REVIEW

Skeletal progenitors and the *GNAS* gene: fibrous dysplasia of bone read through stem cells

Mara Riminucci^{1,2}, Pamela Gehron Robey³, Isabella Saggio^{2,4} and Paolo Bianco^{1,2}

¹Department of Molecular Medicine, La Sapienza University, 00161 Rome, Italy

²Biomedical Science Park San Raffaele, 00128 Rome, Italy

³Department of Health and Human Services, Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland 20892, USA

⁴Department of Genetics and Molecular Biology, Sapienza University of Rome, and Institute for Molecular Biology and Pathology, National Research Council (CNR), 00185 Rome, Italy

(Correspondence should be addressed to P Bianco who is now at Division of Pathology, Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161 Rome, Italy; Email: paolo.bianco@uniroma1.it)

Abstract

Activating mutations of the *GNAS* gene, which causes fibrous dysplasia of bone (FD), lead to remarkable changes in the properties of skeletal progenitors, and it is these changes that mediate the pathological effect of this gene on bone. Mutated skeletal stem cells lose the ability to differentiate into adipocytes, and to maintain *in situ*, and transfer heterotopically, the hematopoietic microenvironment, leading to abnormal bone marrow histology in FD. They overexpress molecular effectors of osteoclastogenesis, thus promoting inappropriate bone resorption leading to fragility of FD bone. They express the phosphate-regulating hormone FGF-23 at normal levels, whose excess in the serum of FD patients correlates with the mass of osteogenic cells within FD lesions, leading to osteomalacia and deformity of the FD bone, and revealing that bone is an endocrine organ regulating renal handling of phosphate. Mechanisms of allelic selection and stem cell selection occur in mutated skeletal stem cells and contribute to the inherent diversity and evolution over time in FD. The definition of the etiological role of *GNAS* mutations marks the watershed between many decades of descriptive observation and the definition of cellular and molecular mechanisms that would explain and hopefully allow for a cure for the disease. Placing stem cells at center stage has permitted substantial advances in one decade, and promises more for the one to come.

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Skeletal stem cells and bone disease

The notion that the human bone marrow includes a second type of stem cell besides hematopoietic stem cells originates from classical studies demonstrating an inherent osteogenic potential of boneless marrow fragments transplanted heterotopically (Tavassoli & Crosby 1968). This potential was later ascribed to adherent, non-hematopoietic, stromal cells, noted for their ability to initiate density-insensitive clonal growth of ‘fibroblastic’ colonies (Friedenstein *et al.* 1970, 1974). The idea that these colony forming unit-fibroblasts (CFU-Fs) would include a subset of putative stem cells was substantiated by the observation that the clonal progeny of a single CFU-F could generate multiple differentiated tissues normally found in the skeleton, i.e. cartilage, bone, hematopoietic supportive stroma, fibrous tissues, and adipocytes. Hence, the hypothesis of a ‘osteogenic’ (Friedenstein *et al.* 1987) or ‘stromal’ (Owen & Friedenstein 1988) stem cell was

formulated, and later resonated in the rediscovery of the ‘osteogenic’ cells as ‘mesenchymal stem cells’ (Caplan 1991, Pittenger *et al.* 1999). Skeletal stem cells (Bianco & Robey 2004, Bianco *et al.* 2006, 2008) are found in the wall of bone marrow sinusoids (Sacchetti *et al.* 2007), where they reside as adventitial reticular cells, a bone marrow-specific adaptation of the ‘mural cell’/pericyte phenotype found in the microvascular wall of all tissues (Bianco *et al.* 2008). They can be prospectively isolated based on expression of CD146/MCAM (Sacchetti *et al.* 2007), a cell adhesion molecule of the immunoglobulin superfamily expressed in adventitial reticular cells in the bone marrow and in pericytes in other tissues. Immunoselection for CD146 allows for isolation of the CFU-Fs, which therefore correspond to CD146-expressing cells in the intact bone marrow, and thus to adventitial reticular cells. *In vivo*, adventitial reticular cells can generate adipocytes (Bianco *et al.* 1988) and bone, and provide structural and functional support to hematopoietic

