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Bone Morphogenetic Protein-2 Is a Regulator of Cell Adhesion

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Bone morphogenetic proteins (BMPs) are a group of peptide growth factors closely related to transforming growth factors-β. The BMPs are suggested to play an essential role in bone development and they are strong candidate molecules to be used clinically to improve fracture healing. BMPs are also involved in the differentiation of several other tissues during embryogenesis. Here, we show that human recombinant BMP-2 regulates cell–matrix interactions by modifying the expression of integrin-type receptors. The synthesis of α3 integrin was down-regulated by BMP-2 in two osteogenic sarcoma-derived cell lines, Saos-2 and HOS, and also in human fetal chondrocytes. BMP-2 had no effect on the expression of α1, α2, α5, α6, and αV integrins. BMP-2 reduced the expression of α3 integrin subunit at mRNA level. Laminin-5 was shown to be the ligand for $\alpha 3\beta 1$ integrin on Saos cells and BMP-2 decreased the ability of Saos cells to attach to it. These results suggest that BMP-2 has a specific effect on the $\alpha 3\beta 1$ integrin-mediated cell adhesion to laminin-5. Given the fact that BMP-2 is expressed in osteosarcomas, in addition to in bone, this mechanism is putatively important especially in bone development and tumors. We also studied the effect of BMP-2 on a human keratinocyte cell line, HaCaT. In HaCaT cells, the expression of α2 integrin was strongly down-regulated by BMP-2, whereas its effect on the expression of α3 integrin was smaller. We suggest that the effects of BMP-2 may be partially mediated by specifically altered cell adhesion.

INTRODUCTION

Cell growth and differentiation are regulated by soluble growth factors and also by extracellular matrix surrounding the cells. These two mechanisms are closely connected to each other. Especially, transforming growth factor-β1 (TGF-β1), the prototype molecule of the TGF-β superfamily, is a well-described regulator of the synthesis and degradation of extracellular matrix [1]. Bone morphogenetic proteins (BMPs) are a group of peptide growth factors closely related to TGFs-β. Seven BMPs have been isolated from vertebrates, namely BMP-2, -3, -4, -5, -6, -7, and -8 [2, 3]. The *Drosophila* homolog to BMPs, the decapentaplegic gene product, is a dorsalizing morphogen and is also implicated in the formation of appendage, eye, and gut [4–6]. Similarly, the vertebrate BMPs are, in addition to initiators of cartilage and bone formation, also regulators of the differentiation of other tissues. Furthermore, receptors for BMP-2 and -4 have been found not only in developing cartilage and bone but in many soft tissues in embryos, as well [7]. The fact that BMPs are involved in normal bone development is the basis for clinical trials developing mechanisms to improve bone fracture healing [8].

TGF-β1, as well as TGF-β2, regulates the interaction of many cell types with extracellular matrix and basement membranes by affecting cell surface expression of integrin-type adhesion receptors [9, 10]. Integrins are composed of two distinct transmembrane glycoprotein subunits, α and β, which are noncovalently linked to each other. Sixteen α chains, 8 β chains, and more than 20 different α/β complexes are known to date [11, 12]. TGF-β1 can increase the expression of all integrin subunits studied in at least some cell lines [10, 13–15]. It cannot usually induce the expression of α subunits not present in untreated cells. In osteogenic sarcoma cell lines it can up-regulate the expression of α2, α5, αV, β1, and β3 integrin subunits [10, 14]. In MG-63 osteosarcoma cells and keratinocytes the expression of α3 integrin is down-regulated by TGF-β [13, 16]. In some endothelial cell lines the expression of αV and β3 integrins is also reduced by TGF-β1 [17]. TGF-β induces the reorganization of collagen fibrils around cells, seen as contraction of floating collagen gels. This phenomenon is due to increased expression of $\alpha 2\beta 1$ collagen receptor integrin [18]. This is one example showing that TGF-β1-related changes in integrin expression also lead to alterations in cell behavior.

In spite of the important role of TGFs-β in the regulation of cell–matrix interaction the effects of BMPs on the cell adhesion apparatus have been mostly unknown. Here, we show that in osteosarcoma-derived cell lines, as well as in chondrocytes and fibroblasts, human recombinant BMP-2 reduces the expression of

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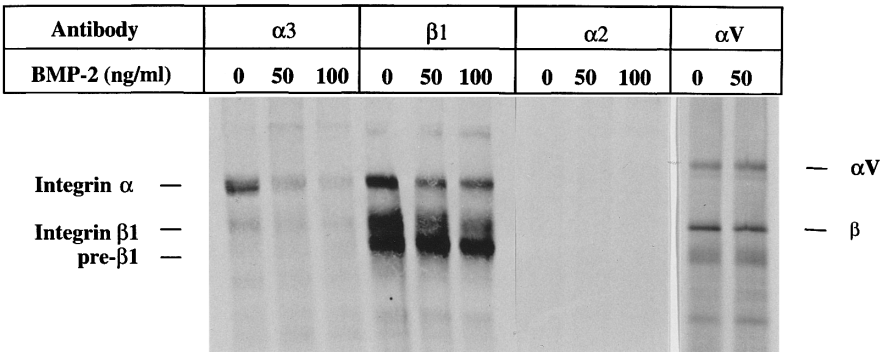

FIG. 1. The effect of BMP-2 on the expression of integrin subunits at protein level in Saos-2 cells. Human osteosarcoma cells, Saos-2, were incubated for 32 h with different concentrations of BMP-2, and the last 8 h with [^{35}S]methionine. Cellular integrins were immunoprecipitated with specific antibodies and analyzed by electrophoresis and fluorography.

