RESEARCH

Runx2 overexpression compromises bone quality in acromegalic patients

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Abstract

Acromegalic patients, characterized by excessive secretion of GH and IGF-1, show a high fracture risk but bone mineral density is a poor predictor for bone fractures in these patients. The effects of an excess of GH/IGF1 on skeleton as well as on osteogenic progenitors, i.e. mesenchymal stem cells, have not been investigated in these patients. We aimed to elucidate the skeletal conditions of acromegalic patients by means of bone microarchitecture analysis and evaluation of MSCs osteogenic commitment. In particular, we performed histomorphometric analyses, and we quantified the expression levels of the osteogenic transcription factor RUNX2 in circulating MSCs. Our results showed an abnormal microarchitecture and demonstrated that bone impairment in acromegalic patients is associated with the upregulation of *RUNX2* expression. Furthermore, osteoblastic activity was significantly reduced in patients under pharmacological treatment, compared to untreated patients. In conclusion, this study demonstrates the key role of *RUNX2* gene overexpression in causing bone impairment in acromegalic patients. It also suggests a therapeutic approach for the improvement of bone quality, focused on the osteoblastic lineage rather than the inhibition of osteoclastic activity.

Key Words

- acromegaly
- mesenchymal stem cells
- RUNX2
- osteogenesis

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Introduction

Acromegaly, a chronic endocrinopathy, is characterized by hypersecretion of growth hormone (GH) and its mediator, insulin-like growth factor-1 (IGF-1), due to pituitary tumors. Overproduction of GH causes a series of clinical symptoms, including osteoarticular manifestations: acromegaly is considered as a cause of secondary osteoporosis. The GH/IGF1 axis represents in fact a major signaling system, among others, for bone development and remodeling. It is known that GH/IGF1 deficiency induces bone loss (Yakar *et al.* 2002) and in active acromegaly, high levels of GH are associated with increased bone mineral density (BMD) (Bolanowski *et al.* 2006). Despite this latter fact, acromegalic patients show a high fracture risk, suggesting that BMD is a poor predictive criterion of bone fractures. Indeed, bone fractures depend on skeletal architecture as well as on bone strength, which is related to structural and qualitative aspects. Recently, it has been shown that high levels of GH in a transgenic mouse model affect bone architecture and mechanical strength (Lim *et al.* 2015) However, the effects of an excess of GH/IGF1 on bone remodeling as well as on osteogenic progenitors, i.e. mesenchymal stem cells (MSCs), are unclear and confusing. MSCs are the leading actors in both types of ossification processes, which occur during embryonic and

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postnatal skeletogenesis. In endochondral ossification, MSC-derived chondrocytes provide a cartilaginous template, which is subsequently replaced by osteoblasts and osteoclasts. The intramembranous ossification process, on the other hand, is characterized by a direct differentiation of MSCs into osteoblasts (Karsenty 2003). In the adult, bone remodeling has a relevant role in maintaining mechanical skeletal integrity; a dynamic equilibrium between bone formation (performed by osteoblasts) and bone resorption (performed by osteoclasts) is required.

Osteogenic differentiation of MSCs is regulated by systemic hormones: parathyroid hormone (PTH), glucocorticoids, estrogens, and by local stimuli: bone morphogenetic proteins (BMPs), transforming growth factor-β (TGF-beta1/2), insulin-like growth factor (IGF), fibroblast growth factor 2 (FGF-2), vascular endothelial growth factors (VEGFs), cytokine modulators (prostaglandins) and the Wnt/β-catenin pathway. All these players can trigger intracellular signaling pathways involved in osteoblastic differentiation by modulating the expression and activity of specific transcription factors (Franceschi *et al.* 2007).

