

Invited Review

Cellular and molecular alterations of osteoblasts in human disorders of bone formation

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Summary. Osteogenesis is a complex process characterized sequentially by the commitment of precursor cells, the proliferation of osteoprogenitor cells, the differentiation of pre-osteoblasts into mature osteoblasts and the apposition of a calcified bone matrix. Recent advances in cell and molecular biology have improved our knowledge of the cellular and molecular mechanisms controlling the different steps of bone formation in humans. Using *ex vivo/in vitro* studies of disorders of bone formation, we showed that the recruitment of osteoprogenitor cells is the most important step controlling the rate of bone formation in both rodents and humans. Accordingly, treatments stimulating osteoblast recruitment were found to increase bone formation in experimental models of osteopenic disorders. Using models of human osteoblastic cells, we identified the profile of phenotypic markers expressed during osteoblast differentiation, and found that hormones and growth factors control osteoblastic cell proliferation and differentiation in a sequential and coordinate manner during osteogenesis *in vitro*. Our recent evaluation of the phenotypic osteoblast abnormalities induced by genetic mutations in the $G_{\alpha s}$ and FGFR-2 genes led to the characterization of the role of these genes in the alterations of osteoblast proliferation and differentiation in humans. These studies at the histological, cellular and molecular levels provided new insight into the mechanisms that are involved in pathological bone formation in humans. It is expected that further determination of the pathogenic pathways in metabolic and genetic abnormalities in human osteoblasts will help to identify novel target genes and to conceive new therapeutic tools to stimulate bone formation in osteopenic disorders.

Key words: Osteoblast, Bone formation, Pathology, Mutations, Human

Introduction

Bone formation is a complex process which involves interactions between cells of the osteoblastic lineage, bone matrix proteins and a variety of local regulatory factors. At the histological level, endosteal bone formation sites appear as complex structures composed of a calcified and uncalcified bone matrix synthesized by mature osteoblasts (Fig. 1). This structure results from complex sequential events involving the recruitment of competent cells, the differentiation into committed cells and the adequate function of mature osteoblasts at the right time and space. The formation of bone matrix requires, therefore, the coordination of several mechanisms controlling cell function and matrix formation. In addition, several interactions occur between marrow stromal cells, osteoblasts, the matrix itself and other bone cells, and the osteoblasts are believed to play a central role in the control of bone remodeling. Thus, abnormalities occurring during the recruitment or differentiation/activity of bone forming cells may lead to local or generalized disorders of bone formation.

For many years, my laboratory has developed studies to delineate the mechanisms controlling the biology and pathology of osteoblasts at the endosteal level. We initially used histological and histomorphometrical methods to analyze bone formation at the endosteal level. The general histological aspects of endosteal bone formation (Marie, 1982) and the regulation of endosteal osteoblasts by minerals, hormones and local factors have been previously reviewed (summarized in Marie et al., 1994). Approaches were then developed to investigate the cellular mechanisms controlling endosteal osteoblast differentiation and osteogenesis in animals and humans (for review, see Lomri and Marie, 1996; Marie, 1998). The aim of the present article was to review recent data on the cellular and molecular alterations of osteoblasts in human disorders of bone formation, based on data obtained in my laboratory and by other investigators. After having described the general methodological approaches used to study the osteoblast function, the

main aspects of osteoblast biology and the recent analysis of human osteoblastic disorders will be summarized.

Methodological approaches to analyse the osteoblast function

Several approaches at the tissue, cellular and molecular levels have been developed to study the function of osteoblasts and bone formation in normal and pathological conditions (Table 1). This includes histological analysis of intact animals, organ cultures of calvaria, osteoblastic cell cultures of periosteal and endosteal origin, analysis of gene expression and, more recently, experiments aimed at inducing deletion or overexpression of specific genes in vitro and in vivo (Table 1). The initial histological and histomorphometric analyses of bone helped to delineate the mechanisms controlling bone formation at the tissue level. These studies proved to be useful to analyse the osteoblast function and alterations with age and diseases in humans (Rasmussen and Bordier, 1974; Meunier et al., 1979; Parfitt et al., 1983). In rodents, histomorphometric studies of bone also led to the analysis of bone formation in normal and pathological conditions (Baylink and Liu, 1979; Wronski et al., 1985) and to the determination of the control of endosteal osteoblasts by calciotropic hormones and minerals (reviewed in Marie et al., 1994).

The development of in vitro models of osteoblastic cell cultures allowed the determination of the control of osteoblasts at the cellular level (Table 1). Periosteal osteoblastic cells derived from calvaria have been widely used as a model to study osteoblast differentiation in rodents (Bellows et al., 1991; Stein and Lian, 1993). In this model, calvaria cells first proliferate, then differentiate and form a mineralized matrix in vitro. We recently developed an original model using human

neonatal calvaria cells, which proved to be useful to study the regulation of human osteogenesis (de Pollak et al., 1997). Osteoblastic cells were also derived from trabecular bone, which allowed the analysis of the biology and pathology of endosteal cells in animals (Lomri et al., 1988; Modrowski and Marie, 1993) and humans (Robey and Termine, 1985; Beresford et al., 1986; Marie et al., 1989a). Thereafter, the development of comparative ex vivo/in vitro analyses of endosteal bone formation and osteoblastic cells proved to be useful