progenitors *in vivo* (Weiss 1976, Westen & Bainton 1979). Upon isolation as CFU-Fs and growth in culture, they generate a progeny of stromal cells that can be heterotopically transplanted in immunocompromised hosts. Heterotopic 'ossicles' are thus generated, in which the hematopoietic microenvironment is established (Fig. 1A). During the development of heterotopic ossicles, a subset of transplanted cells self-renew into adventitial reticular cells of the newly formed bone marrow sinusoids, and reconstitute a compartment of CFU-Fs that can be secondarily passaged as CD146-expressing clonogenic progenitors (Sacchetti *et al.* 2007). Heterotopic ossicles are chimeric systems in which bone and the hematopoietic stroma are of donor origin, while hematopoietic cells and endothelial cells are of host origin. They represent the defining evidence of both potency and self-renewal of skeletal stem cells.

A role for bone marrow stromal cells as mediators/ effectors of disease of bone and bone marrow was long surmised in a variety of conditions including

idiopathic myelofibrosis, hyperparathyroid bone disease, and bone metastasis. Their inherent ability to generate bone (as illustrated by *ex vivo* explantation and *in vivo* transplantation studies) was easily linked, conceptually, to the establishment of heterotopic bone, and stromal changes within the bone marrow space are some of these natural pathological conditions in humans. This had become the proper scenario in which the analysis of a genetic bone disease at the stem cell level was inscribed.

The *GNAS* gene

Human *GNAS* is an imprinted gene locus, located on chromosome 20q13, within which several transcripts and alternative isoforms are encoded (Rao *et al.* 1991, Kehlenbach *et al.* 1994, Hayward *et al.* 1998a). The major *GNAS* transcripts, such as *Gs-α*, *XLas*, *NESP*, and *1A* (alternatively termed *A/B*), are generated from transcript-specific promoters and first exons and share

Figure 1 (A) Combination of human *ex vivo* expanded normal bone marrow stromal cells (BMSCs) with an appropriate scaffold regenerates a complete ectopic ossicle (bone, hematopoietic supportive stroma and marrow adipocytes of donor origin, and hematopoiesis of murine origin) when transplanted into immunocompromised mice. (B) BMSCs derived from fibrous dysplastic (FD) lesions recapitulate the formation of fibrous dysplastic bone and fibrotic tissue (instead of a hematopoietic marrow) in *in vivo* transplants. (C) An FD-causing mutation (*Gs-α*^{R201C}) can be introduced into normal BMSCs by way of lentiviral transduction, resulting in cells that regenerate a FD-like ossicle upon *in vivo* transplantation. (D) Using si*Gs-α*^{R201C} lentiviral vectors in either mutation-transduced or FD BMSCs, only the mutant allele is silenced, resulting in normalization of cAMP production and restoration of BMSC function.

Figure 2 The *GNAS* chromosomal region showing promoters (solid boxes) and first exons specific for each transcript (NESP55, XLas, 1A, and Gs-α) as white boxes and exons common to all *GNAS* transcripts as gray boxes (exons 2–13). NESP55 is expressed from the maternal allele (purple), whereas XLas and 1A are expressed from the paternal allele (blue) due to methylation patterns (meth). In clonogenic bone marrow stromal cells (BMSCs) derived from either normal or FD tissue, NESP is restricted to a subset of clonal cells and is expressed from the maternal allele. However, unlike in other tissues, XLas is expressed from both the maternal and paternal alleles in normal and FD BMSCs (dashed arrows). Gs-α is expressed from both alleles in most (but not all) normal tissues and four different splice variants has been reported: Gs-α-1 (long, with exon 3), Gs-α-2 (short, without exon 3), Gs-α-3 (long form with CAG immediately upstream of exon 4) (codon CAG), and Gs-α-4 (short form with CAG). The R201 FD mutation site is in exon 8. In kidney, thyroid, pituitary, and ovary tissues, Gs-α is only expressed from the paternal allele (black arrow), but in normal and FD BMSCs, overall, it is expressed from both alleles (dashed arrows), although a great deal of asymmetry of expression is noted in clonal BMSC strains.