In this context, the Runt-related transcription factor-2 (*RUNX2*), a Runt DNA-binding domain-containing protein, plays a primary role in the osteoblastic and chondrocytic differentiation processes of MSCs in mice and in humans (Lee *et al.* 1997). It is known that activation of RUNX2 in bone marrow (BM) MSCs induces the expression of a master regulator of osteoblast differentiation, OSTERIX, which in turn activates the expression of downstream genes coding for specific osteoblastic proteins such as collagen type I (COL1A1), bone alkaline phosphatase (bALP) and osteocalcin (OC) (Dalle Carbonare *et al.* 2012). *Runx2*-deficient mice (*Runx2*−/−) die just after birth and show complete absence of bone formation (Komori *et al.* 1997). *RUNX2* overexpression, however, seems to affect osteoblast maturation, suggesting a multifaceted role of this transcription factor in bone formation (Cohen 2009). Although the presence of GH receptors in osteoblasts has been demonstrated (Nilsson *et al.* 1995), few studies so far have investigated the effects of a direct modulation of GH on osteoblasts. Furthermore data concerning *RUNX2* regulation in acromegalic patients are not available. Therefore, in order to gain further understanding of bone changes occurring in acromegaly at the molecular/cellular level, we have investigated *RUNX2* expression in circulating MSCs as well as skeletal microarchitecture in a cohort of acromegalic patients.

Patients and methods

Study subjects

A cohort of 21 acromegalic patients (9 with active disease (ADPs) and 12 normalized after treatment (TPs)) and 10 healthy donors were recruited at Verona University Hospital. Written informed consent was obtained from all participants, and the study was approved by the Ethical Committee of Azienda Ospedaliera Universitaria Integrata of Verona, Italy (number 1538).

The diagnosis of acromegaly was based on high serum GH levels, not suppressible after oral glucose load (in non-diabetic patients), and high plasma IGF-1 levels for age and sex. Acromegaly was excluded or considered cured if the patient had a random GH measurement <1 ng/mL, and, since May 2010, <0.4 ng/mL after oral glucose load (OGTT). The following age-related reference ranges of IGF-1 were for 20–40 years (15–40 nmol/L), for 41–65 years (10–30 nmol/L) and for >65 years (7–25 nmol/L). Age-adjusted IGF-1 values were calculated with age-specific reference ranges for our IGF-1 assay (IGF-1 % = patient's IGF-1/age-specific upper limit × 100) (Table 1). In case of discordant IGF-1 vs GH levels, we measured GH after OGTT, and we considered the patient controlled if the GH was suppressible after OGTT. Serum GH and IGF-1 were determined by chemiluminescent immunometric assay (IMMULITE, DPC, Los Angeles, CA, USA). The analytical sensitivities of GH and IGF-1 assay were 0.01 and 20 ng/mL (2.62 nmol/L), respectively, and the intra-assay precisions were 5.6 and 11.5% in the standard curve range, respectively.

The following bone turnover parameters were evaluated: serum calcium (Roche/Hitachi CV <10%; Roche Diagnostics), serum PTH (DiaSorin Liaison N-tact PTH, CV <10%; DiaSorin, Inc., Stillwater, FL, USA), C-telopeptide of type I collagen (CTX; Elecsys B-CrossLaps/serum assay, CV <20%; Roche Diagnostics) and serum 25-hydroxyvitamin

Table 1 General characteristics of APs (9 active patients (ADPs) and 12 controlled patients (TPs)).

	ADPs (n.9)	**TPs** (n.12)	**Significancy**
Age	58 ± 15	55 ± 12	NS
BMI	32 ± 9	29 ± 5	NS
PTH (pg/mg)	26 ± 11	27 ± 16	NS
Vitamin D (ng/mL)	27.7 ± 6.0	22.6 ± 6.6	NS
Calcium (mg/dL)	9.8 ± 0.4	9.5 ± 0.5	NS
ALP (U/L)	76 ± 23	66 ± 12	NS
GH (μg/mL)	1.4 ± 0.9	0.5 ± 0.5	$P<0.001$
IGF1 (nM/L)	41 ± 11	22 ± 7	$P<0.001$
Creatinine (mg/dL)	0.80 ± 0.12	0.88 ± 0.12	NS
CTX (ng/mL)	0.47 ± 0.25	0.48 ± 0.36	NS

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D (25(OH)D; DiaSorin Liaison, total CV <15%; Diasorin). Phosphate, creatinine and ALP values were obtained using automated standard laboratory methods.

