Basic fibroblast growth factor enhances PPARγ ligand-induced adipogenesis of mesenchymal stem cells

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Received 17 August 2004; revised 1 October 2004; accepted 6 October 2004

Available online 19 October 2004

Edited by Veli-Pekka Lehto

Abstract Mesenchymal stem cells (MSCs) are capable of differentiating into a variety of lineages, including bone, cartilage, or fat, depending on the inducing stimuli and specific growth and differentiation factors. It is widely acknowledged that basic fibroblast growth factor (bFGF) modulates chondrogenic and osteogenic differentiation of MSCs, but thorough investigations of its effects on adipogenic differentiation are lacking. In this study, we demonstrate on the cellular and molecular level that supplementation of bFGF in different phases of cell culture leads to a strong enhancement of adipogenesis of MSCs, as induced by an adipogenic hormonal cocktail. In cultures receiving bFGF, mRNA expression of peroxisome proliferator-activated receptor γ2 (PPARγ2), a key transcription factor in adipogenesis, was upregulated even prior to adipogenic induction. In order to investigate the effects of bFGF on PPARγ ligand-induced adipogenic differentiation, the thiazolidinedione troglitazone was administered as a single adipogenic inducer. Basic FGF was demonstrated to also strongly increase adipogenesis induced by troglitazone, that is, bFGF clearly increased the responsiveness of MSCs to a PPARγ ligand.

Keywords: Cell differentiation; Adipogenesis; Basic fibroblast growth factor; Mesenchymal stem cell; Peroxisome proliferator-activated receptor γ; Thiazolidinedione

1. Introduction

Multipotent mesenchymal stem cells (MSCs) are present in a variety of tissues, including bone marrow, blood, muscle, and adipose tissue [1–4]. MSCs were found to differentiate into cartilage, bone, fat, muscle, and other connective tissues [5,6] depending on culture conditions, which include supplementation of lineage-specific inducing agents as well as hormones and growth factors.

Basic fibroblast growth factor (bFGF) belongs to the family of heparin-binding growth factors [7]. To date, more than twenty FGFs have been discovered and FGFs are known to induce chemotactic, angiogenic, and mitogenic activity and play an important role in early differentiation and developmental processes [8,9]. Basic FGF was reported to influence differentiation of MSCs of various species towards different lineages. Addition of bFGF was shown to enhance chondrogenic and osteogenic differentiation of avian MSCs [10] and to retain the differentiation potential of extensively expanded human MSCs towards both the chondrogenic and osteogenic lineage [11]. Using rat MSCs cultivated under varying conditions, the stimulatory effects of bFGF were repeatedly shown to promote differentiation towards the osteogenic lineage [12–14]. In contrast, the effect of bFGF on the adipogenesis of MSCs is controversially discussed [11,15,16]. However, those studies were not focused on adipogenesis; instead experimental conditions were adjusted to the investigation of osteogenesis or chondrogenesis and adipogenic differentiation was mainly assessed by morphology.

Thus, the first goal of this study was to investigate the effects of bFGF on the adipogenic differentiation of rat MSCs induced by an adipogenic hormonal cocktail consisting of dexamethasone, insulin, 3-isobutyl-l-methylxanthine (IBMX) and indomethacin. Basic FGF was supplemented in different phases of the culture and adipogenesis was characterized on the cellular and the molecular level. Remarkably, in these experiments mRNA expression of the peroxisome proliferator-activated receptor γ2 (PPARγ2), a key transcription factor in adipogenesis in vitro and in vivo [17], was elevated in the bFGF group not only during the course of differentiation, but already prior to adipogenic induction. From this result, it was hypothesized that bFGF can also enhance adipogenesis that is solely induced by a PPARγ ligand. Therefore, the second goal was to determine the effects of bFGF on adipogenic differentiation induced by the thiazolidinedione troglitazone, an acknowledged synthetic ligand of PPARγ [18].

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Abbreviations: bFGF, basic fibroblast growth factor; FACS, fluorescence-activated cell scanning; GLUT4, glucose transporter 4; GPDH, glycerol-3-phosphate dehydrogenase; PPARγ, peroxisome proliferator-activated receptor γ; MSC, mesenchymal stem cell; SCD-1, stearoyl-CoA desaturase-1

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doi:10.1016/j.febslet.2004.10.020

2. Materials and methods

2.1. Materials

If not otherwise stated, chemicals were obtained from Sigma, Steinheim, Germany. Basic FGF was obtained from R&D Systems (Minneapolis, MN, USA). Troglitazone and insulin were kindly provided by Dr. T. Skurk (Deutsches Diabetes Forschungsinstitut, Duesseldorf, Germany) and Hoechst Marion Roussel (Frankfurt am Main, Germany), respectively. Cell culture plastics were purchased from Corning Costar (Bodenheim, Germany).