$\alpha3\beta1$ integrin and, furthermore, it can decrease $\alpha3\beta1$ integrin-mediated cell adhesion to laminin-5. In human keratinocyte cell line, HaCaT, BMP-2 reduces the expression of $\alpha2$ integrin, but has smaller effect on $\alpha3$ integrin.

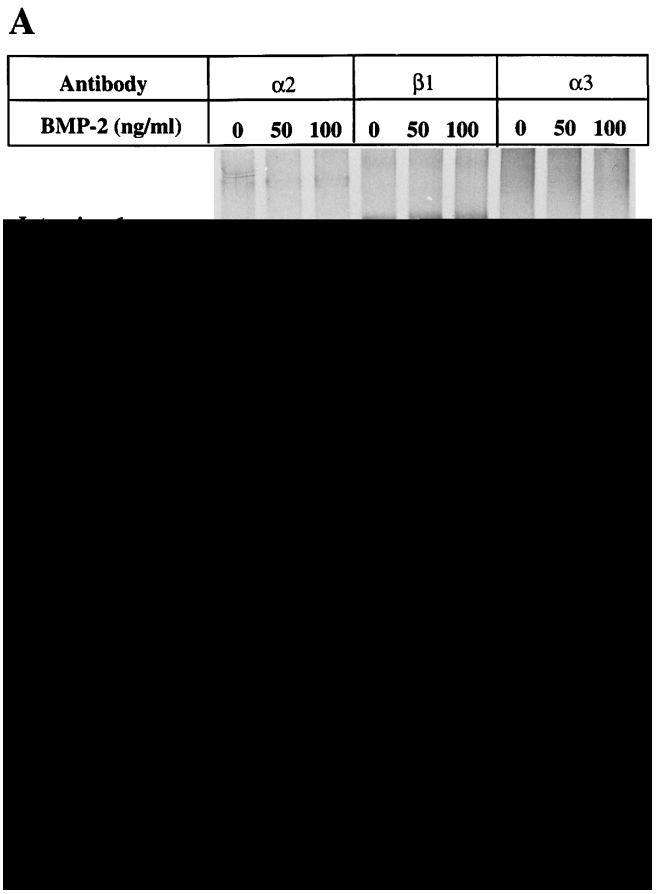

FIG. 2. Effect of BMP-2 on the expression of integrin subunits at protein level in HOS cells. Human osteosarcoma cells, HOS, were incubated for 32 h with different concentrations of BMP-2, and the last 8 h with [^{35}S]methionine. Cellular integrins were immunoprecipitated with specific antibodies and analyzed by electrophoresis and fluorography.

MATERIALS AND METHODS

BMP-2. Human recombinant BMP-2 was from Genetics Institute, Cambridge, MA.

Cell lines. The following human osteosarcoma cell lines were used: MG-63, HOS, and Saos-2. They were obtained from the American Type Culture Collection (Rockville, MD). Human keratinocytes, HaCaT cells, were originally established by Dr. Fusenig (Deutsches Krebsforschungszentrum Heidelberg, Germany; [19]). Human primary fetal chondrocytes were obtained as described previously [20]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 2 m*M* glutamine, 100 IU/ml penicillin-G, and 100 μg/ml streptomycin. For the experiments, cells were maintained in DMEM without FCS for 18 h before BMP-2 was added and the incubation was continued for 24 or 48 h.

Immunoprecipitation and antibodies. Polyclonal rabbit antisera against human $\beta1$ [10], $\alpha2$, and $\alpha3$ integrin subunits [21] and monoclonal antibodies against $\alpha5$ [22] and αV [23] integrin subunits were used in immunoprecipitation assays. Cell cultures were incubated overnight in serum-free medium and then with 50 ng/ml BMP-2 for 16 h. Cells were metabolically labeled with 100 μCi/ml [^{35}S]-methionine (Tran^{35}S-label; ICN Biomedicals, Inc., Irvine, CA) for 8 h in methionine-free minimum essential medium. Cell monolayers were rinsed on ice with a solution containing 150 m*M* NaCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 25 m*M* Tris–HCl (pH 7.4) and then detached by scraping. Cell pellets were obtained by centrifugation at 500*g* for 5 min. Cells were solubilized in 200 μl of the same buffer containing 100 m*M* *n*-octyl-β-D-glucopyranoside (Sigma) on ice with occasional vortexing. Insoluble material was removed by centrifugation at 10,000*g* for 5 min at 4°C. Radioactivity in cell lysates was counted, and an equal amount of radioactivity was used in immunoprecipitation assays. Triton X-100 (0.5% v/v) and bovine serum albumin (0.5 mg/ml) were added to the supernatants, which were then precleaned by incubation with 50 μl of packed protein A–Sepharose (Pharmacia LKB Biotechnology, Inc., Uppsala, Sweden). Supernatants were immunoprecipitated with anti-integrin antibodies for 12 h at 4°C. Immune complexes were recovered by binding to protein A–Sepharose and washing the beads four times with 25 m*M* Tris-buffered isotonic saline (pH 7.4) containing 0.5% Triton X-100 and 1 mg/ml bovine serum albumin and twice with 0.5 *M* NaCl and 25 m*M* Tris–HCl (pH 7.4). The immunoprecipitates were analyzed by electrophoresis on sodium dodecyl sulfate-containing 6% polyacrylamide gels under nonreducing conditions followed by fluorography.

