

Review

Cellular and molecular basis of fibrous dysplasia

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Summary. Recent advances have been made in the cellular and molecular mechanisms involved in monostotic and polyostotic fibrous dysplasia, a rare nonmalignant disease causing bone deformations and fractures. The molecular basis of fibrous dysplasia has been clarified when mutations affecting the stimulatory α subunit of G protein (Gs) have been found in dysplastic bone lesions. The histological analysis of dysplastic lesions revealed that the mutations in $Gs\alpha$ caused abnormalities in cells of the osteoblastic lineage and therefore in the bone matrix. Further *in vitro* analyses of bone cells from mutant dysplastic bone lesions revealed that the abnormal deposition of immature bone matrix in fibrous dysplasia results from decreased differentiation and increased proliferation of osteoblastic cells. Finally, the signaling pathway involved in these osteoblastic abnormalities has been identified. It is now apparent that the constitutive elevation in cAMP level induced by the $Gs\alpha$ mutations leads to alterations in the expression of several target genes whose promoters contain cAMP-responsive elements, such as c-fos, c-jun, Il-6 and Il-11. This in turn affects the transcription and expression of downstream genes and results in the alterations of osteoblast recruitment and function in dysplastic bone lesions. These mechanisms provide a cellular and molecular basis for the alterations in bone cells and bone matrix in fibrous dysplasia.

Key words: Osteoblasts, Bone formation, Fibrous dysplasia, McCune-Albright syndrome, $Gs\alpha$ mutations

Introduction

During the last decade, significant advances have been made in the understanding of cellular and molecular mechanisms involved in the etiology of fibrous dysplasia, a rare nonmalignant bone disease. This has been made possible by the discovery of genetic

mutations expressed in dysplastic cells, and by the identification at the tissue and cellular levels of the resulting cellular abnormalities. In this review, I will summarize the pathophysiological mechanisms responsible for the bone disorders in fibrous dysplasia, based on results obtained in my and other laboratories.

Pathology and histology of fibrous dysplasia

Fibrous dysplasia is a benign disease characterized by focal bone lesions and occurring sporadically. Most patients (70%) present with a single area of dysplasia (monostotic fibrous dysplasia), but some have multiple areas affected (polyostotic fibrous dysplasia). The lesions may be asymptomatic or be present with local pain, deformities, fractures and growth abnormalities during childhood and at puberty (McCune, 1936; Albright et al., 1937; Harris et al., 1962). Several parts of the skeleton may be affected, including femur, tibia, humerus, pelvis, skull and ribs (Harris et al., 1962; Cohen and Howell, 1999). In a small number of cases, fibrous dysplastic lesions may degenerate into malignant tumors, osteosarcoma and chondrosarcoma (Yabut et al., 1988). A minority (about 3%) of polyostotic patients with McCune-Albright syndrome present skin pigmentation called "café-au-lait" lesions (McCune, 1936; Albright et al., 1937), associated with multiple endocrinopathies. This includes sexual precocity, hyperthyroidism, growth hormone excess and acromegaly, hyperprolactinemia and adrenal hyperplasia resulting from autonomous hypersecretion of hormones. Radiologically, the lesions present in the metaphysis and diaphysis of long bones appear cystic and may expand from the marrow cavity to the surrounding cortex. Histologically, lesions of fibrous dysplasia have variable characteristics depending on their location (Grabias et al., 1977; Shenker et al., 1994; Marie et al., 1997; Marie, 1999a; Riminucci et al., 1999). Craniofacial lesions are often present as dense fibrous bone tissue (Riminucci et al., 1999). In long bone, dysplastic lesions are characterized by an accumulation of disconnected trabecular, poorly mineralized bone matrix which is mixed with the normal lamellar bone (Shenker et al., 1994; Marie et al., 1997; Marie, 1999a; Riminucci et al., 1999) (Fig. 1A). The more severe lesions present with

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immature woven bone which is not replaced by mature lamellar bone (Marie et al., 1997; Riminucci et al., 1999). The lack of connectivity and the immature nature of the bone formed results in decreased mechanical properties of the bone, leading to deformations and fractures.