2.2. Cell culture

Marrow stromal cells were obtained from six-week old male Sprague Dawley rats (weight: 170–180 g, Charles River, Sulzfeld, Germany). MSCs were flushed from the tibiae and femora according to the protocol of Ishaug [19]. Cells were centrifuged at 1200 rpm for 5 min. The resulting cell pellet was resuspended in basal medium consisting of DMEM (Biochrom, Berlin, Germany), 10% fetal bovine serum (Gemini Bio-Products Inc., Calabasas, CA, USA), 1% penicillin/streptomycin (Invitrogen, Karlsruhe, Germany), and 50 μg/ml ascorbic acid. Cells were seeded in T75 flasks and cultured at 37 °C and 5% CO_2. Cells were allowed to adhere to the substratum for three days. The flasks were rinsed twice with phosphate-buffered saline (PBS, Invitrogen, Karlsruhe, Germany) in order to remove non-adherent cells. In the following experiments, bFGF was supplemented during different periods of the culture (Fig. 1). Culturing the adherent cells, 12 ml of basal medium with or without 3 ng/ml bFGF was exchanged every 2–3 days until confluence was reached (proliferation phase I, Fig. 1). Cells were passaged once with 0.25% trypsin and EDTA (Invitrogen, Karlsruhe, Germany). For adipogenic differentiation, cells were seeded at a density of 30 000 cells/cm^2 in either six-well plates (RT-PCR) or 24-well plates (glycerol-3-phosphate dehydrogenase (GPDH) activity assay, flow cytometry, and histological staining) and grown to postconfluence for 3 days with or without bFGF (proliferation phase II, Fig. 1). Subsequently, cells were differentiated for 8 days either in the presence or absence of bFGF (differentiation phase, Fig. 1). In detail, to induce adipogenic differentiation, cultures were treated for 3 days with a hormonal cocktail containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (Serva Electrophoresis, Heidelberg, Germany), 10 nM dexamethasone, 60 μM indomethacin and 10 μg/ml insulin, which was added to the basal medium. Subsequently, cultures were maintained for 5 more days in differentiation medium consisting of basal medium supplemented with 10 μg/ml insulin. Alternatively, cells treated with or without bFGF were differentiated by adding exclusively 5 μM troglitazone (instead of the hormonal cocktail) to basal medium. For this purpose, troglitazone was dissolved in dimethylsulfoxide as a 1000-fold stock solution, which was administered with each medium change during the complete course of differentiation (i.e., 8 days) [20].

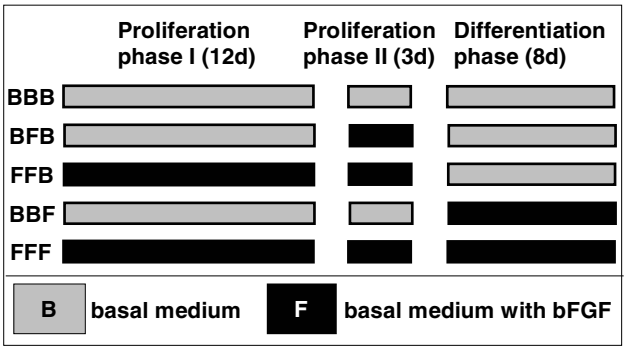

Fig. 1. Basic FGF supplementation in different periods of the cell culture. MSCs were exposed to bFGF only in proliferation phase II for 3 days (BFB), only in both proliferation phases I and II for 15 days (FFB), only in the differentiation phase (BBF), or throughout the complete culture period (FFF). Cells grown in the absence of bFGF (BBB) served as a control. Gray boxes represent basal media without bFGF (B), black boxes represent basal medium supplemented with bFGF (F).

The supplementation scheme of bFGF is depicted in Fig. 1. Briefly, medium supplemented with bFGF is abbreviated with "F", basal medium without bFGF with "B". Cultures treated with bFGF only in proliferation phase II for 3 days are designated as BFB. Addition of the factor during proliferation phases I and II for 15 days is indicated as FFB. Supplementation of bFGF exclusively in the differentiation phase is abbreviated as BBF and bFGF treatment during the complete time of cell culture as FFF. Cells grown in complete absence of bFGF (BBB) served as control. In preliminary experiments (assessment of adipogenesis by Oil Red O staining), bFGF was additionally supplemented only in the proliferation phase I for 12 days. This condition yielded results similar to those of the group receiving bFGF during proliferation phases I and II for 15 days (data not shown); consequently, in order to simplify the experimental design, this group (bFGF in proliferation phase I only) was omitted in the presented study.