a common downstream sequence (Fig. 2). The XLas transcript is expressed in neuroendocrine cells and, to a lesser extent, in other tissues and cell types such as brain, pancreas, adrenal gland, heart, kidney, adipose tissues, and osteogenic cells (Pasolli *et al.* 2000, Plagge *et al.* 2004, Michienzi *et al.* 2007). XLas protein shares with Gs-α the entire carboxyl-terminal region (Kehlenbach *et al.* 1994), and displays some Gs-α-like properties *in vitro* (Klemke *et al.* 2000, Bastepe *et al.* 2002, Linglart *et al.* 2006). NESP expression is highly restricted to neuroendocrine tissues in which the transcript is translated into a chromogranin-like protein, NESP55. The NESP-coding sequence is limited to the first exon, and the resulting protein is unrelated to either Gs-α or XLas (Ischia *et al.* 1997). 1A mRNA is ubiquitously expressed. However, it is not translated into a functional protein, and its function remains as yet unclear. In addition to Gs-α, NESP, XLas, 1A, and splice variants thereof, other less well-known *GNAS* transcripts, such as an antisense transcript, NESPAS, and others (Wroe *et al.* 2000, Holmes *et al.* 2003), have been identified, which add further complexity to the locus (Klemke *et al.* 2001, Freson *et al.* 2003).

The expression of *GNAS* products is epigenetically regulated according to a complex pattern of imprinting (Reik & Walter 2001; Fig. 2). XLas and 1A are paternally transcribed, and their promoters are methylated on the maternal allele. In contrast, the origin of NESP is exclusively maternal, and its promoter is methylated on the paternal allele (Hayward *et al.* 1998a,b, Peters *et al.* 1999). The Gs-α promoter is unmethylated on both alleles. However, recent studies identified an imprinting mark around the 1A promoter, which is thought to suppress the expression of Gs-α from the paternal allele in a tissue-specific manner (Liu *et al.* 2005). Partial imprinting of Gs-α has also been observed in the human renal proximal tubule, anterior pituitary, and thyroid (Hayward *et al.* 2001, Germain-Lee *et al.* 2002, Mantovani *et al.* 2002), which has clinical implications in patients carrying loss-of-function mutations of *GNAS*.

Following transcription, each *GNAS* transcript is alternatively spliced to generate two isoforms (long and short), plus and minus exon 3 respectively. In addition, the serine residue at the end of exon 3 can be spliced in or out to generate a total of two long and two short variants (Fig. 2). Gs-α splicing isoforms have been

the subject of multiple studies. The long and the short Gs- α mRNAs are translated into different molecular weight proteins, the distribution and relative ratio of which vary in different developmental and metabolic conditions (van der Vuurst *et al.* 1998, Ihnatovych *et al.* 2001, Frey *et al.* 2005). Whether the variants are functionally different remains to be determined (Kvapil *et al.* 1994, Novotny & Svoboda 1998, Unson *et al.* 2000, Wenzel-Seifert *et al.* 2001).

GNAS transcripts in skeletal progenitors

Gs- α is expressed in all clonal strains established in culture from single osteoprogenitors, and in each clone, the Gs- α transcripts are spliced to generate comparable amounts of the four isoforms (plus and minus exon 3, plus and minus Ser). Although the specific function of the different Gs- α isoforms is currently unknown, their balanced expression could be a specific feature of undifferentiated skeletal progenitors. Indeed, our recent unpublished data demonstrate that the Gs- α long/short isoform ratio is dramatically reduced during osteogenic and adipocytic differentiation of skeletal progenitors *in vitro*.