Integrin bands were quantified from fluorograms by the Microcomputer Imaging Device Version M4 (Imaging Research, Inc., St. Catharines, Ontario, Canada).

Northern blot hybridizations. Total cellular RNA was isolated by using the guanidium thiocyanate/CsCl method [24]. RNAs were separated in formaldehyde-containing agarose gels, transferred to nylon membranes (Zeta-Probe; Bio-Rad Laboratories, Richmond, CA), and hybridized with ^{32}P-labeled (Amersham, UK) cDNA probes. The following cDNAs were used: human α2 integrin [25], human α3 integrin [26], human αV integrin [27], human α6 integrin [28], human osteonectin [29], human tissue nonspecific alkaline phosphatase [30], human proα1(I) collagen [31], and rat glyceraldehyde-3-phosphate dehydrogenase [32].

Cell adhesion assays. The coating of a 96-well immunoplate (Maxi Sorp; Nunc, Denmark) was done by exposure to 0.2 ml of phosphate-buffered saline (pH 7.4) containing 4–5 μg/cm^2 type I collagen (from lathyric rat skin; Boehringer Mannheim), type IV collagen (native type IV collagen from mouse EHS tumors; Sigma), laminin-1 (mouse EHS; Chemicon), or vitronectin (human plasma; Chemicon) for 12 h at 4°C. To coat the wells with laminin-5, they were exposed to conditioned medium from 804G cells (a kind gift from Dr. M. Hormia, Helsinki, Finland) as described [33]. Bovine serum albumin was used to measure the nonspecific binding. Residual protein absorption sites in all wells were blocked with 1% bovine serum albumin in phosphate-buffered saline for 1 h at 37°C. Confluent cell cultures were detached by using 0.01% trypsin and 0.02% EDTA. Trypsin activity was inhibited by washing the cells with 1 mg/ml soybean trypsin inhibitor (Sigma). Cells (10,000) were suspended in DMEM and transferred into each well and incubated at 37°C for 45 min. Nonadherent cells were removed by rinsing the wells with medium. Adherent cells were fixed with 2% paraformaldehyde, stained with 0.5% crystal violet in 20% ethanol, and washed with distilled water. The immunoplates were allowed to air-dry, and crystal violet in the cell layer was dissolved into 10% acetic acid and measured spectrophotometrically at 600 nm with Multiscan Plus (Lab-Systems).

RESULTS

BMP-2 suppresses the expression of α3 integrin subunit. The effect of BMP-2 on integrin expression was studied by using metabolic labeling with [^{35}S]-methionine, immunoprecipitations with specific antibodies, and electrophoresis. BMP-2 suppressed the synthesis of α3 integrin protein (10% of control; Fig. 1) and had no effect on the other integrin subunits, including α1, αV (Fig. 1), α5, and α6 (not shown). Saos-2 cells are normally negative for $\alpha 2\beta 1$ integrin and BMP-2 could not induce its expression (Fig. 1). An interesting detail in Fig. 1 is that the amount of mature β1 subunit (130-kDa band) is reduced compared to the amount of