Table 1. Methodological approaches to study bone formation and osteoblasts

In vivo/Ex vivo
Histology, Microradiography, Histomorphometry ¹
Immunohistochemistry, In situ hybridization ²
Biochemistry ³
Comparative ex vivo/in vitro analyses ⁴
Cell cultures
Rat/mice calvaria cells ⁵ , Human calvaria cells ⁶
Rat/mice trabecular cells ⁷ , Human trabecular cells ⁸
Rat/mice stromal cells ⁹ , Human stromal cells ¹⁰
Molecular analysis
Gene expression ¹¹
Antisense strategy ¹²
Deletion/overexpression ¹³
Differential display ¹⁴

¹: Baylink and Liu, 1979; Meunier et al., 1979; Parfitt et al., 1983; Wronski et al., 1985; Marie et al., 1994. ²: Bianco et al., 1989; Ikeda et al., 1995. ³: Eastell et al., 1988. ⁴: Marie, 1994, 1995. ⁵: Aubin and Liu, 1996; Canalis et al., 1991; Stein and Lian, 1993. ⁶: De Pollak et al., 1997. ⁷: Lomri et al., 1988; Modrowski and Marie, 1993. ⁸: Beresford et al., 1986; Robey and Termine, 1985; Marie et al., 1989a. ⁹: Maniopoulos, 1988; Malaval et al., 1994; Machwate et al., 1995c. ¹⁰: Beresford et al., 1989; Cheng et al., 1994; Fromigué et al., 1997, 1998. ¹¹: Rodan and Noda, 1991. ¹²: Machwate et al., 1995b; Modrowki et al., 1997. ¹³: Wang et al., 1992; Grigoriadis et al., 1993; Ducy et al., 1997. ¹⁴: Mason et al., 1997; Ryoo et al., 1997; Yotov et al., 1998.



Fig. 1. Histological aspect of mature osteoblasts forming bone matrix (m), along trabecular bone (b) surface. x 250

to identify osteoblastic abnormalities in various pathologies of bone formation in animal models and humans (summarized in Marie, 1994, 1995). More recently, the development of marrow stroma cell cultures allowed to study the differentiation of osteoblasts from their precursors in rat (Maniopoulos et al., 1988; Malaval et al., 1994) and human bone marrow (Beresford, 1989; Cheng et al., 1994; Fromigué et al., 1997). These different models helped to provide information on the sequential events involved in osteoblast recruitment and differentiation. In addition, the molecular analysis of gene transcription *in vitro* and *in situ* led to the identification of the main regulatory factors acting at the different steps of osteoblastic cell proliferation and differentiation (Reviewed in Rodan and Noda, 1991). The recent application of molecular approaches such as mRNA differential display analyses (Mason et al., 1997; Ryoo et al., 1997; Yotov et al., 1998) appears to be promising tool to identify novel genes involved in osteoblast differentiation. Finally, the induction of overexpression or suppression of genes *in vitro* using antisense strategies, or *in vivo* in transgenic mice, and the skeletal changes induced by genetic mutations in mice and humans led to the identification of genes that determine skeletal patterning, mesenchymal cell determination and osteoblast differentiation in normal and genetic diseases (Table 2). Altogether, these methodologies provided complementary informations and led to significant progress in the biology and pathology of osteoblasts and bone formation at the tissue, cellular and molecular levels.

Osteoblast biology and bone formation

Bone development

Skeletal formation results from complex events including skeletal patterning during development,

determination and commitment of cells of the osteoblastic lineage, proliferation of pre-osteoblastic cells and their differentiation into mature osteoblasts. Developmental studies showed that skeletal patterning is directed by Hox genes, that are expressed transiently and locally during development (Morgan and Tabin, 1993). Skeletal defects produced by gain or loss of Hox genes indicate that these genes regulate limb patterning by inducing mesenchymal cell proliferation and condensation (Johnson and Tabin, 1997). Multiple factors may be involved in the early steps of skeletal cell engagement into a specific lineage. The induction of mesenchymal cell commitment toward cartilage or bone differentiation during development appears to be under the control of particular local inductors. Signals such as Fibroblast Growth Factors (FGFs), Sonic hedgehog (shh) and Bone Morphogenetic Proteins (BMPs) appear to influence the growth and differentiation of skeletal cells during development (reviewed in Johnson and Tabin, 1997). Members of the Transforming Growth Factor- β (TGF- β) family, such as BMPs appear to play a key role in the control of mesenchymal condensation (Lyons et al., 1991; Wozney, 1992). These factors may act, in part, by inducing or repressing the expression of tissue-specific transcription factors that play a role in the

Table 2. Genetic mutations in osteoblastic cells inducing phenotypic alterations in bone formation.

MUTATION	ALTERATION
Col-1	osteogenesis imperfecta
msx-2	craniosynostosis
Gsa	fibrous dysplasia
twist	craniosynostosis
M-CSF	osteopetrosis
FGFR-1, -2	craniosynostosis

Only a few mutations affecting cells of the osteoblastic lineage are indicated.