Circulating mesenchymal stem cells (cMSCs)

cMSCs were obtained from 21 acromegalic patients and 10 controls as previously reported (Valenti *et al.* 2011). Briefly, peripheral blood mononuclear cells (PBMCs) obtained with the Ficoll Paque procedure were mixed with 4 mL peripheral blood of the same patient and incubated with 'RosetteSep' (Human Mesenchymal Stem Cell Enrichment cocktail; Stem Cell Technologies) to obtain cMSCs by negative selection according to manufacturer's instruction.

Analysis of cell phenotype

To compare pre- and post-depleted samples, we analyzed RNA expression of CD3, CD14, CD19, CD45 and CD34 hematopoietic markers as previously reported (Valenti *et al.* 2008). We performed the expression analysis of MSCs positive markers CD73 and CD105 as well. This method allows the phenotypic analysis of cells obtained with stringent stem cell purification techniques (Thoma *et al.* 1994).

Osteogenic differentiation of mesenchymal stem cells

Sera, obtained from 10 mL of fresh blood by centrifugation at 400 ***g***, were harvested and frozen in aliquots at −80°C until use. We used hMSCs (PromoCell) to analyze the effects of sera on osteogenic differentiation. We chose commercial MSCs in order to avoid confounding effects of different circulating growth factors as well as cytokines. Sera pools (controls, patients with active disease/ADPs and treated patients/TPs) were obtained mixing equal serum volumes from all individuals. Serum was added to medium at 10% concentration with and w/o beta glycerophosphate. Cells were then plated at a density of 5×10^4 cells per well into 48-well plates and cultured for one week (for *RUNX2* expression) or two weeks (for *SPARC* expression), before gene expression analysis.

Real-time PCR

PCR was performed in a total volume of 20 µL containing 1× Premix Ex Taq (2×), 1× Rox Reference Dye (50×) and 20 ng of cDNA; probe sets for CD3, CD14, CD19, CD45, CD34, *RUNX2*, beta-actin and beta-2 microglobulin genes were obtained from Assay-on-Demand Gene Expression Products (RUNX2, Hs00231692_m1; SPARC, Hs00234160_m1; CD3, Hs00174158_m1; CD14, Hs02621496-s1; CD19, Hs00174333_m1; CD45, Hs00174541_m1; CD34, HS00156373_m1; CD73, Hs00159686_m1, CD105, Hs00923996_m1; ACTB, 4326315E; B2M, 4326319E; Applied Biosystems). Real-time RT-PCR reactions were carried out in two-tube system and in multiplex.

The amplification conditions included 30 s at 95°C (initial denaturation), followed by 50 cycles at 95°C for 5 s (denaturation) and at 60°C for 31 s (annealing/extension). Thermocycling and signal detection were performed with ABI Prism 7000 Sequence Detector; (Applied Biosystems). Signals were detected according to the manufacturer's instructions.

We selected the ΔRn in the exponential phase of amplification plots to determine the Ct values. ΔΔCt values were then calculated compared to control as previously reported (Livak & Schmittgen 2001).

To normalize mRNA expression for sample-to-sample in RNA input, quality and reverse transcriptase efficiency, we amplified the *ACTB* and *B2M* housekeeping genes. *ACTB* and *B2M* endogenous/internal control genes were abundant and remained constant proportionally to total RNA among the samples.

Ct data

Ct values for each reaction were determined using TaqMan SDS analysis software. For each amount of RNA tested, triplicate Ct values were averaged. Because Ct values vary linearly with the logarithm of the amount of RNA, this average represents a geometric mean.

Bone densitometry and fracture evaluation

Each patients underwent densitometry at the lumbar spine and at the hip.

Bone densitometry was performed with Hologic QDR 4500 Acclaim (Hologic Inc., Waltham, MA, USA).