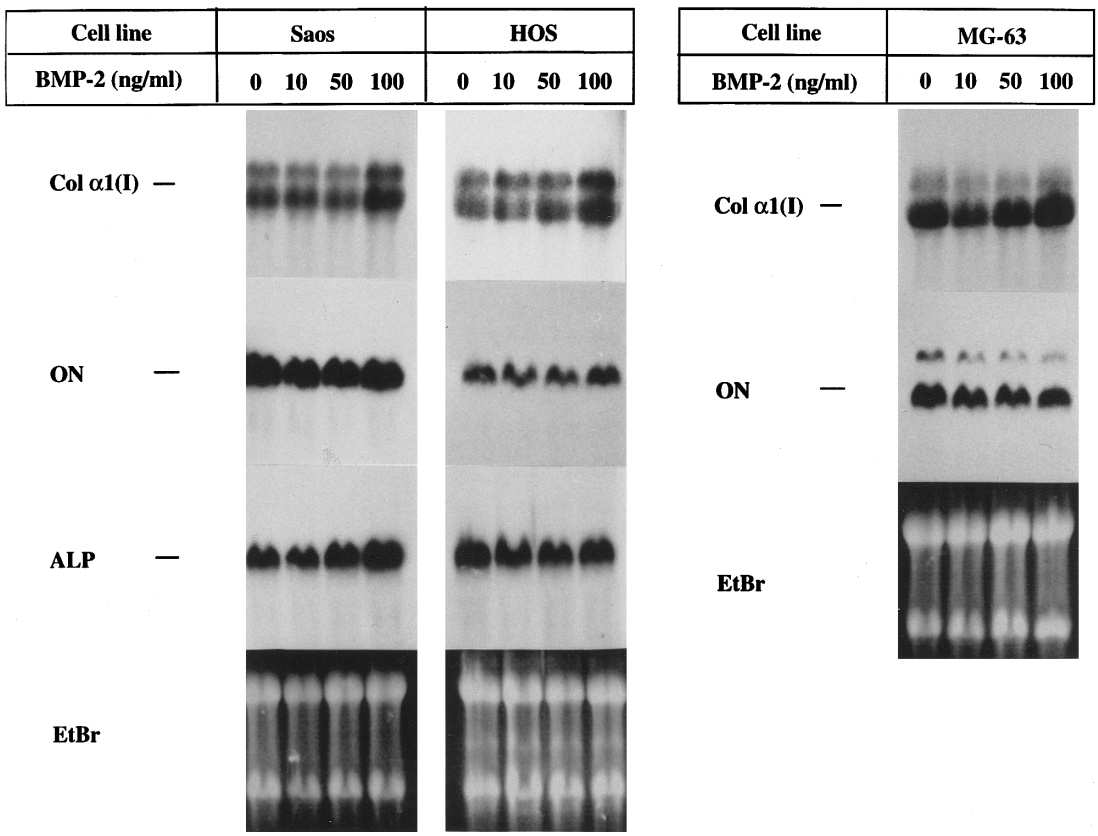

FIG. 3. The effect of BMP-2 on the expression of bone differentiation markers or bone extracellular matrix molecules at mRNA level. Saos-2, HOS, and MG-63 cells were treated for 24 h with 10, 50, or 100 ng/ml BMP-2 in serum-free medium. Total RNA was isolated and Northern blotting on nylon membranes was done. In hybridization the following cDNAs were used: osteonectin (ON), alkaline phosphatase (ALP), and α1(I) collagen.

precursor $\beta 1$ (110-kDa band). This is in accordance with our previous observation that in mesenchymal cells the maturation rate of the precursor $\beta 1$ integrin is dependent on the total number of α subunits available [10, 34]. In Saos-2 cells, when the amount of their most abundant α integrin subunits is decreased, at the same time the rate of maturation of the $\beta 1$ subunit pool seems to be decelerated. The effect of BMP-2 on the integrin expression in other osteogenic cell lines, HOS and MG-63, was also studied by using immunoprecipitations. HOS cells express a very similar pattern of integrins compared to Saos-2 cells. BMP-2 also had a similar effect on their integrin synthesis; it decreased the synthesis of $\alpha 3$ integrin subunit (18% of control), but had no effect on the other integrins (Fig. 2). The integrin pattern on MG-63 cells is different from the two other cell lines: The major $\beta 1$ integrin on MG-63 cells is $\alpha 3\beta 1$ heterodimer. There are also small amounts of $\alpha 2\beta 1$ and $\alpha 5\beta 1$ integrins present, but $\alpha 1\beta 1$ integrin is not usually detectable [10, 35]. BMP-2 had no effect on their integrin expression (not shown). TGF-β is a potent regulator of collagen gene expression also in the osteogenic cell lines, unlike BMP-2 (Fig. 3).

BMP-2-induced decrease in the expression of $\alpha 3\beta 1$ integrin is not associated with altered expression of bone differentiation markers. To study whether the short treatment of Saos cells with BMP-2 alters the expression of bone differentiation markers or bone extracellular matrix molecules we used Northern blot hybridizations and measured the mRNA levels of $\alpha 1$(I) collagen, osteonectin, and alkaline phosphatase. The BMP-2 concentration of 100 ng/ml could slightly induce the expression of alkaline phosphatase (1.5-fold) and $\alpha 1$(I) collagen (1.1-fold), whereas smaller concentrations (10 and 50 ng/ml) were ineffective (Fig. 3). Similar results were seen when another human osteosarcoma-derived cell line, HOS, was used (Fig. 3), whereas in a third cell line, MG-63, BMP-2 had no effect (Fig. 3). This cell line expresses alkaline phosphatase mRNA in such a low level that they could not be measured by Northern hybridizations (not shown).