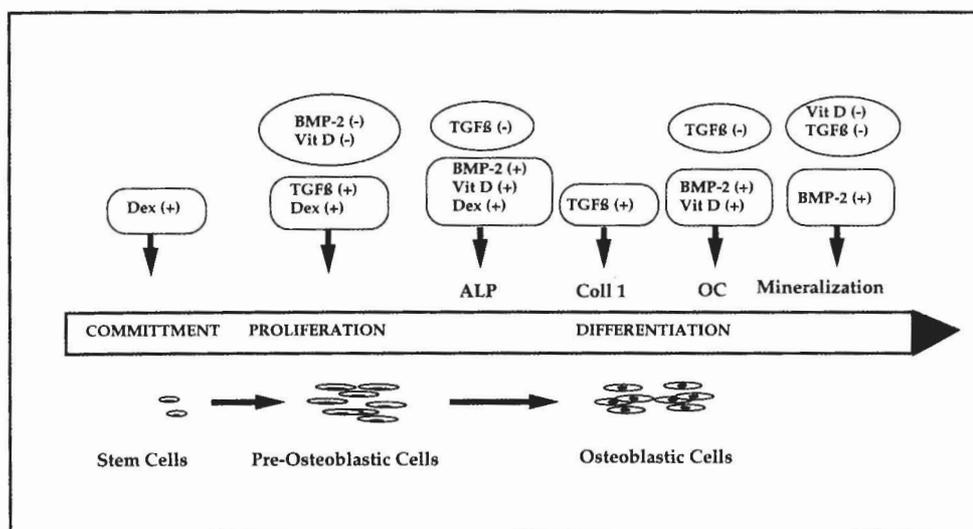


Fig. 2. Schematic representation of the developmental sequence of bone formation showing the main regulatory factors controlling osteoblast differentiation in the human bone marrow stroma (Fromigué et al., 1997, 1998 for details).

determination of precursor cells towards osteoblasts (Lian et al., 1996). Recent data indicate that *Osf2/Cbfl*, a specific factor inducing osteoblast differentiation, is expressed in osteoblast precursors or in mature osteoblasts and that local inducing factors such as BMP-7 induces *Osf2* (Ducy et al., 1997). However, the role and regulation of *Osf2* during human osteoblastic cell differentiation are not yet known.

Bone formation

The sequence of events characterizing the differentiating process leading the stem cell to differentiate into osteoblast has been identified. In endosteum, osteoblasts derive from mesenchymal stem cells in the mesenchyme or in the marrow stroma (Owen, 1985). Once engaged into the osteoblastic lineage, osteoblast precursor cells proliferate and then differentiate progressively into pre-osteoblasts, then into mature post-mitotic osteoblasts (Fig. 2). In rat calvaria cells, the initial decline in osteoblastic cell growth is followed by the progressive expression of markers of differentiation *in vitro* (Stein and Lian, 1993) and *in vivo* (Machwate et al., 1995a). The expression of phenotypic markers during rat calvaria cell differentiation has been in part established (reviewed in Aubin and Liu, 1996). In humans, studies of endosteal cells (Marie et al, 1989a), calvaria cells (de Pollak et al, 1996, 1997; Lomri et al, 1997; Debais et al, 1998) and marrow stromal cells (Fromigué et al, 1997,

1998) led us to depict a general scheme of expression of phenotypic markers expressed during human osteoblastic cell differentiation (Table 3). Pre-osteoblasts express markers such as alkaline phosphatase (ALP), osteopontin and collagen type I (Coll I) whereas mature post-mitotic osteoblasts express osteocalcin (OC) and contribute to the synthesis, organization, deposition and mineralization of the bone matrix. Such expression profile that characterizes the osteoblast phenotype during human osteoblast differentiation may be compared to the phenotype markers expressed during rat osteoblast differentiation (Aubin and Liu, 1996).

The switch between cell proliferation and differentiation is an important step controlling bone formation (Lian et al., 1991) and may be controlled by transcription factors such as *c-fos*. The osteocalcin promoter contains several AP-1 sites which bind *fos* and *jun* heterodimers. *c-fos* is expressed by proliferating cells (Owen et al., 1990a; Machwate et al., 1995a), is induced by the mitogenic factor TGF- β (Machwate et al., 1995b), and its expression precedes osteogenic differentiation during osteogenesis (Closs et al., 1990; Machwate et al., 1995a), suggesting that *fos/jun* interactions may play a role in the switch between proliferation and differentiation (Lian et al., 1991). Studies in transgenic mice (Grigoriadis et al., 1993) also support a role for *c-fos* in the induction of bone formation. Although *c-fos* may control in part the onset of differentiation, it is likely that multiple classes of transcription factors are involved in the induction of specific genes at the onset and during the development of osteoblast differentiation (Lian et al., 1996). Genes such as members of helix-loop-helix (HLH) DNA-binding proteins may be involved in the mechanisms leading to activation of transcription factors and regulation of genes such as osteocalcin presenting E-box (Lian et al., 1996). For example, *msx-2* may be involved to modulate the expression of the osteoblast phenotype since *msx-2* is a transcriptional regulator of the osteocalcin promoter (Towler et al., 1994). This is suggested by the observation that *msx-2* mutation engenders premature cranial ossification in humans (Jabs et al., 1993). Other transcription factors, however, may modulate osteoblast differentiation (Ogata and Noda, 1991; Tamura and Noda, 1994).

After the initial induction of differentiation, several steps are probably critical for the normal development of bone formation. Pre-osteoblasts adhere to the extracellular matrix, express osteoblast markers, synthesize, deposit and mineralize bone matrix components (for a review, see Gehron-Robey, 1989). Bone matrix proteins containing the RGD sequence promotes osteoblast attachment or spreading (Grzesik and Gehron-Robey, 1994). In addition, a GHK sequence present in the α_2 (I) chain of human collagen, thrombospondin and osteonectin (Lane et al., 1994) also promotes human or rat osteoblast attachment and modulates human osteoblast phenotype (Godet and Marie, 1995). Cell-matrix interactions may also play an important role in the

Table 3. Phenotypic osteoblast markers in human osteoblastic cells.