2.3. GPDH activity assay

GPDH activity was measured using a protocol adapted from Pairault and Green [21]. In brief, cells washed with PBS were scraped in lysis buffer containing 50 mM Tris, 1 mM EDTA, and 1 mM β-mercaptoethanol on ice. Subsequently, the resulting suspension was sonicated with a digital sonifier (Branson Ultrasonic Coporation, Danburg, CT, USA). Cell lysates were centrifuged for 5 min at 13 200 rpm at 4 °C. Aliquots of the supernatant were mixed with a solution containing 0.1 M triethanolamine, 2.5 mM EDTA, 0.5 mM β-mercaptoethanol, 120 μM reduced nicotinamide adenine dinucleotide (NADH) (Roche, Mannheim, Germany), and 200 μM dihydroxyacetonephosphate. Enzyme activity was monitored by measurement of the disappearance of NADH at 340 nm over 4.2 min. Enzyme activity was normalized to the protein content of each sample. Proteins were determined by the method of Lowry et al. [22]. Proteins were precipitated using 12% trichloroacetic acid. In alkaline solution, proteins were solubilized and complexed with a mixture of disodium tartrate, copper sulfate and folin-ciocalteu reagent (all Merck, Darmstadt, Germany). Absorption was measured at 546 nm after 30 min incubation.

2.4. Oil Red O staining

Cells were washed once with PBS and fixed with 10% formaldehyde (Merck, Darmstadt, Germany) overnight. Cells were covered with 3 mg/ml Oil Red O for 2 h. Excess dye was removed with PBS and finally, cells were fixed with 10% formaldehyde.

2.5. Flow cytometry

This method was carried out using a protocol adapted from Gimble et al. [23]. Cells were carefully harvested by treatment with 0.25% trypsin/EDTA and centrifuged at 200 g at 4 °C for 5 min. After washing the pellet with PBS, cells were centrifuged as described above and resuspended in PBS containing the lipophilic fluorescent dye Nile Red. Cells were incubated for 30 min on ice. Samples were analyzed with a FACSCalibur flow cytometer (Becton–Dickinson, Heidelberg, Germany). Nile Red fluorescence was measured on the FL2 emission channel through a 585 ± 21 nm band pass filter, following excitation with an argon ion laser source at 488 nm. For each sample, 10^4 cells were collected. To determine the number of adipocytes in each sample, a selection marker M1 was set in histograms. The amount of adipocytes was assessed by determining the percentage of cells within the M1 region.

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was harvested from the cells with Trizol reagent (Invitrogen, Karlsruhe, Germany) and isolated according to the manufacturer's instructions. First-strand cDNA was synthesized from total RNA by using random hexamers (Roche Diagnostics, Mannheim, Germany) and Superscript II RNase H Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). Samples were incubated at 42 °C for 50 min and heated afterwards at 70 °C for 15 min to inactivate the enzyme. Subsequently, PCR was performed with Sawady Taq-DNA-Polymerase (PeqLab, Erlangen, Germany); initial denaturation occurred at 94 °C for 120 s, final extension at 72 °C for 30 s for each set of primers. The amplification was carried out using the following specific oligonucleotides:

PPARγ2: 5′-GAGCATGGTGCCTTCGCTGA-3′/5′-AGCAAGGCACTTCTGAAACCGA-3′

GLUT4: 5′-AGCAGCTCTCAGGCATCAAT-3′/5′-CTCAAAGAAGGCCACAAAGC-3′
SCD-1: 5′-CGGGATCACCGCGCCCACCACAAGT-3′/5′-CCACGGACCCCAGGGAAACCAGGATG-3′
18S: 5′-TCAAGAACGAAAGTCGGAGGTTCG-3′/5′-TTATTGCTCAATCTCGGGTGGCTG-3′

18S rRNA served as control. Conditions set for the investigated genes were: 94 °C for 45 s, 62 °C for 45 s, 72 °C for 1 min (36 cycles) for PPARγ2; 94 °C for 45 s, 56 °C for 45 s, 72 °C for 1 min (32 cycles) for glucose transporter 4 (GLUT4); 94 °C for 45 s, 62 °C for 45 s, 72 °C for 1 min (36 cycles) for stearoyl-CoA desaturase-1 (SCD-1); and 94 °C for 45 s, 56 °C for 45 s, 72 °C for 1 min (25 cycles) for 18S rRNA. Reverse transcription and PCR were performed using a Mastercycler Gradient (Eppendorf AG, Hamburg, Germany). The PCR products were analyzed by electrophoresis on 2% agarose gels, stained with ethidium bromide. Finally, the gels were subjected to imaging and densitometric scanning of the resulting bands under UV light ($\lambda = 312$ nm) using a Kodak EDAS 290 (Fisher Scientific, Schwerte, Germany).