WHO criteria for the diagnosis of PO were used (normal: *T*-score higher than −1 S.D.; osteopenia: *T*-score between −1 and −2.5 S.D.; osteoporosis: *T*-score lower than −2.5 S.D.).

To establish the presence of vertebral fractures, antero-posterior and latero-lateral X-ray of dorsal and lumbar spine were performed. A qualitative analysis was made by a specialized radiology and subsequently a quantitative evaluation, according to Genant score, was conducted from D4 to L4.

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Briefly, posterior, medium and anterior vertebral heights were measured and an incident fracture was diagnosed if there was at least 20% of difference between them. Fractures were divided into three different degrees: mild (a reduction of the heights ratio between 20% and 25%), moderate (a reduction of 25–40%) and severe (a reduction higher than 40%).

All non-vertebral fractures were recorded from patient interviews or from clinical files.

Histomorphometry

Iliac crest bone biopsy was performed in 13 patients (6 ADPs and 7 TPs). The samples were fixed in 70% ethanol and embedded undecalcified in methyl-methacrylate resin (Merck 800590). Bone sections were cut by using a microtome (Polycut S, Leica Microsystems) equipped with a carbide-tungsten blade, stained with Goldner's stain and mounted on microscope slides for histomorphometric measurements. The sections were prepared as previously described (Bedogni *et al.* 2012). Measurements were performed by means of an image analysis system consisting of an epifluorescent microscope (Leica DM2500) connected to a digital camera (Leica DFC420 C) and a computer equipped with a specific software for histomorphometric analyses (Bone 3.5, Explora Nova, France). Histomorphometric parameters were reported in accordance with the ASBMR Committee nomenclature (Dempster *et al.* 2013). All thickness/depth results (O.Th, MAR, E.De, W.Th) were corrected for obliquity of sections by multiplying by $\pi/4$. In addition, to better describe osteoblast activity, we calculated the number of Ob actively contributing to bone formation (ObS/BS), Ob density (Ob.N/Ob.S; the number of Ob per unit of Ob.S) and Ob vigor index (Ob.S*MAR/Ob.N) using the stereological data to reflect the total bone formed per Ob.

We selected 13 biopsies taken at the autopsy in patients aged 60±6 years as previously described (Dalle Carbonare *et al.* 2008). The dynamic parameters of turnover have been compared with normal values reported in previous studies (Melsen & Mosekilde 1978, Melsen *et al.* 1978).

Statistical analysis

Results are expressed as mean±S.D. The Mann–Whitney test was used for parameters which did not show a linear distribution (e.g. PTH, ALP and IGF1: Table 1). For the analysis of treatment responses (data related to the treatment of hMSCs cells with sera), multiple ANOVA measurement followed by Bonferroni as *post hoc* analysis was performed. A probability value of less than 0.05 was considered statistically significant. Spearman correlation coefficient and regression curve estimations were performed when indicated. Analyses were applied to experiments carried out at least three times. Statistical analyses were performed using SPSS for Windows, version 22.0 (SPSS).

Results

Patient characteristics

Characteristics of study patients are summarized in Table 1.

None of the patients showed alterations in renal function or in bone metabolism, except for vitamin D, which was slightly below the normal range in both ADPs and TPs (n.v. >30 ng/mL). BMD values at lumbar spine and femur were normal, expressed as *T* score and also corrected for age (*Z* score).

Histomorphometric data

Acromegalic patients showed reduced values of (BV/TV: 15.9±5.7% vs 18.7±1.2% $P<0.05$), trabecular thickness (TbTh: 78.0±6.8 µm vs 82.9±8.2 µm $P<0.05$), with increased trabecular separation (TbSp: 586±183 µm vs 372±125 µm $P<0.05$) compared to sex- and age-matched healthy controls. In addition, microarchitecture analysis showed reduced values of direct (NdN/TV: 1.4±0.8 vs 2.0±0.2 NdN/TmN: 0.4±0.1 vs 0.6±0.2, $P<0.05$) and indirect parameters (MSV 0.49±0.47/mm^3 vs 0.12±0.22 $P<0.05$) (Fig. 1).