MARKER	IMMATURE CELL	MATURE OSTEOBLAST
<i>General</i>		
ALP	±	+
Stro-1	+	-
<i>Matrix proteins</i>		
Col-III	-	-
Col-I	++	++
OP	++	+
ON	+	+
OC	-	+
BSP	-	+
Thrombospondin	nd	+
Biglycan	nd	±
Decorin	nd	±
<i>Local Factors/Receptors</i>		
PTHrP	++	+
PTH/PTHrP-R	+	++
FGF-2	+	nd
FGFR-1	+	nd
FGFR-2	+	nd
L1	nd	±
TNF α	nd	±
IL6	nd	++
GM-CSF	nd	+

Data obtained from our studies of human endosteal osteoblasts (Marie et al., 1989a), human bone marrow stromal cells (Fromigué et al., 1997; Oyajobi et al., 1997) and calvaria cells in humans (de Pollak et al., 1997; Lomri et al., 1997; Debais et al, 1998). ±, +, ++: weak, clear, marked expression, respectively. nd: not determined.

induction of the osteoblast phenotype (Lynch et al., 1995). It is likely that the transcription of phenotypic genes is mediated by complex interactions between integrins, cytoskeletal proteins and components of signal transduction pathways (summarized in Lomri and Marie, 1996). In addition to cell-matrix interactions, cell to cell communication processes by cell adhesion proteins, recognition molecules or gap junctions are likely to be important for the coordinate induction of gene expression in osteoblasts (Civitelli, 1995). Finally, the osteogenesis process is completed by mineralization of the matrix. In vivo, this process requires appropriate alkaline phosphatase activity in osteoblasts (Garba and Marie, 1985). Collagen and non-collagenous proteins may contribute in part to the initiation of bone mineralization through their calcium-binding properties (Gehron-Robey, 1989). Although osteocalcin expression increases prior to calcification in vitro (Collin et al., 1992), this protein does not seem to play a major role in matrix calcification since abrogation of osteocalcin expression in knock-out experiments does not lead to defective bone mineralization (Ducy et al., 1996).

Regulation of bone formation

Many hormones and growth factors may affect cell recruitment and differentiation (reviewed in Canalis et al., 1991; Baylink et al., 1993; Martin et al., 1993; Marie and de Vernejoul, 1993; Mundy, 1995). The proliferation of osteoblast precursor cells is important since the rate of bone formation in both rodents and humans is mainly dependent on the recruitment of osteoblasts (reviewed in Marie and de Vernejoul, 1993; Marie, 1995). Among the multiple growth factors controlling bone cell proliferation, the most important are probably those that are present locally, or are released from the matrix, such as Insulin-like growth factors (IGFs), TGF- β s and FGFs that may act in an autocrine or paracrine way to stimulate cell proliferation. Granulocyte Macrophage Stimulating Factor (GM-CSF) may also act as autocrine growth factor for osteoblastic cells (Modrowski et al., 1997). The cellular mechanisms involved in the biological effects of these factors are now more clearly understood (reviewed in Siddhanti and Quarles, 1994).

The maturation of osteoblastic cells is controlled by several local and hormonal factors which act at several steps during the sequence of differentiation. Some growth factors, such as TGF- β , IGFs and BMPs are capable of stimulating the differentiation of periosteal or endosteal osteoblasts, while others (Epidermal Growth Factor, Platelet Derived Growth Factor, FGFs) induce inhibitory effects on osteoblast differentiation (Canalis et al., 1991; Mundy, 1995; Marie, 1997a). Hormones may affect osteoblastic cell differentiation by inducing direct effects on phenotypic genes, or by affecting the production and bioavailability of growth factors (Rodan and Noda, 1991; Lian and Stein, 1993). In the model of rat calvaria, some factors affect the differentiation processes and modulate the development of the bone cell

phenotype differently depending on the maturation state of the osteoblast (Li et al., 1996). In the model of human calvaria, we found that several factors affect osteoblast differentiation differently at distinct steps of maturation (Debiais et al., 1998; Hay et al., 1998). In the model of human marrow stromal cells, we recently found that the differentiation of osteoblast precursor cells is modulated by factors such as dexamethasone, TGF- β , vitamin D, and BMP-2 in a sequential and complementary fashion (Fromigué et al., 1997, 1998) (Fig. 2). These results indicate that multiple hormonal and local factors act in a coordinate manner at different stages of maturation to control osteoblast proliferation and differentiation and osteogenesis.

This brief survey of osteoblast biology points to the fact that osteoblasts and bone formation are controlled at multiple steps, starting from cell determination and leading to cell differentiation and function. A variety of local factors and osteotropic hormones control the osteoblast differentiation process by acting on the commitment and recruitment of undifferentiated cells, or by influencing the expression or repression of phenotypic markers. Thus, abnormalities in stem cell recruitment and/or differentiation may lead to reversible or irreversible alterations of bone formation.

Alterations in osteoblasts and bone formation

While genetic alterations of genes involved in skeletal patterning usually lead to alterations in cell determination and skeletal development (reviewed in Erlebacher et al., 1995), alterations of local or hormonal factor production or signaling result in abnormalities in osteoprogenitor cell proliferation or function.

Alterations in skeletal patterning and cell determination

There are few examples of alterations of developmental genes leading to abnormalities in cell determination and/or skeletal patterning (reviewed in Jacenko et al., 1994). Altered expression of Hox genes lead to abnormal skeletal patterning (Krumlauf, 1994). Deletion of BMPs, that are involved in skeletal patterning, are mostly lethal in mice, whereas mutations in Growth Differentiating Factor-5, a BMP-related protein, leads to skeletal abnormalities (Kingsley et al., 1992; King et al., 1994). Mutations in Cbfa1, a transcription factor of the runt family, are associated with cleidocranial dysplasia in human (Mundlos et al., 1997). Cbfa1 is involved in early skeletal patterning and is essential for osteoblast differentiation (Komori et al., 1997; Mudlos et al., 1997; Otto et al., 1997). Deletion of the specific osteoblast transcription factor Osf2/Cbfa1 in mice leads to the lack of osteoblast differentiation, which indicates that this factor is involved in the determination of mesenchymal cells into osteoblasts (Ducy et al., 1997). These effects are likely to result from alterations in the determination of target cells that are unable to differentiate properly.