BMP-2 down-regulates $\alpha 3$ integrin subunit expression at mRNA level. In MG-63 cells the regulation of both $\alpha 2$ and $\alpha 3$ subunits by TGF-$\beta 1$ takes place at the mRNA level [10]. Here, we studied the effects of BMP-2 on integrin expression by Northern blot hybridization

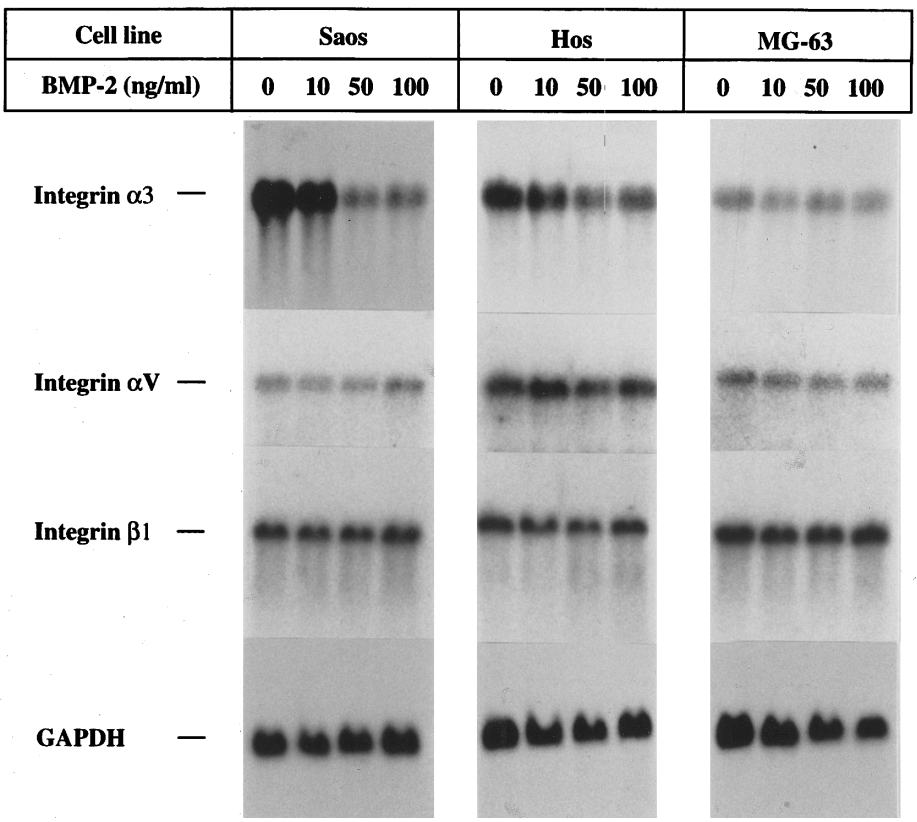

FIG. 4. The effect of BMP-2 on the expression of $\alpha 3$, αV, and $\beta 1$ integrin subunits in human osteosarcoma-derived cell lines, Saos-2, HOS, and MG-63. Cells were treated for 24 h with 10, 50, and 100 ng/ml BMP-2 in serum-free medium. Total RNA was isolated and Northern blotting on nylon membranes was done.

in Saos-2, MG-63, and HOS cells. BMP-2 decreases the expression of $\alpha 3$ integrin subunit at the mRNA level in Saos-2 (20% of control) and HOS cells (70% of control) (Fig. 4). The concentration needed was 50 ng/ml. There was no effect on the expression of the integrin subunits in MG-63 cells.

BMP-2 suppresses the expression of $\alpha 2$ and $\alpha 3$ integrin subunits in human keratinocyte cell line, HaCaT, and $\alpha 3$ integrin expression in human fetal chondrocytes. To test whether the effect of BMP-2 on integrin expression was limited to osteosarcoma cells we tested its effect also on human fetal chondrocytes and the human keratinocyte cell line HaCaT. In chondrocytes BMP-2 decreased the mRNA levels of $\alpha 3\beta 1$ integrin (45% of control; Fig. 5). In HaCaT cells, however, the down-regulation of $\alpha 3$ integrin mRNA levels was smaller (55% of control), as was also the decrease in $\alpha 3$ integrin synthesis studied at protein level (70% of control; Fig. 6). Instead, in HaCaT cells BMP-2 strongly decreased the mRNA levels (20% of control) and protein synthesis of $\alpha 2$ integrin (30% of control; Fig. 6). BMP-2 decreased the expression of $\beta 1$ integrin mRNA levels, as well (not shown). The precursor $\beta 1$ integrin pool was almost missing in HaCaT cells, whereas precursor $\alpha 3$ integrin pool was prominently present, suggesting that in epithelial cells the number of $\beta 1$ subunits instead of α subunits might be rate limiting.

Saos-2 cells use $\alpha 3\beta 1$ integrin in cell adhesion to laminin-5. Integrin-type receptors mediate the adhesion of cells to most matrix proteins. The most important ligand of $\alpha 3\beta 1$ integrin may be laminin-5 [36]. In the absence of isolated laminin-5 we used conditioned medium from 804G cells containing laminin-5, but no other adhesion proteins for HaCaT cells [33]. In Fig. 7 we show that HaCaT cell adhesion to laminin-5 can be prevented by using a specific anti-$\alpha 3$ antibody (P1B5), and that HaCaT cells seem to use $\alpha 3\beta 1$ integrin also in adhesion to laminin-1. HaCaT cell adhesion to fibronectin or type I collagen was not affected by this antibody. In Saos-2 cells the antibody against $\alpha 3$ integrin could block their attachment to laminin-5, whereas it had no effect on the cell adhesion to fibronectin, laminin-1, or type I collagen (Fig. 7). Thus, in Saos-2 cells the ligand-binding specificity of $\alpha 3\beta 1$ integrin was found to be narrow when compared to HaCaT cells.