Osteoblast abnormalities in fibrous dysplasia

The histological analysis of dysplastic lesions suggest the existence of abnormalities in the formation of osteoprogenitor cells (Marie et al., 1997; Marie, 1999a; Riminucci et al., 1999). Indeed, the number of immature osteoblasts is low. Moreover, cells located along the bone surface are not oriented and elongated, and are distinct from normal cuboidal mature osteoblasts (Fig. 1A,B). The marrow cavity is filled with elongated, immature, alkaline phosphatase-positive cells, and this cellular invasion does not allow the normal development of hematopoietic and adipogenic populations (Fig. 1C). These histological characteristics indicate that the pre-osteoblastic population is increased whereas the population of mature cells is decreased. Defective osteoblast differentiation is also evidenced by the abnormal collagen organization in the immature bone. The collagen fibers are not parallelly oriented, a characteristic of immature woven bone. The collagen fibers sometimes appear perpendicular to the bone surface, showing a comb-like structure (Fig. 1A). These abnormalities found in both monostotic and polyostotic lesions reflect a disorganization of collagen fibers synthesized by dysplastic cells (Marie et al., 1997; Riminucci et al., 1999). In addition to the abnormal

formation and orientation of collagen, non-collagenous protein synthesis is affected. Osteonectin level is increased, whereas osteopontin and bone sialoprotein are decreased in dysplastic bone, reflecting the immature composition of the matrix (Marie et al., 1997; Riminucci et al., 1999; Sakamoto et al., 1999). The reduced content in some of these bone proteins in the matrix may reduce the adherence of osteoblasts, leading to cell retraction and disorientation (Riminucci et al., 1999). As a result of the disorganized immature bone matrix, osteocytes arising from dysplastic osteoblasts are more numerous than normal, and are located within large lacunae. They also do not form a well organized network through intercellular extensions present in the canaliculi within the bone matrix (Fig. 1B). In addition to these alterations of the osteoblast lineage, there are zones of active bone resorption in dysplastic bone that are characterized by the presence of multiple osteoclasts actively resorbing the mineralized matrix and forming local lytic areas (Fig. 1B). Some of the histological characteristics found in fibrous dysplasia (marrow invasion, hyper-resorption, immature bone, lytic areas) are also found in severe hyperparathyroidism and other cases of high turnover of bone (Rasmussen and Bordier, 1974).

Cellular basis of fibrous dysplasia

The availability and development of bone cell cultures obtained from dysplastic lesions allowed us to identify the cellular abnormalities in osteoblasts in both monostotic and polyostotic fibrous dysplasia. We found that dysplastic cells show an increased proliferation rate associated with elevated intracellular cyclic AMP