2.7. Statistics

Fluorescence-activated cell scanning (FACS) data, GPDH data, and RT-PCR quantification are expressed as means ± standard deviation. Single-factor analysis of variance (ANOVA) was used in conjunction with a multiple comparison test (Tukey's test) to assess statistical significance at a level of $P < 0.01$ for FACS and GPDH data and of $P < 0.05$ for RT-PCR data.

3. Results

In cultures that did not receive adipogenesis-inducing agents, no lipid droplets were observed in the absence of bFGF (Fig. 2 "BBB-not induced", Table 1) and only very few lipid droplets were detected in the presence of bFGF (Fig. 2 "FFF-not induced", Table 1). Moreover, the activity of GPDH, a key enzyme in lipid biosynthesis, was virtually undetectable in the absence of inducing agents, irrespective of bFGF supplementation (Fig. 3).

In order to investigate the modulating effects of bFGF, cultures stimulated by hormonal inducers were supplemented with bFGF in different phases of the cell culture (Fig. 1). In cultures without bFGF (BBB-control), MSCs only weakly gave rise to adipocytes after induction with a hormonal cocktail (Fig. 2): Only about 2% of cultured cells differentiated into adipocytes, as determined by FACS analysis (Table 1). Exposure to bFGF enhanced the adipogenesis of MSCs in all cases, as determined 8 days after induction by Oil Red O staining and Nile Red flow cytometry of differentiated adipocytes (Fig. 2). Supplementation with bFGF only during the proliferation phase II (BFB) and during the proliferation phases I and II (FFB) yielded a 2.8-fold and 6-fold increase of the fraction of adipocytes, respectively, as compared to BBB-control (Table 1). The latter resulted in adipocytes containing the largest lipid droplets of all groups investigated (Fig. 2). Addition of bFGF in the differentiation phase only (BBF) resulted in a 2.3-fold increase of the adipocyte fraction (Table 1), whereas bFGF supplementation during the complete culture (FFF) yielded the largest increase, i.e., 9.4-fold. Measurement of the GPDH activity supported the observations with regard to effects of bFGF (Fig. 3). In all induced cultures supplemented with bFGF, a significant increase of GPDH activity was detected as compared to the BBB control group. The highest values were again determined for experimental groups FFB and FFF.

Expression of the adipocyte-specific genes PPARγ2, a key transcription factor, GLUT4, a glucose transporter and late