BMP-2 decreases the ability of Saos cells to attach to laminin-5. Saos-2 cells were kept for 48 h in serum-free medium with 10 ng/ml BMP-2 before the adhesion of cells to surfaces coated with type I and IV collagens, fibronectin, vitronectin, or laminin-1 was tested. As shown in Table 1 BMP-2 did slightly increase the adhesion of Saos cells to these substrata. However, BMP-2 reduced the ability of Saos cells to attach to laminin-5. This reduction was even more obvious when the number of laminin-5-adherent cells was compared to the number of cells attached to other matrices (Table 1).

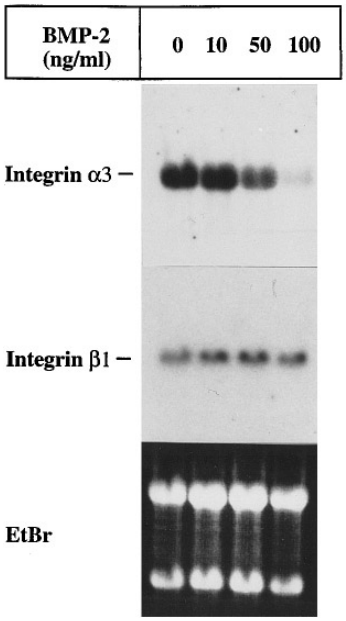

FIG. 5. The effect of BMP-2 on the expression of $\alpha 3$ integrin subunit in human chondrocytes. Cells were treated for 24 h with different concentrations of BMP-2 in serum-free medium. Total RNA was isolated and Northern blotting was done on nylon membranes.

DISCUSSION

The data presented here show that BMP-2 is a regulator of cell adhesion. It seems to modify the expression of integrin-type adhesion receptors and the attachment of cells to laminin. Laminins are a family of connective tissue proteins, found predominantly in basement membranes. The association of different α, β, and γ subunits into heterotrimers gives rise to the different laminins [37]. Laminin-1 is the prototype molecule of the family and most information about the biology of laminins is based on studies using laminin-1. Laminin-5 is composed of $\alpha 3$, $\beta 3$, and $\gamma 2$ subunits. It was first described as epiligrin, kalinin, or nicein. It is essential for the formation of hemidesmosomes and its mutation causes one form of epidermolysis bullosa [38]. In wound healing the fact that laminin-5 is expressed under migrating keratinocytes, while many other basement membrane proteins are not present, suggests that cells might use laminin-5 as a platform in migration [39]. In cell culture, however, keratinocytes cannot migrate on laminin-5 [40], whereas some cancer cells are able to do it [41].

To date nine integrin heterodimers have been reported to interact with laminin-1 (reviewed in Ref. [42]). Some of them might not recognize the other laminins, e.g., in some cell lines the collagen receptor integrins $\alpha 1\beta 1$ and $\alpha 2\beta 1$ bind to laminin-1, but the domain recognized by $\alpha 1\beta 1$ integrin is missing from other lami-