Alterations in osteoblastic cell proliferation and function

Some alterations in transcription factors may lead to abnormal osteoblast proliferation. For example, over expression of *c-fos* which plays a role in the recruitment of osteoblasts (Grigoriadis et al., 1993; Machwate et al., 1995b), leads to osteosarcomas, indicating that *c-fos* is involved mainly in osteoblastic cell proliferation. Many abnormalities in the recruitment of pre-osteoblastic cells may lead to metabolic disorders in bone formation. This is the case of aging, mechanical unloading and abnormal production of calciotropic hormones. Aging in humans induces a trabecular bone loss resulting from a permanent imbalance between bone resorption and formation activities (Parfitt et al., 1983). Histomorphometric analysis of bone formation showed that the age-related decrease in the amount of bone matrix synthesized results in a local reduction of bone formation and in thinning of trabeculae even if the bone turnover rate may increase with age (Lips et al., 1978; Parfitt, 1990). In experimental models, age-related bone loss is associated with a decreased number of osteogenic cells (Liang et al., 1992; Khan et al., 1995). In humans, the proliferative capacity of osteoblastic precursor cells also declines with age (Evans et al., 1990; Fedarko et al., 1992a). Thus, aging is associated with diminished bone formation because a lower number of osteoblasts cannot synthesize a sufficient amount of matrix. The defective recruitment of osteoprogenitor cells and the relative decline in bone formation occurring with aging may result from an insufficient local production of growth factors. For example, IGF concentrations decrease in aged bone in humans (Nicolas et al., 1994). Moreover, the osteoblast responsiveness to growth factors may decrease with age (Pfeilschifter et al., 1993; Kato et al., 1995). Altogether, these data point to a role of growth factors in the decreased bone formation associated with aging (reviewed in Marie, 1997b).

Estrogen deficiency occurring after menopause or ovariectomy leads to an acceleration of bone turnover due to excessive bone resorption with regards to bone formation (Wronski et al., 1985). The pathogenic pathways involved in the increased bone resorption in ovariectomized mice have been clarified (Manolagas and Jilka, 1995). In contrast, the mechanisms causing imbalance between bone resorption and formation in estrogen deficiency are not well understood. We demonstrated that the increased bone formation in estrogen deficient rats is related to an increased recruitment of osteoblast precursor cells arising from the marrow stroma (Modrowski et al., 1993). In postmenopausal women, we found that the increased bone formation results from an increased osteoblastic cell proliferation in patients with high bone turnover (Marie et al., 1989b). Since these changes were not related to increased production of cytokines by osteoblasts (Marie et al., 1993), the increased recruitment of osteoblastic cells in estrogen deficiency may arise from an increased release of growth factors from the resorbed matrix. In

contrast, postmenopausal women with a low bone turnover have a decreased proliferative capacity of endosteal osteoblastic cells (Marie et al., 1989b). Similar results were found in osteoporotic males (Marie et al., 1991), which indicates that the recruitment of osteoblasts from progenitor cells is the most important limiting step controlling bone formation at the tissue level (summarized in Marie, 1994, 1995). The above observations suggest that bone formation in estrogen deficiency can be increased by factors acting on osteoblast recruitment. Interestingly, TGF- β concentrations are decreased in ovariectomized rat bone (Finkelman et al., 1992) and TGF- β (Kalu et al., 1993) increases marrow stromal cell growth (Long et al., 1995). Fluoride treatment can also increase bone formation in ovariectomized rats (Modrowki et al., 1992) and postmenopausal women (Marie et al., 1992) by increasing the recruitment of osteoblastic cells. In aged ovariectomized rats, we also found that the administration of the growth factor IGF-I improved bone formation and bone mass, mainly by increasing the number of bone forming cells (Muller et al., 1994). This stresses the importance of stimulating osteoblastic cell recruitment to improve bone formation in osteopenic disorders (reviewed in Marie, 1995).

Unloading is another pathological state leading to alteration of osteoblast recruitment. Mechanical loading is an important modulator of bone mass and unloading results in decreased bone formation and bone loss (Skerry, 1997). Mechanical loading stimulates bone formation, presumably by inducing several intracellular events in bone cells (Jones et al., 1991; Ingber, 1997). Mechanical forces may induce mechanical signals to the cells by inducing modifications in stretch-sensitive ion channels, or in integrins coupled to cytoskeleton and associated signaling (for review see Shyy and Chien, 1997). Physical forces of gravity, stress, fluids or strain may thus modulate the expression of several early genes, growth factors and intracellular signaling processes in bone cells, resulting in changes in transporters (Mason et al., 1997) or growth factor production in mechanosensitive bone lining cells or osteocytes (Klein-Nulend et al., 1995; Zhuang et al., 1996) and alterations in bone cell recruitment and/or function. We studied the cellular effects of skeletal unloading in the model of hindlimb weightlessness in rats. In this model, unloading results in reduced endosteal bone formation with no change in bone resorption (Globus et al., 1986). We demonstrated that unloading induced a marked inhibitory effect on the proliferative capacity of osteoblastic cell recruitment in the endosteum and the marrow stroma (Machwate et al., 1993). Hypokinesia was also found to reduce the osteogenic capacity of precursor cells in the marrow stroma (Keila et al., 1994). This demonstrates that unloading induces a marked reduction in osteoblast recruitment, resulting in a marked reduction in bone formation. In order to increase the capacity of osteoblastic cells to proliferate in unloaded bones, we evaluated the therapeutic effects of growth factors. Some