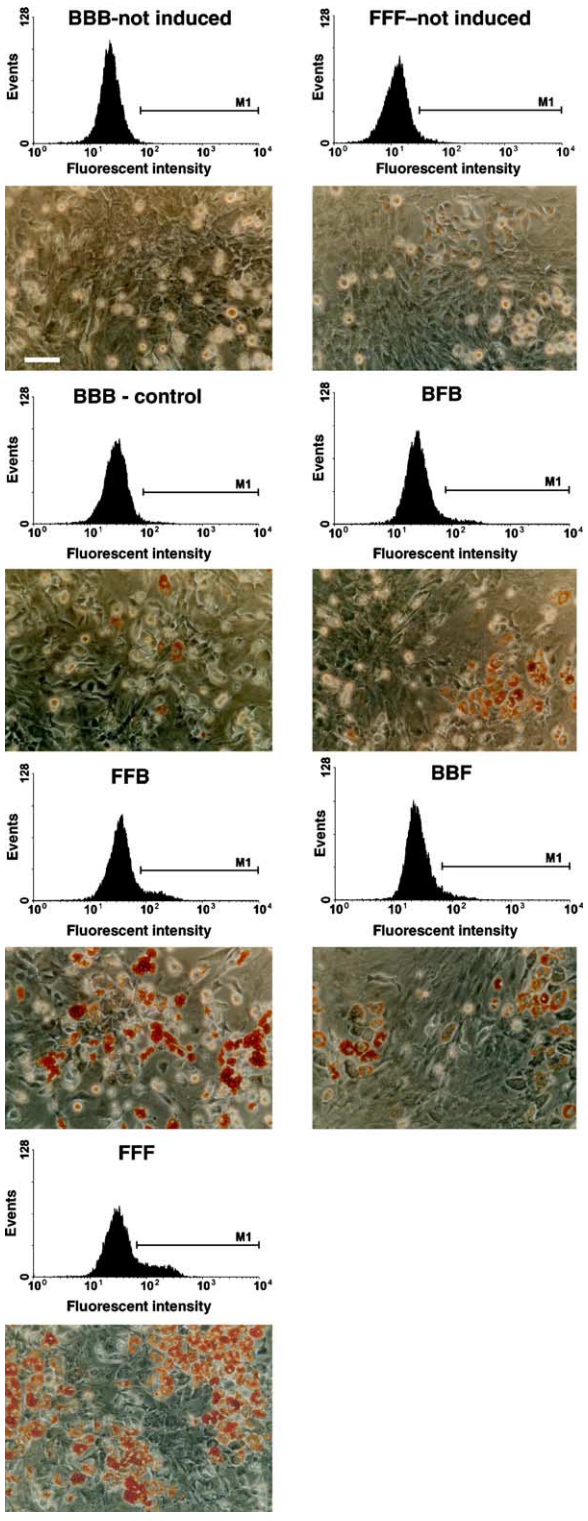

Fig. 2. Adipogenesis of MSCs on day 8 of differentiation: assessment by Oil Red O staining and Nile Red flow cytometry (for quantification data, see Table 1). The groups designated as "not induced" were cultivated without induction by the hormonal cocktail (BBB-not induced: in the absence of bFGF; FFF-not induced: in the presence of bFGF). All other groups were hormonally induced. BBB-control was cultivated in the absence of bFGF and served as a control group. Other cultures were treated with bFGF in proliferation phase II for 3 days (BFB), in proliferation phases I and II for 15 days (FFB), in the differentiation phase (BBF), and in complete culture (FFF), respectively. Scale bar: 50 μm.

Table 1
Quantification of flow cytometry analysis

Experimental group	FACS (% cells in M1)	FACS (relative)
BBB-not induced	0.34 ± 0.06	–
FFF-not induced	1.43 ± 0.65	–
BBB (induced)-control	2.23 ± 0.42	1.00
BFB (induced)	6.21 ± 0.95*	2.78
FFB (induced)	13.46 ± 1.19**	6.03
BBF (induced)	5.07 ± 0.64*	2.27
FFF (induced)	20.90 ± 1.15***	9.36

To determine the number of adipocytes in each sample, a selection marker M1 was set in histograms (see Fig. 2). Column 2 represents the quantification of differentiated adipocytes expressed as a percentage of total cells in culture, column 3 shows the relative increase as compared to the control group without bFGF (BBB induced-control). Tukey's test ($n = 4$) indicated a statistically significant increase as compared to the control group (BBB) (*), compared to BBB, BFB and BBF (**), and compared to all groups (***).

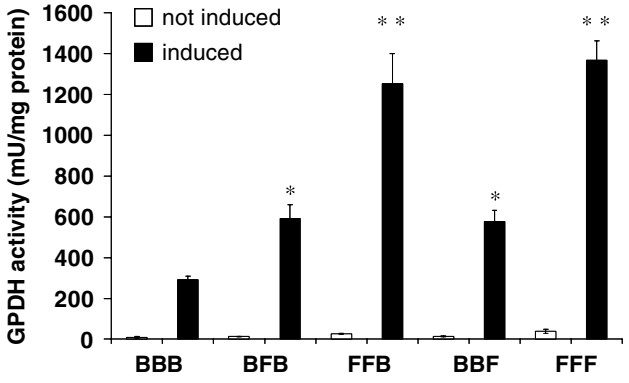

Fig. 3. Measurement of GPDH activity on day 3 of differentiation. Cultures were treated with bFGF in proliferation phase II for 3 days (BFB), in proliferation phases I and II for 15 days (FFB), in the differentiation phase (BBF), and in complete culture (FFF), respectively. Cells grown in the absence of bFGF served as control (BBB). GPDH activity was determined in not induced cultures (□) and in induced cultures (■). Tukey's test ($n = 4$) indicated a statistically significant increase as compared to control group (BBB) (*) and as compared to BBB, BFB and BBF (**).

marker of adipogenesis, and SCD-1, a key enzyme in the synthesis of unsaturated fatty acids and also a late marker of adipogenesis, was determined on the mRNA level by RT-PCR (Fig. 4). Selected experimental groups were investigated in order to further elucidate the contribution of bFGF to adipogenic differentiation, i.e., the group receiving bFGF throughout the entire proliferation phase (FFB) was compared to the BBB control group.

