik, C.A. O'Brien, A.N. Economides, N. Stahl, R.L. Jilka, S.C. Manolagas, Essential requirement of BMPs-2/4 for both osteoblast and osteoclast formation in murine bone marrow cultures from adult mice. antagonism by noggin, J. Bone Miner. Res. 15 (2000) 663–673.
[17] W. Singhatanadgit, I. Olsen, Endogenous BMPR-IB signaling is required for early osteoblast differentiation of human bone cells, In Vitro Cell. Dev. Biol. Anim. 47 (2011) 251–259.
[18] M.E. Bolander, Regulation of fracture repair by growth factors, Proc. Soc. Exp. Biol. Med. 200 (1992) 165–170.

[19] P. Krejci, J. Prochazkova, V. Bryja, A. Kozubik, W.R. Wilcox, Molecular pathology of the fibroblast growth factor family, Hum. Mutat. 30 (2009) 1245–1255.
[20] R.A. Jackson, V. Nurcombe, S.M. Cool, Coordinated fibroblast growth factor and heparan sulfate regulation of osteogenesis, Gene 379 (2006) 79–91.
[21] Z. Huang, E.R. Nelson, R.L. Smith, S.B. Goodman, The sequential expression profiles of growth factors from osteoprogenitors [correction of osteroprogenitors] to osteoblasts in vitro, Tissue Eng. 13 (2007) 2311–2320.
[22] D.M. Ornitz, P.J. Marie, FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease, Genes Dev. 16 (2002) 1446–1465.
[23] X. Du, Y. Xie, C.J. Xian, L. Chen, Role of FGFs/FGFRs in skeletal development and bone regeneration, J. Cell. Physiol. 227 (2012) 3731–3743.
[24] M.A. Fang, C.A. Glackin, A. Sadhu, S. McDougall, Transcriptional regulation of alpha 2(I) collagen gene expression by fibroblast growth factor-2 in MC3T3-E1 osteoblast-like cells, J. Cell. Biochem. 80 (2001) 550–559.
[25] L.N. Luong, J. Ramaswamy, D.H. Kohn, Effects of osteogenic growth factors on bone marrow stromal cell differentiation in a mineral-based delivery system, Biomaterials 33 (2012) 283–294.
[26] G. Sapkota, C. Alarcon, F.M. Spagnoli, A.H. Brivanlou, J. Massague, Balancing BMP signaling through integrated inputs into the Smad1 linker, Mol. Cell. 25 (2007) 441–454.
[27] A. Suzuki, J. Guicheux, G. Palmer, Y. Miura, Y. Oiso, J.P. Bonjour, J. Caverzasio, Evidence for a role of p38 MAP kinase in expression of alkaline phosphatase during osteoblastic cell differentiation, Bone 30 (2002) 91–98.
[28] O. Ghali, C. Chauveau, P. Hardouin, O. Broux, J.C. Devedjian, TNF-alpha's effects on proliferation and apoptosis in human mesenchymal stem cells depend on RUNX2 expression, J. Bone Miner. Res. 25 (2010) 1616–1626.
[29] V. Devescovi, E. Leonardi, G. Ciapetti, E. Cenni, Growth factors in bone repair, Chir. Organi Mov. 92 (2008) 161–168.
[30] D. Chen, X. Ji, M.A. Harris, J.Q. Feng, G. Karsenty, A.J. Celeste, V. Rosen, G.R. Mundy, S.E. Harris, Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages, J. Cell Biol. 142 (1998) 295–305.
[31] M. Zhao, S.E. Harris, D. Horn, Z. Geng, R. Nishimura, G.R. Mundy, D. Chen, Bone morphogenetic protein receptor signaling is necessary for normal murine postnatal bone formation, J. Cell Biol. 157 (2002) 1049–1060.
[32] M. Hughes-Fulford, C.F. Li, The role of FGF-2 and BMP-2 in regulation of gene induction, cell proliferation and mineralization, J. Orthop. Surg. Res. 6 (2011) 8.
[33] M. Phimphilai, Z. Zhao, H. Boules, H. Roca, R.T. Franceschi, BMP signaling is required for RUNX2-dependent induction of the osteoblast phenotype, J. Bone Miner. Res. 21 (2006) 637–646.
[34] H.J. Ahn, W.J. Lee, K. Kwack, Y.D. Kwon, FGF2 stimulates the proliferation of human mesenchymal stem cells through the transient activation of JNK signaling, FEBS Lett. 583 (2009) 2922–2926.
[35] J. Guicheux, J. Lemonnier, C. Ghayor, A. Suzuki, G. Palmer, J. Caverzasio, Activation of p38 mitogen-activated protein kinase and c-Jun-NH2-terminal kinase by BMP-2 and their implication in the stimulation of osteoblastic cell differentiation, J. Bone Miner. Res. 18 (2003) 2060–2068.
[36] P.J. Marie, Fibroblast growth factor signaling controlling bone formation: an update, Gene 498 (2012) 1–4.
[37] H. Sowa, H. Kaji, T. Yamaguchi, T. Sugimoto, K. Chihara, Activations of ERK1/2 and JNK by transforming growth factor beta negatively regulate Smad3-induced alkaline phosphatase activity and mineralization in mouse osteoblastic cells, J. Biol. Chem. 277 (2002) 36024–36031.
[38] K. Nakayama, Y. Tamura, M. Suzawa, S. Harada, S. Fukumoto, M. Kato, K. Miyazono, G.A. Rodan, Y. Takeuchi, T. Fujita, Receptor tyrosine kinases inhibit bone morphogenetic protein-Smad responsive promoter activity and differentiation of murine MC3T3-E1 osteoblast-like cells, J. Bone Miner. Res. 18 (2003) 827–835.
[39] Y.F. Huang, J.J. Lin, C.H. Lin, Y. Su, S.C. Hung, C-Jun N-terminal kinase 1 negatively regulates osteoblastic differentiation induced by BMP2 via phosphorylation of Runx2 at Ser104, J. Bone Miner. Res. 27 (2012) 1093–1105.
[40] L.C. Fuentealba, E. Eivers, A. Ikeda, C. Hurtado, H. Kuroda, E.M. Pera, E.M. De Robertis, Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal, Cell 131 (2007) 980–993.
[41] S. Ohba, F. Yano, U.-i. Chung, Tissue engineering of bone and cartilage, IBMS BoneKEy 6 (2009) 405–419.
[42] N. Maegawa, K. Kawamura, M. Hirose, H. Yajima, Y. Takakura, H. Ohgushi, Enhancement of osteoblastic differentiation of mesenchymal stromal cells cultured by selective combination of bone morphogenetic protein-2 (BMP-2) and fibroblast growth factor-2 (FGF-2), J. Tissue Eng. Regen. Med. 1 (2007) 306–313.