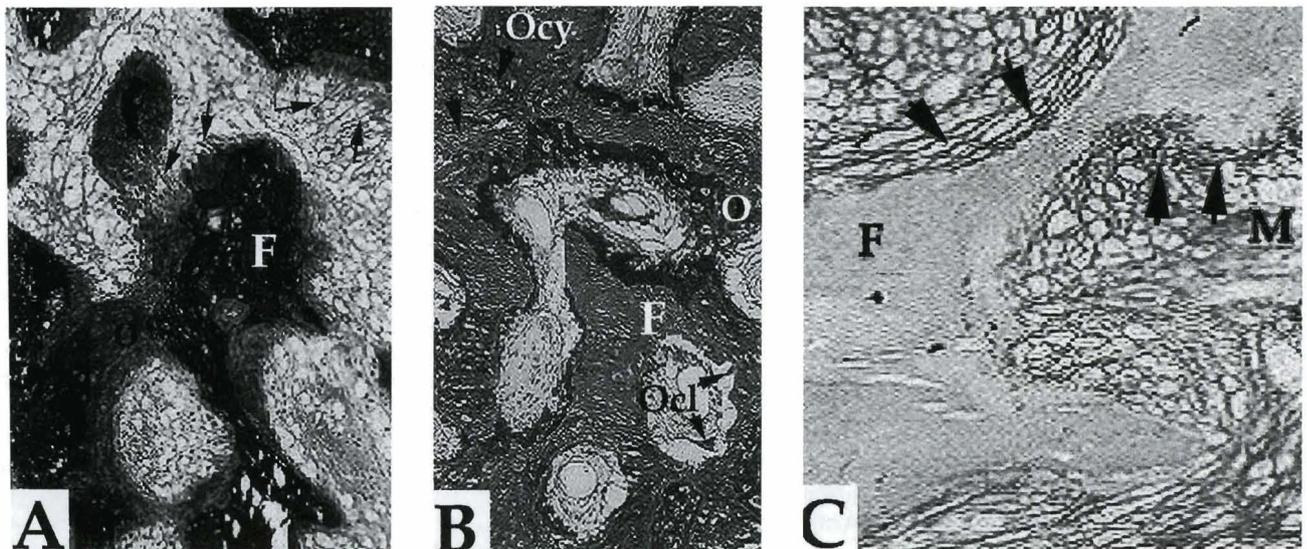


Fig. 1. Histological abnormalities induced by $G_{s\alpha}$ mutations in polyostotic fibrous dysplasia in the McCune-Albright syndrome (**A**, **C**) and in monostotic fibrous dysplasia (**B**). Bone lesions are characterized by immature fibrotic bone (F), irregular osteoid (o) and disorganized collagen which presents a comb-like structure (A: arrows). Mature osteoblasts are rare and numerous osteocytes (B: Ocy) are found in large lacunae within the immature bone. Bone resorption by osteoclasts (B: Ocl) is locally increased. The marrow (M) space is invaded by immature osteoprogenitor cells which present alkaline phosphatase activity (C: arrows). A, B, x 125; C, x 250

(cAMP) levels (Marie et al., 1997). Osteoblastic cells isolated from the more severe bone lesions, composed of immature bone matrix, show a less differentiated phenotype and increased proliferative rate compared to cells from the less affected lesions composed of a mixture of mature and immature bone matrix. This indicates that the increased cell proliferation is associated with the less differentiated osteoblast phenotype in the dysplastic lesions. In addition to the increased cell proliferation, dysplastic cells show decreased differentiation, as evidenced by the alteration of osteocalcin mRNA expression and protein synthesis (Marie et al., 1997; Sakamoto et al., 1999; Hopyan et al., 1999). However, dysplastic cells respond normally to 1,25-dihydroxyvitamin D which enhances osteocalcin expression in osteoblasts (Marie et al., 1997). The expression of Runx2/Cbfa1, a master transcription factor known to stimulate osteocalcin expression (Karsenty, 2000), was found to be increased in dysplastic bone cells (Sakamoto et al., 1999). One possible explanation for the increased Runx2/Cbfa1 expression with regard to the reduced osteocalcin expression is that dysplastic cells express high levels of Msx2, a transcription factor that is expressed early during osteoblast differentiation and that inhibits osteocalcin expression (Newberry et al., 1997). The concomitant decrease in cell differentiation and increased proliferation of pre-osteoblastic cells in dysplastic lesions lead to a rapid deposition of an immature, poorly organised woven bone characteristic of the bone lesion at the histological level (Marie et al., 1997; Riminucci et al., 1999).

Molecular basis of fibrous dysplasia

The molecular basis of fibrous dysplasia has been clarified when mutations affecting the stimulatory α subunit of G protein (Gs) have been found in dysplastic bone lesions. In the cell membrane, adenylate cyclase is coupled to hormonal receptors by a G protein which binds ATP (Fig. 2). This protein is composed of α , β and γ subunits. When inactivated, the α -subunit binds GTP. When a ligand binds to the receptor, the α -subunit is released from the remaining $\beta\gamma$ protein complex. This process is reversed through GTP hydrolysis by the intrinsic GTPase activity of the G_{α} protein (Spiegel et al., 1992). The α chain of the G protein binds and hydrolyses GTP, and thereby activates adenylate cyclase that produces cAMP (Fig. 2). Mutations in the G_{α} have been initially identified in multiple endocrinopathies (Landis et al., 1989; Lyons et al., 1990). In these pathologies, a constitutive activation of G_{α} leads to accumulation of cAMP in endocrine tissues, leading to increased cell proliferation and hormonal secretion (Klibanski, 1990; Landis et al., 1990; Spada et al., 1990). This was found in autonomous thyroid and pituitary tumors, as a result of somatic mutations of G_{α} (arginin201cystein or glutamin227leucin). These activating mutations inhibit the GTPase activity of G_{α} , resulting in increased adenylate activity and increased