Gene expression of PPARγ2 was assessed one day before induction (cells grown to confluence), one day and three days after induction (Fig. 4A). Expression of PPARγ2 was increased on day one and day three after induction, as compared to samples harvested one day prior to induction (Fig. 4A). PPARγ2 expression was elevated in the group receiving bFGF (FFB) as compared to the control group (BBB); this trend was observed not only one day and three days after, but also one day before induction (Fig. 4A).

Additionally, gene expression of late markers of adipogenic differentiation, GLUT4 and SCD-1, was assessed on day three after induction (Fig. 4B). Again, quantification showed that differentiation under the influence of bFGF (FFB) led to an increased expression of both markers as compared to the control group (BBB) (Fig. 4B).

When using troglitazone as a single inducer (instead of the hormonal cocktail), adipogenic differentiation of MSCs was also observed. In detail, only weak adipogenesis was detected in the absence of bFGF (Fig. 5B). Again, the exposure of MSCs to bFGF throughout the entire culture period resulted in a strong enhancement of adipogenesis, which was detected by Oil Red O staining (Fig. 5D) and GPDH activity (Fig. 6). In general, after induction with troglitazone, intracellular lipid droplets were distinctly smaller compared to cultures induced with the hormonal cocktail (Fig. 5), a previously described phenomenon [24]. Apart from that, with regard to Oil Red O staining and GPDH activity, troglitazone cultures were comparable to corresponding cultures employing the hormonal cocktail. Hence, adipogenic differentiation was enhanced by bFGF to the same extent under both inducing conditions (Figs. 5 and 6).

4. Discussion

Adipogenesis is a complex process involving several transcription factors and signal transduction pathways and is affected by a variety of environmental conditions like growth and differentiation factors [17]. In this study, we show that the growth factor bFGF can strongly enhance adipogenesis of MSCs induced by a widely used hormonal cocktail consisting of dexamethasone, IBMX, indomethacin, and insulin.

Besides other features of adipogenic differentiation (Figs. 2, 3 and 4B), bFGF increased the expression of PPARγ2, a key transcription factor in adipogenesis [17]. As this effect was observed even prior to adipogenic induction (Fig. 4A), the hypothesis that bFGF can also enhance adipogenic differentiation that is induced by a PPARγ ligand alone was explored. Indeed, bFGF enhanced adipogenesis solely induced by troglitazone, a commonly recognized PPARγ ligand, to the same extent as that induced by the hormonal cocktail (Figs. 5 and 6).

The effects of bFGF on the hormonal cocktail-induced adipogenesis of MSCs are inherently more complex to explain. The inducing effects of dexamethasone and IBMX are still not well understood and are controversially discussed [17]. However, indomethacin is commonly acknowledged to be a PPARγ ligand [25]. Furthermore, other experiments using only IBMX and indomethacin as adipogenic inducers showed enhancing effects of bFGF similar to those observed in the experiments employing the complete hormonal cocktail (data not shown). Therefore, the function of indomethacin as PPARγ ligand suggests that, again, the increase of PPARγ2 levels by bFGF likely contributed to the observed effects.

The obtained results are in agreement with earlier morphological observations using rat MSCs [16]. The seemingly contradictory results of a different report obtained with human MSCs showing no effect of bFGF treatment may be reasonably explained by distinctly different adipogenic inducing schemes [11]. In contrast to our induction phase of 3 days, Tsutsumi et al. used a similar hormonal cocktail for 25 days and determined comparable results for groups with and without bFGF supplement [11]. Additionally, preliminary experiments in our laboratory using human MSCs yielded results