growth factors were previously found to stimulate bone formation in vivo. For example, local injections of TGF- β stimulate periosteal bone formation (Noda and Camillière, 1989; Marcelli et al., 1990). The administration of EGF (Marie et al., 1990) or FGF-2 (Martin et al., 1997) also increases endosteal bone formation. In addition, the increased expression of TGF- β 2 in osteoblasts results in increased osteoprogenitor cell proliferation and differentiation in transgenic mice (Erlbacher and Drynck, 1996). In unloaded rats, we found that the systemic administration of IGF-I increased bone formation and prevented partially the endosteal bone loss, an effect related to an increased recruitment of pre-osteoblastic cells in the endosteum and marrow stroma (Machwate et al., 1994). We also found that the systemic administration of TGF- β 2 completely prevented the endosteal bone loss in unloaded rats, as a result of increased proliferation of ALP-positive pre-osteoblastic cells in the marrow stroma, and of increased expression of type I collagen by mature osteoblasts in the metaphysis (Machwate et al., 1995c). In contrast, the administration of BMP-2, which

stimulated the differentiation of osteoblasts but reduced pre-osteoblast recruitment, had no beneficial effect on bone formation and bone mass in this model (Zerath et al., 1997). These findings suggest that growth factors that are capable of increasing the number of osteoblast precursor cells are able to improve bone formation in osteopenic animals. This raises the possibility that growth factor administration may be useful to increase bone formation in osteopenic disorders. However, although treatments with some growth factors in humans were found to increase bone formation, this also may induce other undesirable effects on bone resorption (summarized in Marie, 1997b).

Genetic mutations affecting osteoblasts

Several genetic mutations affecting bone matrix proteins have been described. Mutations affecting collagen type II, IX, X or XI were found to induce multiple abnormalities in cartilage and bone development (reviewed in Jacenko et al., 1994; Erlbacher et al., 1995). In humans, mutations in the

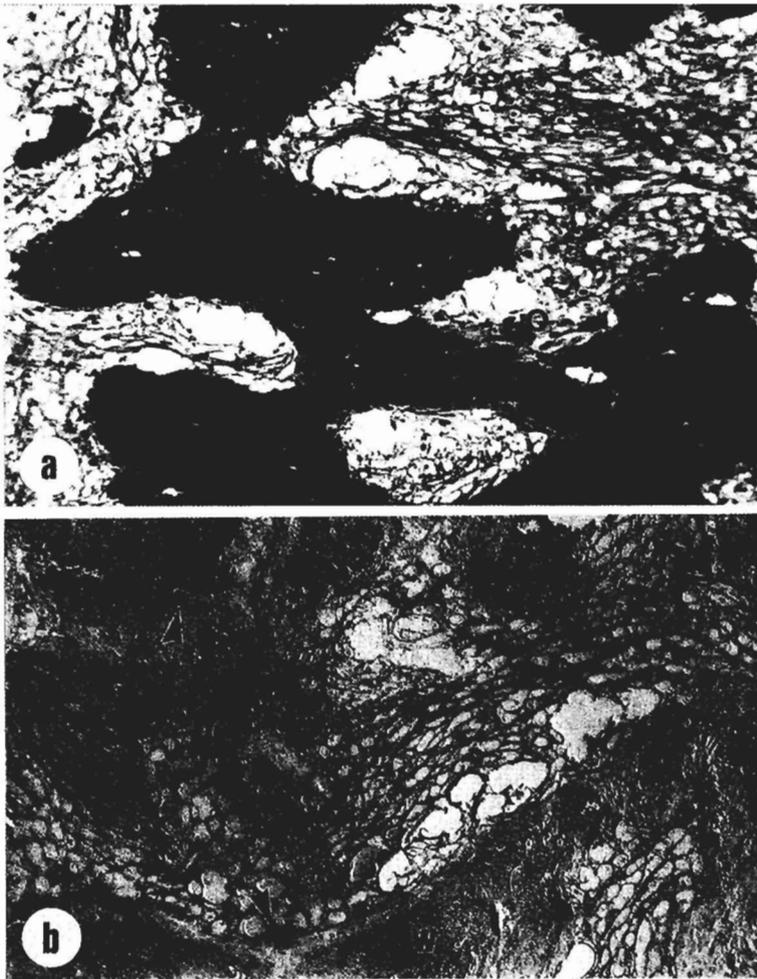


Fig. 3. Increased bone formation induced by the $Gs\alpha$ mutation in a patient with McCune Albright syndrome (MAS). Histological sections of a dysplastic bone lesion showing in **a**, woven (w) immature bone, few osteoblasts (o), and in **b**, alkaline phosphatase positive mesenchymal cells (c) depositing collagenous fibers along the bone surface (arrowheads). (Marie et al., 1997). x 250

genes coding for type I collagen induce skeletal disorders such as osteogenesis imperfecta (for a review, see Prockop, 1992). In vitro studies indicate that the production and processing of type I collagen are altered in cultured bone cells of patients with osteogenesis imperfecta (Fedarko et al., 1992b). However, osteoblast differentiation does not appear to be affected in bone cells from these patients despite the defective collagen type I metabolism (Morike et al., 1993). Besides mutations affecting structural bone matrix components, gene mutations affecting cell signaling (c-src, c-fos) are associated with impaired osteoclast function and osteopetrosis (Soriano et al., 1991; Wang et al., 1992). Mutations affecting the production of factors released by osteoblasts may alter cell differentiation. For example, the defective production of macrophage colony-stimulatory factor (M-CSF) affects the differentiation of osteoclasts and osteopetrosis in mice (Yoshida et al., 1990). In some osteopetrotic rats, aberrant gene expression in osteoblasts correlates with abnormalities in matrix composition and osteoclast development (Shaloub et al., 1991; Jackson et al., 1994). In humans, a defect in osteoblasts was also found to induce osteopetrosis (Lajeunesse et al., 1996). Thus, genetic

alterations of the osteoblast function, including those contributing to the differentiation of osteoclasts, may have important effects on bone development.