In normal fetal and post-natal bone, as well as in fibrous dysplasia (FD) tissue, Gs- α transcripts are biallelic in origin, and the amount of the transcript generated from each allele is, overall, similar (Mantovani *et al.* 2004). In individual skeletal progenitors isolated *in vitro*, conversely, the transcriptional activity of the two Gs- α alleles may be significantly different (Michienzi *et al.* 2007). Each allele can be selectively expressed or conversely, almost completely, silenced in a random fashion, independent of parental origin of each allele (Fig. 2). This pattern of regulation of expression of the two Gs- α alleles has never been reported in other systems, and its mechanism remains to be elucidated. However, it can neither be referred to as imprinting, which specifically involves maternal or paternal genes (Constancia *et al.* 1998), nor be referred to as allelic expression imbalance, which is an inherited trait and therefore consistent in different cells from the same patient (Yan *et al.* 2002).

Of the other GNAS transcripts, XLas, 1A, and NESP are all expressed in skeletal progenitors, although the expression of NESP seems to be restricted to a small subset of clones. Interestingly, while NESP and 1A transcripts retain the monoallelic origin observed in other tissues, XLas displays, in cultured skeletal progenitors, a biallelic pattern of transcription, never previously reported in human cells (Michienzi *et al.* 2007; Fig. 2). In summary, the overall imprinting status of the gene in skeletal cells seems to be slightly different from that observed in other human tissues. In addition, multiple GNAS transcripts are expressed in skeletal

stem cells and undifferentiated progenitors, but the GNAS transcript portfolio is remarkably heterogeneous among different clonogenic cells, even beyond the variable allelic origin of Gs- α itself. Further studies should elucidate how this complexity is modulated during differentiation events.

Fibrous dysplasia of bone and GNAS mutations

Activating missense mutation of the GNAS gene causes FD of bone (Weinstein *et al.* 1991, Schwindinger *et al.* 1992) (FD, OMIM 174800), and when more broadly distributed, a genetic, non-inherited multi-organ disease, which in addition to bone involves, in variable combination, a constellation of endocrine organs, the melanocyte system, the liver, and likely a broader range of tissues in which the clinical expression of the underlying mutation is either minor or not readily recognized (Bianco *et al.* 2003). The combination of FD, endocrinopathy, and skin pigmentation is classically known as the McCune–Albright syndrome (Albright *et al.* 1937, 1938), but additional syndromic pictures exist, such as Mazabraud's syndrome, in which FD combines with muscular myxomas (Henschen & Fallvon 1926). Replacement of Arg201 with either His or Cys in the mutated protein results in a ~30-fold reduced intrinsic GTPase activity, translating into its constitutive activation (Bourne *et al.* 1989). Constitutively active Gs- α stimulates adenylyl cyclase, resulting in overproduction of cAMP and subsequent abnormal cellular responses.

Of the entire range of organ lesions, the skeletal lesions remain the most serious and the least treatable. Attention to skeletal stem cells and their role in the pathogenesis of FD was drawn by the observation that the fibrosis that characterizes the abnormal bone marrow of FD bone, like the one seen in hyperparathyroid bone disease, is in fact comprised of an excess of cells with phenotypic features of bone marrow stromal cells (Riminucci *et al.* 1997, 1999). GNAS-mutated CFU-Fs can indeed be isolated from the abnormal, fibrotic bone marrow of FD lesions, like normal CFU-Fs are isolated from normal bone marrow. It was then natural to reason that transplantation of mutated skeletal progenitors isolated from FD lesions would generate heterotopic ossicles recapitulating the abnormal histology of FD bone and fibrotic bone marrow, much like transplantation of normal skeletal progenitors recapitulates organogenesis of normal bone. Transplantation of bone marrow stromal cell strains derived from FD tissue generated FD-like ossicles, in which abnormal woven bone similar to FD bone is deposited by donor cells, and a fibrotic bone marrow develops, which like the fibrotic bone marrow

of FD lesions does not accommodate hematopoietic cells, nor does it include bone marrow adipocytes (Bianco *et al.* 1998, 2000; Fig. 1B). Recapitulation of FD histology in ossicles generated by transplantation of mutated skeletal progenitors proved the principle of FD as a 'stem cell disease', and provided a humanized *in vivo* model of the disease based on stem cell transplantation.