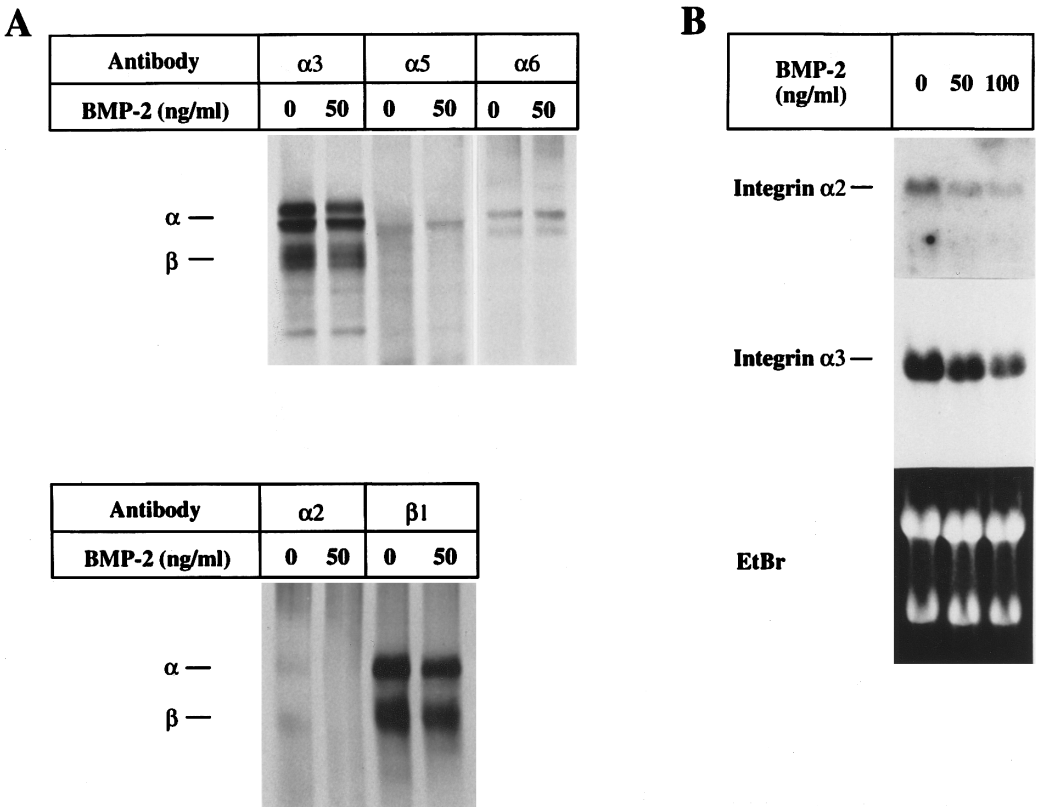

FIG. 6. The effect of BMP-2 on the expression of integrin subunits $\alpha 2$ and $\alpha 3$ in human keratinocytes, HaCaT cells. (A) HaCaT cells were incubated for 32 h with 50 ng/ml BMP-2, the last 8 h with [^{35}S]methionine. Cellular integrins were immunoprecipitated with specific antibodies and analyzed by electrophoresis and fluorography. (B) Cells were treated for 24 h with different concentrations of BMP-2 in serum-free medium. Total RNA was isolated and Northern blotting was done on nylon membranes.

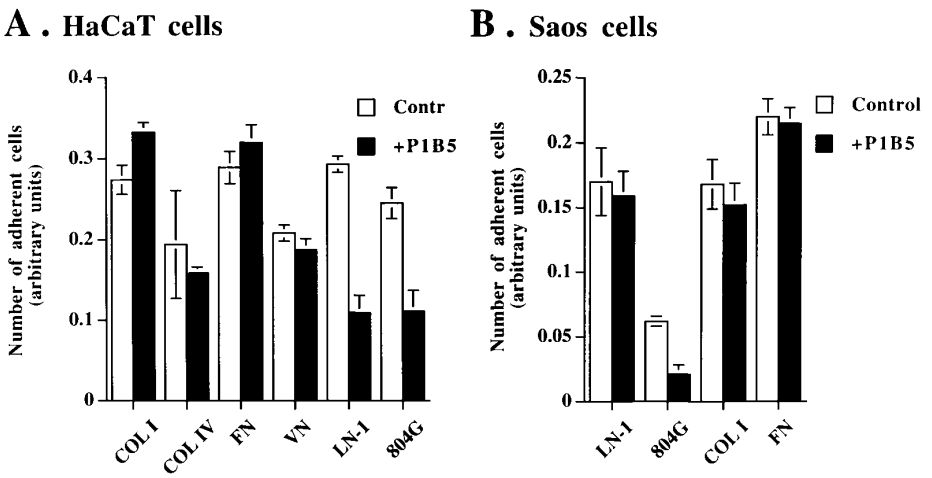

FIG. 7. Cell adhesion of human keratinocyte HaCaT cells (A) and human osteosarcoma Saos-2 cells (B) with or without a functional antibody against $\alpha 3$ integrin (P1B5). Cells were treated for 48 h with 10 or 50 ng/ml BMP-2 in serum-free medium. They were detached and replaced on different matrices with P1B5. After 1 h the nonadherent cells were washed out and the adherent ones were stained with crystal violet. Cell-bound stain was dissolved and measured spectrophotometrically. Matrices tested were laminin-1 (LN-1), laminin-5 (804G conditioned medium), fibronectin (FN), type I collagen (COL I), and vitronectin (VN).

TABLE 1

Effect of BMP-2 on Saos-2 Cell Adhesion to Various Matrix Molecules

Experiment 1

Substrate	Number of adherent cells[a]		Number of adherent cells compared to number of vitronectin-adherent cells[a]	
	Control	BMP-2	Control	BMP-2
Type I collagen	0.10 ± 0.03	0.19 ± 0.02 (190%)	0.59 ± 0.16	0.84 ± 0.07 (142%)
Type IV collagen	0.07 ± 0.01	0.12 ± 0.01 (171%)	0.39 ± 0.07	0.54 ± 0.03 (138%)
Fibronectin	0.20 ± 0.01	0.27 ± 0.01 (135%)	1.21 ± 0.04	1.19 ± 0.05 (100%)
Vitronectin	0.17 ± 0.01	0.23 ± 0.03 (135%)		
Laminin-1	0.17 ± 0.01	0.27 ± 0.02 (159%)	0.98 ± 0.17	1.18 ± 0.10 (120%)
Laminin-5 (804G medium)	0.054 ± 0.003	0.037 ± 0.01 (69%)	0.32 ± 0.03	0.16 ± 0.04 (50%)

Experiment 2

Substrate	Number of adherent cells[b]	
	Control	BMP-2
Laminin-5 (804G medium)	195 ± 84	123 ± 32 (63%)

[a] Arbitrary units, based on the amount of crystal violet bound to adherent cells.
[b] Cell numbers per microscopic field.