cAMP levels (Spiegel et al., 1993; Spiegel, 2000) (Fig. 2). In the McCune-Albright syndrome, activating mutations were found in exon 8 of the G_{α} gene in patients with endocrinopathies (Weinstein et al., 1991; Ringel et al., 1996). In this syndrome, somatic mutations of G_{α} were found at variable levels in several endocrine or non endocrine tissues, leading to a mosaic distribution of the somatic mutation early during embryogenesis, and resulting in multiple and variable endocrinopathies later on (Cutler et al., 1989; Shenker et al., 1993, 1994; Malchoff et al., 1994; Levine, 1999). The mutations consist of the substitution of cysteine (R201C) or histidin (R201H) by arginin at position 201 in G_{α} . Another mutation such as R201G (Shenker et al., 1995; Candelieri et al., 1997; Riminucci et al., 1997) or mutations which do not affect G_{α} have also been found in patients with fibrous dysplasia (Gessl et al., 1994). However, fibrous dysplasia is not associated with aberrant chromosomal recombinations (Dal Cin et al., 2000).

Mutations of G_{α} have been found by RT-PCR analysis in dysplastic bone lesions (Shenker et al., 1995; Candelieri et al., 1997; Riminucci et al., 1997). The culture of osteoblastic cells isolated from dysplastic bone lesions allowed us to show the expression of R201C and R201H mutations in dysplastic bone cells

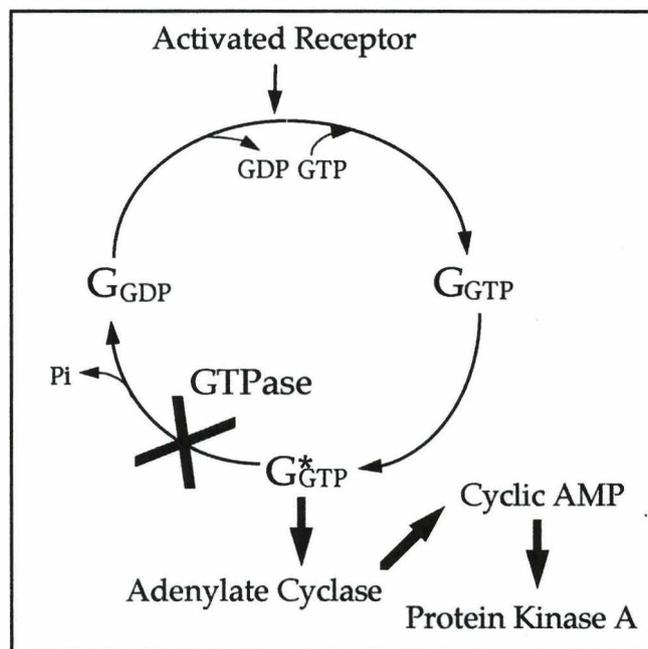


Fig. 2. Schematic representation of the effects of G_{α} mutation on adenylate cyclase activity. Adenylate cyclase is coupled to hormonal receptors by the G_{α} protein. When the receptor is activated, the α chain of the protein binds and hydrolyses GTP to GDP, thereby activating adenylate cyclase which leads to cAMP production. G_{α} -activating mutations (G_{α}^*) inhibit the GTPase activity, resulting in increased adenylate cyclase activity and increased cAMP accumulation. This leads to activation of protein kinase A and phosphorylation of a number of target molecules.

(Shenker et al., 1995). These mutations are expressed in both monostotic and polyostotic fibrous dysplasia (Shenker et al., 1995; Alman et al., 1996) and represent the molecular basis of the cellular abnormalities in the two types of fibrous dysplasia. Interestingly, further analyses indicated that not all bone cells within the dysplastic lesions express such mutations (Riminucci et al., 1997). This mosaic distribution allows cell survival because the mutations are lethal (Happle, 1986). It is therefore believed that $G\alpha$ mutations which appear early during embryogenesis induce the generalized abnormalities found in the McCune-Albright syndrome, whereas the somatic mutations appearing later in life induce more restricted pathologies such as thyroid and pituitary adenomas, and monostotic fibrous dysplastic lesions. Thus, the endocrine and skeletal lesions associated with these mutations reflect a variable phenotypic expression of the mutations resulting from a somatic mosaicism of a same molecular alteration (Ringel et al., 1996; Spiegel, 1996; Cohen and Howell, 1999). In dysplastic lesions, $G\alpha$ mutations are expressed in osteoprogenitor cells present in the marrow stroma (Marie et al., 1997; Bianco et al., 2000). The frequency of expression of mutations is higher in polyostotic than in monostotic fibrous dysplasia (Stanton et al., 1999). In terms of functional activity, dysplastic cells from monostotic and polyostotic lesions are able to be osteogenic in vitro in the presence of glucocorticoids which stimulate osteoblastic cell differentiation. However, mutated dysplastic cells are not osteogenic in vivo, probably because of the lethality induced by the mutation. Only mixed mutated and non mutated cells reconstituting the natural mosaic are able to form dysplastic lesions in vivo (Bianco et al., 1998). This indicates that $G\alpha$ mutations are responsible for the formation of dysplastic bone lesions, and that the presence of mutated and nonmutated cells is required for the development of the lesion (Bianco et al., 1998).