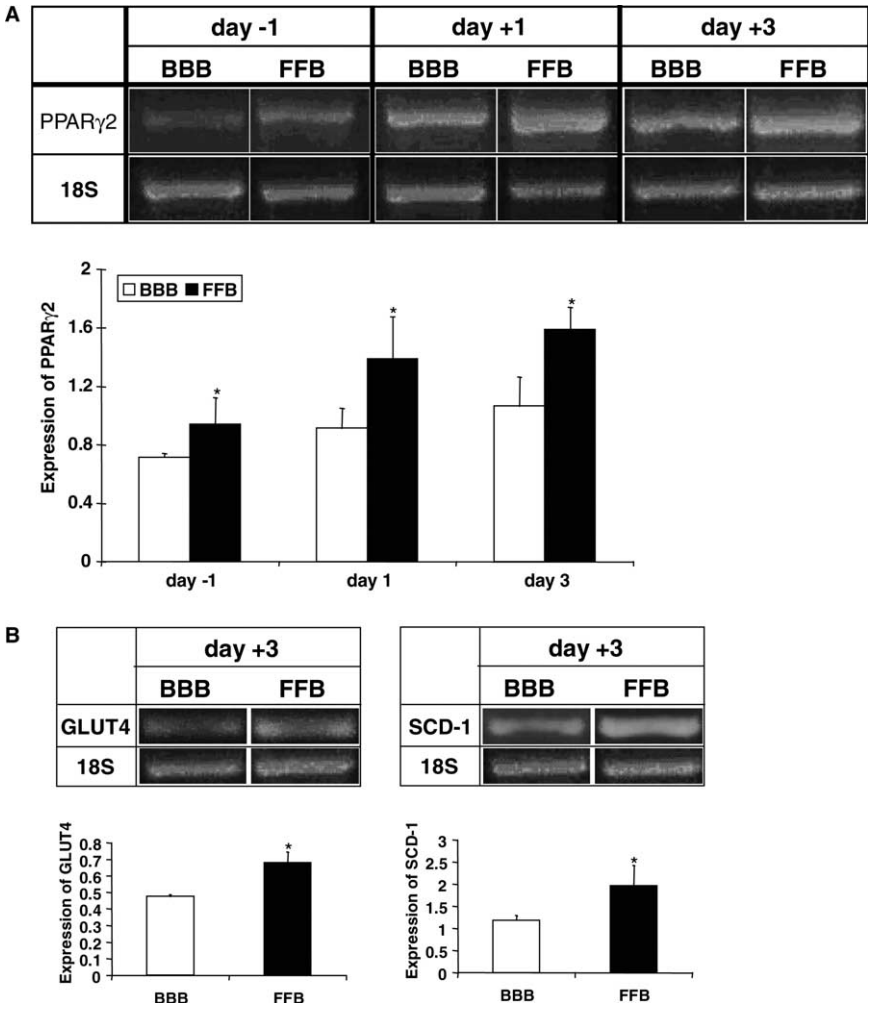

Fig. 4. Assessment of expression of adipocytic genes PPARγ2, GLUT4, and SCD-1 using RT-PCR technique. Two experimental groups (BBB and FFB) were exemplarily investigated to demonstrate the effect of bFGF. Three independent experiments were conducted; representative results of one experiment are shown here. Additionally, data from semi-quantitative image analysis are depicted. Statistically significant difference between BBB and FFB group is indicated by *. (A) The expression levels of the transcription factor PPARγ2 were determined one day before, one day after and three days after induction with the hormonal cocktail. 18S RNA was used as control. (B) Gene expression of late markers of adipogenic differentiation, GLUT4 and SCD-1, was determined on day 3 after induction.

similar to our data from the rat MSCs, suggesting that the effects of bFGF on adipogenic differentiation are not species-specific.

In the following, possible mechanisms are discussed through which bFGF may enhance adipogenesis and result in elevated PPARγ2 expression levels, respectively. First, bFGF may cause a preferential proliferation of a subpopulation of MSCs [26,27], e.g., one with enhanced expression of PPARγ2. In this regard, the theoretically possible contribution of differentiated adipocytes, which are present in the bone marrow [28], can be excluded: Mature adipocytes do not attach to the substratum during cell isolation due to their buoyancy [29] and, in the case they did so nonetheless, they would be identified by their typical phenotype (lipid droplets); furthermore, they have been demonstrated to be virtually unable to proliferate on culture plastic substrate [30]. An alternative explanation for enhanced adipogenesis is that bFGF may exert a direct effect on differentiation or commitment level [26,27], e.g., by directly inducing or at least maintaining expression levels of PPARγ2 mRNA of MSCs. Basic FGF elevated adipogenesis the most following supplementation throughout the complete culture period. The addition of bFGF only during the proliferation phase (3 days or 15 days) also resulted in a distinct increase in differentiation. These data may be explained with both hypotheses, either the preferential proliferation of a subpopulation or by direct effects on the commitment level. However, the enhancing effects of bFGF on adipogenesis after supplementation to postconfluent cells (BBF) (Figs. 2 and 3) rather support the idea that a direct effect on the commitment level of MSCs contributes, at least in part, to the observed extent of adipogenesis. As a possible mechanism for a direct effect on the commitment level, Prusty et al. have suggested the MEK/ERK signaling pathway to play an important role in adipogenesis [31]. Basic FGF is known to be a potent activator of the MEK/ERK pathway [8]. Short-term exposure of bFGF (6 h) to 3T3-L1 preadipocytes promoted adipogenesis by phosphorylation of ERK1/2 and resulted in enhanced PPARγ and C/EBPα gene expression, irrespective of the presence of MEK1 inhibitors [31]. However, long-term treatment (more than 12–24 h) of these cells with bFGF, as done in this study, led to an