Recently, genetic mutations were found to induce alterations in osteoblast recruitment or differentiation. Mutations in transcription factors such as *msx-2* (Jabs et al., 1993; Satokara and Maas, 1994) or *twist* (El Ghouzzi et al., 1997) were shown to induce premature ossification of the skull in mice and humans. Although these early genes may affect calvaria osteoblastic cell differentiation, the phenotypic mechanisms induced by these mutations are not yet known. Mutations affecting growth factor signaling may also induce alterations in osteoblast differentiation. Recent examples are given by mutations in FGF receptors (FGFRs) which induce skeletal abnormalities in humans. Alterations in FGFR-3 induce abnormal cartilage formation (Rousseau et al., 1994) whereas mutations in FGFR-1 and -2 induce abnormalities in cranial ossification (summarized in Wilkie et al., 1995a). However, the phenotypic changes induced by these genetic alterations in osteoblastic cells remain mostly unknown. We recently analyzed the cellular phenotypic of FGFR-2 mutations in Apert syndrome, a syndrome characterized by premature

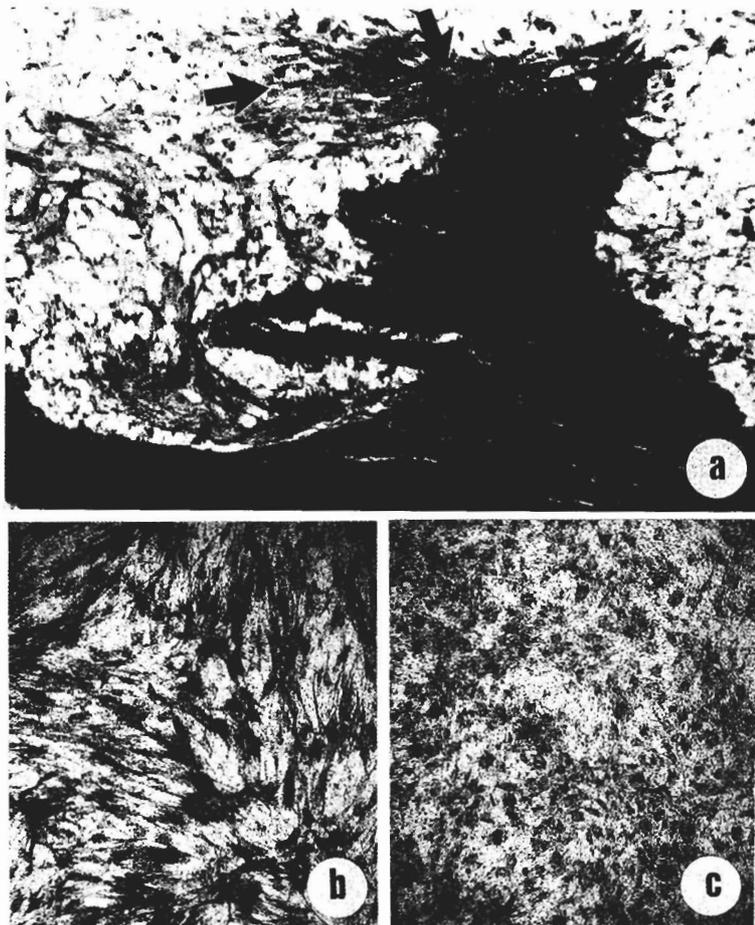


Fig. 4. Increased subperiosteal bone formation induced by an FGFR-2 mutation in a fetus with Apert syndrome. **a.** Histological section of subperiosteal calvaria bone lesion showing increased subperiosteal bone formation (arrows). Cultured calvaria osteoblastic cells with the FGFR-2 mutation showed an increased number of alkaline phosphatase positive cells (**b**) compared to normal cells (**c**). (Lomri et al., 1998). x 250

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fusion of cranial sutures induced by mutations in the extracellular domain of the FGFR-2 receptor (Wilkie et al., 1995b). In this syndrome, we found by histological analysis that the Ser252Trp in FGFR-2 induces an increased subperiosteal pre-osteoblastic cell population in fetal calvaria (Fig. 3a). We also found that calvaria cells isolated from infants and fetuses with this mutation have an increased number of alkaline phosphatase positive osteoprogenitor cells, indicating an expansion of the pre-osteoblastic cell population (Fig. 3b vs c). In addition, we showed that the expression of phenotypic markers and osteogenic function are increased in mutant fetal calvaria cells (Lomri et al., 1998). These data indicate that this FGFR-2 mutation increases the differentiation pathway of subperiosteal calvaria cells into pre-osteoblasts, leading to increased osteoblast phenotype and premature calvaria ossification during fetal development. This example shows that a single mutation in a growth factor receptor in human osteoblastic cells may induce marked changes in the commitment of osteoblast precursor cells and bone formation.