Molecular mechanisms of fibrous dysplasia

The formation of dysplastic bone lesions resulting from $G\alpha$ mutations involves several mechanisms. In theory, activating mutations of $G\alpha$ should induce stimulation of adenylate cyclase and increased cAMP accumulation (Fig. 2). Accordingly, we found that $G\alpha$ mutations induce a constitutive accumulation of cAMP in osteoblastic cells in the McCune-Albright syndrome (Marie et al., 1997) and this was confirmed by other groups (Candelieri et al., 1997; Bianco et al., 1998). The downstream events leading to the abnormal cellular phenotype in osteoblastic cells are linked to cAMP signaling. The signaling pathways induced by cAMP are known to tightly control gene expression (Borrelli et al., 1992). cAMP accumulation activates protein kinase A which then phosphorylates transcription factors that are members of the cAMP-response-element-binding-protein (CREB) family. This leads to binding of these proteins to DNA domains called cAMP-responsive

element (or CRE) in the promoter of target genes (Montminy et al., 1990). This pathway controls the expression of multiple genes in several endocrine and nonendocrine tissues. For example, in pituitary cells, the increased activity of PKA induced by $G\alpha$ -activating mutations stimulate the promoter activity of prolactin and growth hormone (Tian et al., 1994; Gaiddon et al., 1995), leading to endocrinopathies associated with the mutation. In osteoblastic cells, a rise in cAMP induced by $G\alpha$ mutations also induce multiple effects (Fig. 3). One of the more rapid effects is cell retraction which is observed in dysplastic cells (Riminucci et al., 1997). This may be explained by the rapid disassembling effect of cAMP on cytoskeletal proteins leading to cell retraction (Lomri and Marie, 1990). The rise in cAMP in mutated dysplastic cells may also be responsible for the decreased expression of bone matrix proteins, such as osteopontin (Noda and Rodan, 1989) whose promoter contains cAMP-responsive elements (Kopp et al., 1989; Kerr et al., 1993). The rise in cAMP is also likely to be involved in the increased bone resorption found in dysplastic lytic lesions. Such local stimulation of bone resorption is found in severe cases of hyperparathyroidism in which high levels of parathyroid hormone induces elevated intracellular cAMP levels in osteoblasts (Lomri and Marie, 1990) and results in increased osteoclastic bone resorption by increasing bone resorbing cytokines (see below).

The elevation in cAMP induced by the $G\alpha$ is an important mechanism leading to bone cell abnormalities