Skeletal progenitors as mediators of FD pathogenesis

Mutated skeletal progenitors directly mediate specific facets of FD pathology. For example, greatly enhanced bone resorption noted in FD lesions is rooted into an enhanced osteoclastogenesis, mediated by osteogenic cells. Molecular mediators of these effects include IL6, which was shown to be produced in excess, and regulated by glucocorticoids, in bone marrow stromal cells isolated from FD lesions (Stanton *et al.* 1999). Both basal and induced production of IL6 appears to be different in clonal mutated skeletal progenitors compared to non-mutated cognates, and non-osteogenic cells within the local FD milieu can also contribute to local IL6 levels (Riminucci *et al.* 2003b). IL6 is also abundantly expressed in FD lesions *in situ*. Interestingly, FD lesions are noted for osteoclastogenic events that occur ectopically; i.e. within the fibrotic intertrabecular space and away from bone surfaces, and such ectopic osteoclastogenic foci were noted as sites of high expression of IL6 in stromal cells (Riminucci *et al.* 2003b). More recently, studies using lentiviral transduction of bone marrow stromal cells revealed a potent and rapid up-regulation of RANKL as one of the most prominent effects induced by mutated *Gs-α* (Piersanti *et al.* 2010), suggesting that direct interaction of mutated stromal cells with osteoclast progenitors may be involved in initiating osteoclastogenesis (and particularly, ectopic osteoclastogenesis) within FD lesions.

A crucial feature of FD lesions, directly linked to abnormal bone fragility and risk fracture, indeed represents the ultimate expression of a change in skeletal progenitors brought about by *Gs-α* mutation and excess cAMP production. Likewise, following sporadic reports on FD-associated osteomalacia (Ryan *et al.* 1968, Dent & Gertner 1976, Dachille *et al.* 1990), systematic studies on the histopathology of FD demonstrated the occurrence of severe mineralization defects in the majority of FD lesions (Bianco *et al.* 2000, 2003, Corsi *et al.* 2003; Fig. 3A), and of generalized osteomalacia in a subset of patients. Attempts to elucidate the pathogenetic mechanisms of FD-associated osteomalacia identified the phosphaturic factor, FGF-23, as a major player in the disorder (Riminucci *et al.* 2003a, Kobayashi *et al.* 2006) and osteogenic cells as a major

source of FGF-23 in the human body (Riminucci *et al.* 2003a; Fig. 3B). Inappropriate increases in serum FGF-23 levels cause a phosphate-wasting syndrome (reduced renal phosphate reabsorption, hypophosphatemia, and low levels of 1,25-(OH)₂-D₃) leading to rickets/osteomalacia, in a variety of settings (Shimada *et al.* 2001, Quarles 2003, Yu & White 2005). In inherited hypophosphatemic rickets, increased serum levels of FGF-23 result either from missense mutations of the *FGF-23* gene, which reduce the clearance of the protein (Autosomal dominant hypophosphatemic rickets, ADHR 2000), or from mutations of genes encoding other bone-derived factors such as *Phex* (X-linked hypophosphatemia (XLH; Jonsson *et al.* 2003)) and *DMP-1* (autosomal recessive hypophosphatemic rickets, ARHR (Feng *et al.* 2006)) which, through as yet unknown mechanisms, regulate serum levels of FGF-23. In oncogenic osteomalacia, the phosphate-wasting disorder is a paraneoplastic expression sustained by massive secretion of the molecule by mesenchymal neoplastic cells. Analysis of FGF-23 production in skeletal progenitors *in situ* (Fig. 3B) and *in vitro* leads to the recognition that increased FGF-23 in FD does not reflect a regulatory effect of *GNAS* on *FGF-23* gene expression. Comparable levels of the molecule are produced *in vitro*, at the single cell level, by mutated FD cells and normal osteoprogenitors (Riminucci *et al.* 2003a), and serum levels of FGF-23 in FD patients therefore correlate with disease burden (Riminucci *et al.* 2003a; Fig. 3C). Thus, in the range of bone and mineral disorders associated with excess FGF-23, the osteomalacia occurring in FD represents a unique type of phosphate-wasting syndrome emanating from the abnormal expansion of FGF-23-producing, non-neoplastic skeletal cells. On the other hand, the identification of production of FGF-23 by cells of osteogenic lineage including skeletal progenitors revealed the skeleton as an endocrine organ regulating renal handling of phosphate.