Another example of a mutation affecting osteoblast maturation is provided by the McCune-Albright syndrome (MAS). In this syndrome, monostotic or polyostotic lesions of fibrous dysplasia are induced by activating missense mutations of the gene encoding the α subunit of G_s , the G protein that stimulates cAMP formation. This mutation (substitution of Arg²⁰¹ with either Cys or His) leads to abnormal $G_s\alpha$ protein, inhibition of the GTPase activity and constitutive activation of adenylate cyclase (Spiegel et al., 1993; Shenker et al., 1994). In this syndrome, bone lesions are characterized by woven ossified tissue, a marked increase in bone matrix formation rate and extensive marrow fibrosis. Until recently, the cellular phenotype

induced by this mutation in osteoblastic cells was not known. We analyzed the tissue and cellular events resulting from activating mutations of the $G_s\alpha$ gene in patients with MAS or with monostotic fibrous dysplasia. We first reported that osteoblastic cells isolated from the bone surface in polyostotic and monostotic lesions express missense mutations in the $G_s\alpha$ gene with substitution of His or Cys for Arg in position 201 (Shenker et al., 1995). This finding was later confirmed at the tissue level (Riminici et al., 1997), suggesting that the mutation in bone cells may be responsible for the fibrous dysplastic lesions. We then showed by histomorphometric analysis that few morphologically mature osteoblasts and numerous immature alkaline phosphatase-positive cells deposit an excessive and disorganized collagenic matrix in dysplastic lesions (Fig. 3). In cultured osteoblastic cells from the dysplastic areas, the increased intracellular basal cAMP production increased cell growth and decreased osteocalcin production (Marie et al., 1997), indicating that the activating mutation of $G_s\alpha$ increased the proliferation of mesenchymal osteoprogenitor cells, resulting in accelerated matrix deposition in fibrous dysplastic lesions. These lesions are reminiscent to those found in tertiary autonomous hyperparathyroidism where very high levels of parathyroid hormone (PTH) lead to the formation of fibrotic lesions and immature woven bone (Rasmussen and Bordier, 1974). Excessive PTH production is known to result in increased proliferation of endosteal osteoblastic cells and increased bone formation in patients with primary (Marie and de Vernejoul, 1993) and secondary hyperparathyroidism (Marie et al., 1989c). In normal or osteopenic animals, intermittent PTH administration also induces stimulation of osteoprogenitor cell proliferation (Nishida et al., 1994) and induces c-fos expression in bone cells in vivo (Lee et al., 1994), suggesting that osteoblastic cell proliferation is increased by cAMP. Several mechanisms may be involved (for further discussion, see Marie et al., 1997). The expression of c-fos, which appears to play a critical role in the control of osteoblastic cell growth (see above) appears to be increased in bone lesions of patients with fibrous dysplasia (Candelière et al., 1995), suggesting that high cAMP levels induced by the constitutive activation of adenylate cyclase by mutations in the $G_s\alpha$ gene, or by increased PTH levels, may increase the proliferation of mesenchymal osteoprogenitor cells by inducing c-fos expression. These examples of pathological bone formation in humans emphasize that identification of the cellular and molecular alterations induced by genetic mutations in osteoblastic cells may allow to delineate the mechanisms involved in disorders of bone formation (Fig. 5).

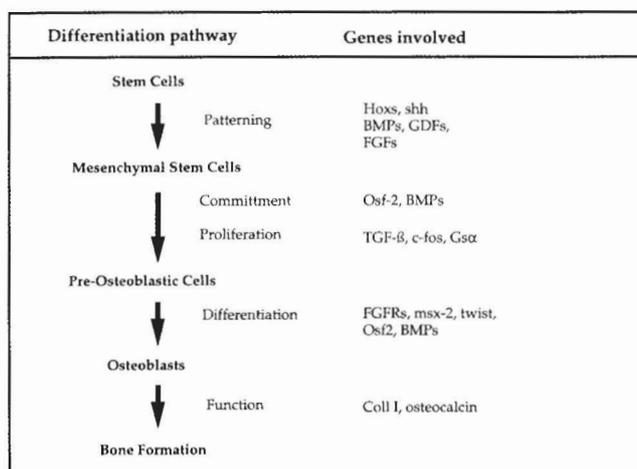


Fig. 5. Genes believed to be implicated in the pathogenic pathway of osteoblast differentiation in some disorders of bone formation, based on the phenotype induced by gene deletion, overexpression or mutations in mouse or humans (see text for details).

Conclusions

The recent advances in the cellular and molecular biology of osteoblasts in normal and pathologic conditions helped to provide new insight into the

mechanisms controlling endosteal bone formation in normal and pathological conditions in humans. As summarized above, the comparative studies of human endosteal bone-forming cells and bone formation in human diseases revealed that the rate-limiting step in bone formation is the recruitment of osteoblasts. In addition, the availability of models of human osteogenesis *in vitro* led to the determination that hormones and growth factors act on cell proliferation and differentiation in a differential and sequential way. Moreover, recent studies on the alterations on human osteoblast phenotype induced by mutations led to the identification of the role of some genes in cell proliferation and differentiation. A number of questions remain however unanswered concerning the mechanisms controlling recruitment and differentiation of osteoblasts in humans. For example, the determination of early genes involved in human osteoblast recruitment from stem cells needs to be established. One approach to establish this goal may be to study the differential gene expression in immature and mature human osteoblastic cells. Another possibility would be to determine the cellular and molecular mechanisms inducing the phenotypic osteoblastic abnormalities in local genetic disorders of bone formation in humans. These approaches, currently developed in my laboratory, are likely to provide new insights into the cellular and molecular processes controlling bone formation *in vivo*, which may lead, in the long term, to the development of novel strategies to induce new bone formation in osteopenic disorders.

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