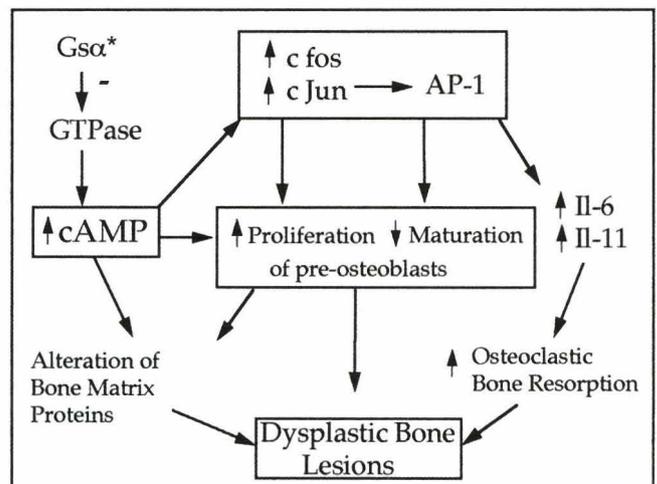


Fig. 3. Molecular mechanisms involved in fibrous dysplasia. The mutations in $G\alpha$ ($G\alpha^*$) result in increased intracellular cAMP levels that modulate the expression of several genes whose promoter contains a cAMP-responsive element (CRE). This pathway leads to abnormal production of bone matrix proteins and to increased c-fos expression, leading to abnormal recruitment and function of osteoblastic cells, and to abnormal bone matrix production. The cAMP overproduction induced by the mutation also leads to increased production of interleukin-6 and -11 (IL-6, IL-11) which in turn stimulate osteoclastic bone resorption. These molecular mechanisms induced by the $G\alpha$ mutations result in the formation of dysplastic bone lesions.

in fibrous dysplasia (Fig. 3). One mechanism involves *c-fos*, a protein that forms heterodimers with proteins of the *jun* family, forming a complex that binds to specific sequences in the promoter of several genes (Angel and Karin, 1991). The *c-fos* promoter contains a cAMP-responsive element (Sassone-Corsi et al., 1988), and is therefore a potential target for $G\alpha$ mutations in fibrous dysplasia. Indeed, $G\alpha$ -activating mutations induce *c-fos* overexpression in vitro (Sassone-Corsi et al., 1988). In endocrine cells, the $G\alpha$ -induced stimulation of *c-fos* transcription involves multiple sequences such as CRE and the serum responsive element SRE (Gaiddon et al., 1994). In bone, $G\alpha$ mutations increase *c-fos* expression and *c-jun*, which acts by binding *c-fos* and forms the AP-1 heterodimer complex. In endocrine cells, mutations of $G\alpha$ increase *c-jun* and Jun B expression (Gaiddon et al., 1994). Histological analysis demonstrated an increased expression of both *c-fos* and *c-jun* in dysplastic lesions (Sakamoto et al., 1999) which may in turn induce abnormal expression of genes controlled by AP-1 (Angel and Karin, 1991; Sassone-Corsi, 1995). Members of the AP-1 family are important factors regulating bone formation. For example, deletion or overexpression of *c-fos* in transgenic mice induces severe bone abnormalities (Grigoriadis et al., 1995). *c-fos* over-expression in mice results in increased bone formation, with characteristics similar to those found in dysplastic bone lesions (Grigoriadis et al., 1995). In vitro, AP-1 controls genes that are involved in osteoblast differentiation, and *c-fos* expression modulates the expression of osteoblast genes such as osteocalcin and osteopontin whose promoter contains AP-1 sites (Lian et al., 1991). In situ hybridization and immunohistochemical analyses showed that *c-fos* mRNA and protein levels are high in bone lesions of dysplastic patients with $G\alpha$ mutations (Candelieri et al., 1995). Thus, *c-fos* overexpression in fibrous dysplasia may be involved in the molecular mechanisms leading to the bone lesions. *c-fos* is often overexpressed in human osteosarcomas (Wu et al., 1990). Moreover, experimental studies showed that *c-fos* can induce oncogenic transformation in mouse osteoblasts (Grigoriadis et al., 1993, 1995). Thus, *c-fos* overexpression may be part of the mechanisms leading to tumor development in some cases of fibrous dysplasia (Yabut et al., 1988). Several mechanisms can be evoked to explain how *c-fos* overexpression can induce the cellular abnormalities found in dysplastic bone lesions. The increased *c-fos* protein by cAMP may induce cells to enter into the G1 phase of the cellular cycle (Herschman, 1991). This is consistent with the finding that *c-fos* is expressed by immature osteoblasts and controls osteoblast proliferation and differentiation (Lian et al., 1991; Machwate et al., 1995a,b). It seems therefore possible that the increased osteoblastic cell proliferation and the abnormal osteoblast differentiation observed in fibrous dysplasia results in part from the increased expression of *c-fos* and other members of the AP-1 family.