nins [43]. Laminin-5 has at least three different cellular receptors, namely $\alpha3\beta1$, $\alpha6\beta1$, and $\alpha6\beta4$ integrins. Integrin $\alpha3\beta1$ might also interact with the RGD sequence in fibronectin and probably also in denatured collagens. However, laminin-5 may be the most important ligand for $\alpha3\beta1$ integrin [36]. Here, we suggest that the ligand-binding function of $\alpha3\beta1$ integrin is dependent on the cell line. In HaCaT keratinocytes it seems to be a receptor for both laminin-1 and laminin-5, but in Saos cells it binds to laminin-5 only. In the cell adhesion assays we have taken advantage of the fact that rat 804G cells secrete laminin-5 to their culture medium, and that the conditioned medium from these cells does not contain significant amounts of other adhesion molecules [33].

BMP-2 decreased Saos cell adhesion to laminin-5 by down-regulating the expression of $\alpha3\beta1$ integrin. Other growth and differentiation factors can regulate the expression of integrins, as well, and the modification of cell–matrix interactions is probably an important target of growth factor action, which often leads to further changes in cell phenotype and behavior. Previously, platelet-derived growth factor has been shown to induce the expression of $\alpha2$, $\alpha3$, $\alpha5$, and $\beta1$ integrin subunits [44, 45]. The effect of TGF-β on $\alpha3\beta1$ integrin expression is cell-line dependent. In fibroblasts TGF-β increases $\alpha3\beta1$ integrin expression [10], but in MG-63 osteosarcoma cells [13] and keratinocytes [16] TGF-β decreases it. The intracellular molecular pathways regulating the expression of $\alpha3$ integrin gene are not known. The effect of BMP-2 was seen at the mRNA level. Similarly, the effect of TGF-β on $\alpha3$ integrin gene expression is pretranslational [13]. Many integrin genes are regulated at the level of transcription, but the essential promoter elements of $\alpha3$ integrin subunit gene are unknown.

In osteogenic sarcoma cells the effect of BMP-2 on integrins was limited to the decrease in $\alpha3\beta1$ integrin expression. Down-regulation of $\alpha3\beta1$ integrin caused decreased cell adhesion to laminin-5, suggesting that BMP-2 may be an important regulator of cell–basement membrane interaction. In Saos cells $\alpha3\beta1$ integrin was not a receptor for laminin-1, whereas its interactions with other laminin subtypes are incompletely studied and it is very possible that its ability to bind laminins is not limited to laminin-5. The expression patterns of different laminin subtypes during bone development must be known better before it is possible to speculate about the occasions where cell–laminin interaction may be important. Interestingly, many osteosarcomas express BMP-2 [46]. BMP-2 is expressed also in human adenocarcinoma cells [47], malignant fibrous histiocytomas, and spindle cell sarcomas [46]. It is an important possibility that BMP-2 regulates the migration and invasion of malignant cells. Previously, laminin-5 has been shown to promote carcinoma cell migration [41] and different integrin-type receptors are likely to play an essential role in the process. Furthermore, the fact that the integrins generate signals regulating the expression of matrix metalloproteinases (MMPs) [22] emphasizes their role in cancer cell invasion [48]. Indeed, we have shown previously that anti-

bodies against $\alpha 3$ integrin subunit stimulate the expression of MMP-9 (92-kDa gelatinase) in keratinocytes [49].

The fact that BMP-2 regulated the expression of $\alpha 3\beta 1$ integrin also in cell lines other than Saos and HOS, namely human primary chondrocytes and HaCaT keratinocytes, makes it possible to suggest that this is a more general phenomenon during development. Neither is the role of BMP-2 limited to the regulation of bone formation, but it is also involved in the differentiation of many other tissues [50]. In epithelial HaCaT cells BMP-2 had only a small effect on the expression of $\alpha 3$ integrin, whereas it decreased more significantly the amount of $\alpha 2$ integrin subunits. Integrin $\alpha 2\beta 1$ is a receptor for several collagen types [51] and laminin-1 [52]. This effect is different to one previously described for TGF-β, which can increase the expression of $\alpha 2\beta 1$ integrin and decrease the expression of $\alpha 3\beta 1$ integrin in epidermal keratinocytes [16]. TGFs-β and BMP-2 bind to distinct cell surface receptor systems, but the mechanisms of the receptor action and also the activation of downstream elements have similarities [53]. However, their effects on integrin expression seem to be independent and different. Furthermore, the effect of BMP-2 on integrin expression is cell-type specific, and it seems to decrease the expression of integrins rather than to enhance it. Given the importance of integrins in processes like cell migration, adhesion, and differentiation, this phenomenon may have interesting consequences. Furthermore, programmed cell death can be triggered during development also by altered or lost adhesion of cells to their matrix [54]. In embryonic tissues BMPs regulate the developmentally programmed cell death, inducing, for example, in chick limb the interdigital apoptosis required for normal embryogenesis [55]. In general the molecular mechanisms of BMP action during embryogenesis are incompletely known. Our data suggest that some of the BMP-related effects might be mediated by altered cell adhesion.

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