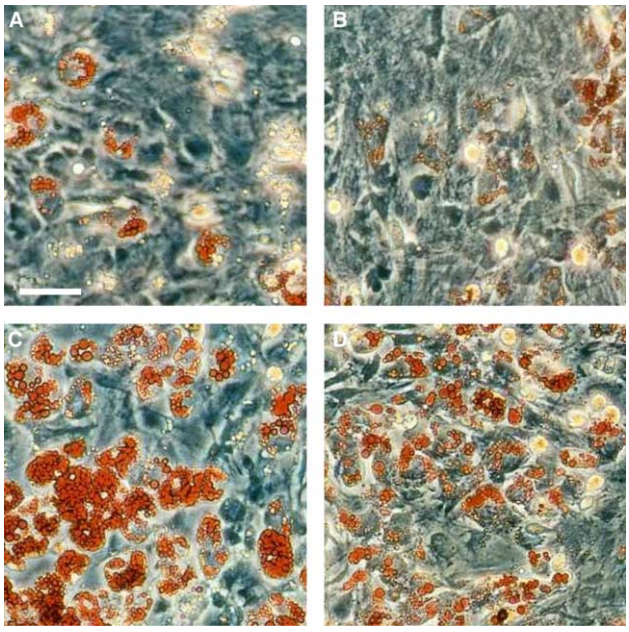

Fig. 5. Oil Red O staining of differentiated adipocytes on day 8 of differentiation. MSCs were induced with the hormonal cocktail in the absence (A) and in the presence of bFGF (during complete culture) (C) or with troglitazone in the absence (B) and the presence of bFGF (during complete culture) (D), respectively. Scale bar: 50 μM.

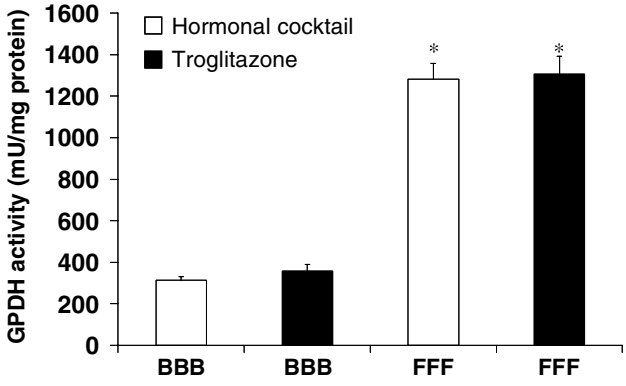

Fig. 6. Measurement of GPDH activity on day 3 of differentiation. MSCs were induced with either the hormonal cocktail (□) or with troglitazone alone (■). For each induction condition, cells were cultivated either in the absence (BBB) or in the presence of bFGF (during complete culture) (FFF). Asterisks indicate significantly elevated values as compared to the control group (BBB) ($n = 3$).

inhibition of adipogenic differentiation of 3T3-L1 cells [31]. Overall, the identification of the mechanism(s) by which bFGF exerts its effects on adipogenesis of MSCs may only be elucidated in full by cloning MSCs. Separated MSCs might allow the exact determination of the response of different subsets of cells to bFGF and adipogenic inducers.

Nevertheless, this study underlines the outstanding role of PPARγ in the adipogenic conversion of MSCs, demonstrated by the distinct responsiveness of MSCs to the PPARγ ligand troglitazone and the strikingly increased responsiveness provoked by bFGF. This finding is unexpected because thiazolidinediones alone are not sufficient to stimulate efficient differentiation, but require to be associated with glucocorticoids, insulin and/or IBMX in most culture systems including preadipocytic systems [32–35]. Thus, MSCs and especially bFGF-treated MSCs appear to respond in a different way to PPARγ activators such as troglitazone as compared to preadipocytic cells.

Moreover, bFGF may play a crucial role in the fate of bone marrow cells: It has been previously shown that under appropriate culture conditions bFGF can enhance osteogenic differentiation [12–14], and it is demonstrated here that given the required adipogenic environment bFGF also enhances adipogenesis. Thus, depending on microenvironmental stimuli and status of lineage-specific transcription factors, bFGF present in bone marrow [36] may function as a regulator in the control of adipogenesis and osteogenesis of MSCs. Furthermore, in several studies an inverse relationship between adipogenic and osteogenic differentiation in MSC cultures was demonstrated [37–39]. Therefore, bFGF may modulate the origin and progression of osteoporosis due to its capability to sensitize MSCs for an enhanced differentiation into either osteoblasts or adipocytes.

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