As discussed above, localized lytic lesions are found

in dysplastic lesions. At least two cytokines may be responsible for this local increase in bone resorption in fibrous dysplasia. Osteoprogenitor cells bearing the R201H $G\alpha$ mutation show a constitutive increase in interleukin-6 (IL-6) production (Yamamoto et al., 1996). In addition, less differentiated cells present in the bone lesions produce more IL-6 levels (Stanton et al., 1999). Inhibition of cAMP levels in mutant cells reduce IL-6 production, suggesting that the $G\alpha$ mutation-induced increased cAMP levels results in IL-6 overexpression (Yamamoto et al., 1996). This is consistent with the finding that transfection of the mutated $G\alpha$ in osteoblastic cells induces IL-6 production (Motomura et al., 1998). The IL-6 promoter presents cAMP-responsive elements, as well as AP-1 and NF- κ B sites (Kishimoto, 1989). The $G\alpha$ mutation increases binding of AP-1, nuclear factor NF-IL-6, NF- κ B and CREB to the IL-6 promoter, which can thus account for the increased expression of this cytokine in dysplastic osteoblasts (Motomura et al., 1998). The implication of IL-6 in the increased bone resorption in dysplastic cystic lesions (Yamamoto et al., 1996) is supported by the finding that, in other bone diseases characterized by increased bone resorption such as postmenopausal osteoporosis and Paget's disease, increased IL-6 stimulates osteoclast differentiation and bone resorption (Jilka et al., 1992; Roodman, 1992). Interestingly, glucocorticoids were found to decrease IL-6 production by dysplastic cells and to reduce bone resorption, which may be of potential therapeutic interest in the treatment of fibrous dysplasia (Stanton et al., 1999). IL-11 production was also found to be increased in mutated dysplastic cells (Yamamoto et al., 1996), suggesting that this bone resorbing cytokine may also be involved in the lytic lesions in this syndrome. Recent data indicate that local molecules produced by stromal cells and osteoblasts play a major role in osteoclast differentiation and bone resorption (Marie et al., 2000; Hofbauer et al., 2000). Such molecules are involved in the increased bone resorption induced by several local factors such as IL-6 and IL-11 (Hofbauer et al., 2000). It is thus possible that these molecules may be the final step of IL-6- and IL-11-induced bone resorption induced by $G\alpha$ mutations in fibrous dysplasia.

The cellular alterations observed in fibrous dysplasia may involve other mechanisms. Two studies reported an increased level for estrogen expression in osteoblasts in both monostotic fibrous dysplasia and McCune-Albright syndrome (Kaplan et al., 1988; Pensler et al., 1990). This was not linked to the endocrine status of the subjects and could thus reflect abnormal regulation of these receptors (Pensler et al., 1990). This abnormality may contribute to the bone pathology in this syndrome because estrogens stimulate the proliferation of osteoblastic cells in part by increasing the expression of local growth factors such as IGF-I and TGF- β (Rickard et al., 1999) which are important factors controlling osteogenesis (Marie, 1999b). It is not known, however, whether the expression of these growth factors is increased locally in

fibrous dysplasia. Finally, a rise in the expression of the parathyroid hormone related peptide (PTHrP) has been recently reported in osteoblastic cells of patients with the McCune-Albright syndrome. A treatment with 1,25-dihydroxy-vitamin D3 can reduce the production of PTHrP in vitro as well as bone markers in this disease. It is thus possible that a local increase in the expression of PTHrP, which acts on bone cells in a similar way to PTH, could contribute to the bone cell alterations in the McCune Albright syndrome (Fraser et al., 2000).

Conclusion

The discovery that Gs α mutations are expressed in dysplastic bone cells provided a basis for the cellular and molecular mechanisms involved in the osteoblast alterations observed in fibrous dysplasia. It is now apparent that the abnormal osteoblastic cell proliferation and differentiation observed in fibrous dysplasia result from increased cAMP accumulation, leading to alteration in the expression of target genes including c-fos, c-jun, Il-6, Il-11, which in turn modulate the transcription and expression of downstream genes and result in the alterations of osteoblast and osteoclast recruitment and function in the dysplastic bone lesions. These pathways provide a cellular and molecular basis for the bone alterations in fibrous dysplasia. Further analysis of the molecular pathways activated by the Gs α mutations in dysplastic bone cells may lead to the identification of target genes for cellular therapies and allow us in the future to develop a therapeutic approach of the skeletal alterations in fibrous dysplasia.

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