Kinetics of *GNAS*-mutated stem cells *in vivo*

Consumption of skeletal progenitors that occur over time in mice with targeted expression of a constitutively active PTH/PTHrP receptor (signaling through *Gs-α*) in bone suggests that cAMP signaling might mediate a significant alteration in the population kinetics of skeletal progenitors. In as yet unknown mechanisms, activating *GNAS* mutations also seem to alter the stem cell-dependent kinetics of the osteogenic lineage in humans. FD and McCune–Albright syndrome (MAS) are somatic mosaic states, in which cells with either normal or disease genotypes coexist in different tissues and organs. Each clinical FD lesion consists itself of a mosaic of normal and mutated stem/progenitor cells,

Figure 3 (A) Fibrous dysplastic bone is characterized by osteomalacia, as demonstrated by von Kossa staining that reveals poor mineralization and a great excess of osteoid material. (B) The osteomalacic nature of FD bone is caused by expression of the phosphate-regulating hormone, FGF-23, by malfunctioning osteoblastic cells (arrows) and cells in the fibrotic tissue (ft) within the lesions, as demonstrated by *in situ* hybridization. (C) While activating *Gs-α* mutations do not lead to an up-regulation of FGF-23, serum levels of FGF-23 correlated with disease burden, which reflects abundance of activated malfunctioning osteogenic cells and their precursors in fibrous dysplastic lesions.

rather than simply representing a uniformly mutated piece of a mosaic organism or organ (Bianco *et al.* 1998). Relative frequencies of normal and mutated stem/progenitor cells vary in individual lesions, patients, and in points of time in each patient (Kuznetsov *et al.* 2008; Fig. 4C). In a small cohort of patients, the frequency of mutated clonogenic stem/progenitor cells appeared to be negatively correlated with patient's age (Fig. 4D), and was plausibly related to the high rate of apoptotic demise of osteogenic cells observed within FD lesions (Kuznetsov *et al.* 2008; Fig. 4A and B). At least in a proportion of 'aged' lesions observed past the age of skeletal maturity, mutated stem/progenitor cells are not detectable, and it is conceivable that this phenomenon reflects the ultimate clearance of mutated progenitors over time in some lesions. Interestingly, progressive remodeling of FD lesions into bone with normal architecture and clearance of fibrosis with restoration of hematopoietic

marrow (Fig. 4E) also occur in a proportion of 'aged' FD lesions, in which mutated osteoprogenitors cannot be identified. Upon clearance of mutated CFU-Fs, non-clonogenic stromal cells carrying the disease genotype remain detectable within a lesional site. However, it is only in the presence of mutated stem/progenitor cells that bone marrow stromal cell strains recapitulate FD lesions upon xenotransplantation, suggesting that it is only retention of stem/progenitor cells that can maintain an FD lesion *in vivo*. Studies in larger cohorts of patients, specific analysis of the behavior of lesions at different skeletal sites (e.g. craniofacial versus appendicular), or with distinct overall histological patterns (Riminucci *et al.* 1999), and correlation with additional determinants of disease or of general hormonal status (e.g. excess of specific hormones, menopause, and gender) are needed to elucidate how these 'normalization' phenomena are inscribed into the general natural history of the disease. Clearly, lesions that keep

Figure 4 (A) In FD lesions obtained from young patients, a large number of apoptotic bodies are noted within the tissue (arrows). (B) Further evidence of apoptosis is demonstrated by TUNEL staining, suggesting extensive cell death within the lesions. (C) When the number of skeletal stem cells is estimated by determining the colony forming efficiency of normal and FD BMSCs, there is a huge increase in colony forming unit-fibroblasts (CFU-Fs) from FD lesions compared to normal bone in patients under the age of 30; yet, the number decreases dramatically in patients over the age of 30, and is not statistically different from normal, suggesting a biological change within the lesion. (D) When the percent of mutant clones (arising from mutant CFU-Fs) was enumerated compared to normal clones, it was determined that there is a sharp decline as a function of age, with no mutant clones being identified in older patients, reflecting their demise due to apoptosis. (E) While mutant BMSCs are lost as a function of age, normal ones persist, and remodel lesional tissue into normal bone (stained black with von Kossa) and support the establishment of normal hematopoiesis.

progressing over the age of 40 are commonly seen clinically, and unfortunately FD cannot be seen as a self-limited disease in any way. Nonetheless, these ‘normalization’ events represent a striking example of stem cell replacement therapy in bone, brought about by nature itself. They remind us that stem cell therapy is feasible in FD, as it is an option obviously chosen by nature itself. However, FD bone can only be replaced by normal bone, through stem cell-fueled remodeling, in a time window that extends over decades, due to the naturally low rate of turnover in bone. On a different note, it seems worth remarking, also, that remodeling of bone per se does not imply an adverse bearing on the evolution of FD lesions. In spite of its likely role in initiating and establishing the lesion, bone remodeling also has a role, at least in some cases, in reverting dysplastic bone back into normalized cancellous bone. Whereas current drug therapy remains anchored in the use of anti-remodeling agents, a more precise and focused understanding of the actual evolution of individual lesions at the time of pharmacological intervention might contribute to refine therapeutic choices and decisions.

Transfer and analysis of the FD phenotype in stem cells

Specific aspects of the role of *GNAS* mutation in dysregulating the biology of skeletal progenitors elude feasibility through study of FD-derived cells. Global analyses of transcriptome changes downstream of *GNAS* mutation, or elucidation of early responses induced in stem cells by *GNAS* mutations, are difficult, or impossible, respectively, using clinical material. The fundamental cellular phenotype can be transferred and reverted in stem cells using lentiviral technologies, which provide a complementary approach and circumvent these hurdles (Fig. 1C). The relevance of these strategies for analysis of the impact of *GNAS* mutations in stem cells rests with the controlled flexibility they allow in the exposure of stem cells to the disease gene. Thus, they allow for dissection of its early and late effects to match them to different differentiation stages, or to monitor cell responses, such as the induction of adaptive enhancement of phosphodiesterase activity and expression, in a controlled way. The same technologies,

combined with strategies for allele-selective RNA interference, offer an option for attempting gene correction in skeletal stem cells (Fig. 1D). The idea of correcting a genetic disease in bone by correcting the underlying gene dysfunction in stem cells is both natural and supported by remarkable success histories in other areas of medicine and other kinds of stem cells (reviewed in Riminucci *et al.* (2006)). The FD-causing mutations are dominant, gain-of-function mutations in a gene that is otherwise indispensable and ubiquitously expressed. Silencing the disease gene is required, rather than gene replacement. Silencing must be specific for the mutated allele, as Gs- α function is indispensable, and the mutated allele only differs from the wild type by 1 base. This posits the most complex scenario one can conceive for gene therapy. Nonetheless, proof of principle for the feasibility of gene correction in FD has been provided. Expression of short hairpin RNA-interfering sequences through lentiviral vectors in human skeletal stem cells that are either derived from natural FD lesions, or transduced with lentiviral construct encoding the causative mutated Gs- α , results in the loss of mutated Gs- α and in the abrogation of cAMP overproduction (Piersanti *et al.* 2010; Fig. 1D). As directed from lentiviral vectors, silencing is stable, and can be made completely specific for the mutated Gs- α , leaving expression of the wild-type allele unscathed. In addition, the block of adipogenesis induced by mutated Gs- α in skeletal progenitors is reversed by lentiviral-directed, mutation-specific RNAi, indicating that at least some functional effects that specifically relate to the progenitor cell nature of the target cells (adipogenesis) downstream not only of the mutation per se, but also of the fundamental cellular phenotype (cAMP overproduction) can indeed be reverted at least *in vitro*.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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