The analysis of cortical parameters showed increased mean cortical thickness (1436.86±587.8 mm vs 1065.1±530.4 mm, $P<0.05$) and porosity (11.9±1.1% vs 4.7±1.4%, $P<0.001$) with respect to sex- and age-matched normal controls. The evaluation of turnover showed a significant increase of bone formation rate, which was calculated as mineral apposition rate × mineralizing surfaces/bone surfaces (0.03±0.01 vs 0.13±0.07, $P<0.01$).

To evaluate the relationship between disease activity and bone turnover, we compared acromegalic patients with active disease (ADPs) with those receiving a treatment and obtaining hormone normalization (TPs). As expected, ADPs showed increased bone turnover, associated with increased cortical thickness and porosity, TPs instead presented normal bone turnover, with persisting cortical thickness and porosity. Interestingly, in TPs patients,

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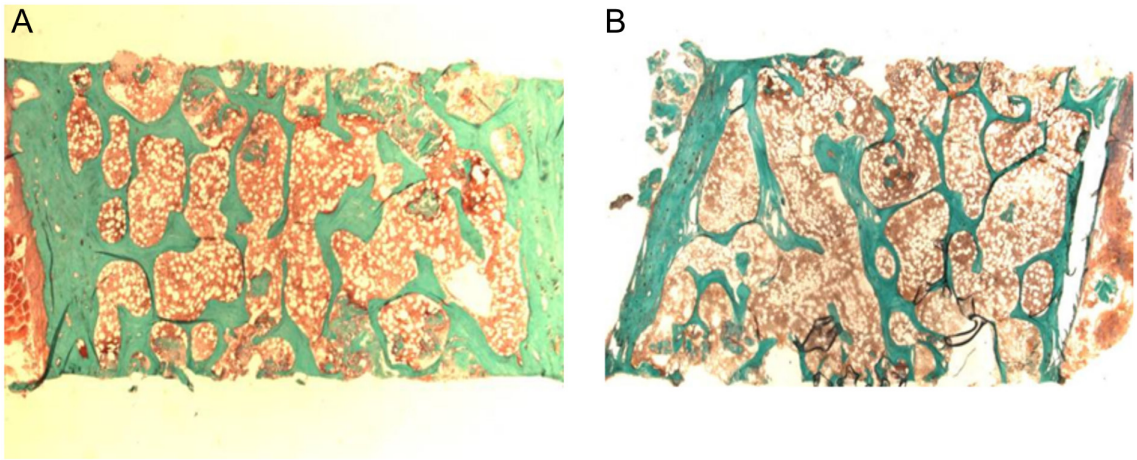

Figure 1
Representative histomorphometric images from: (A) acromegalic patient and (B) healthy donor. Acromegaly is characterized by increased cortical thickness and porosity, with altered bone microarchitecture, compared to donor (see also histomorphometric data in 'Results' section).

parameters of osteoblastic activity were significantly reduced compared to ADPs patients. In fact, osteoblast number (ObN/BS 0.09±0.01 vs 0.16±0.01, $P<0.001$), active osteoblasts (ObS/BS 2.8±0.4 vs 6.4±1.3, $P<0.01$) and osteoblast vigor (ObVg 45.9±6.8 vs 65.7±16.9, $P< 0.05$) in the presence of reduced osteocyte number (OsteocyteN 0.32±0.13 vs 0.51±0.07, $P<0.01$) were significantly different in the two groups.

RUNX2 gene expression in circulating mesenchymal stem cells

In order to evaluate the levels of osteogenic commitment in acromegalic patients, we analyzed the expression levels of the osteogenic transcription factor RUNX2 in MSCs. Circulating MSCs obtained from acromegalic patients (APs) and healthy donors (HDs) expressed cluster differentiation markers in similar percentages. The cells expressed low levels of CD14, CD45 and CD34 after depletion; they were negative for CD3 and CD19 in both APs and HDs (Table 2). The expression values of CD14, CD45 and CD34 were not significantly different for APs and HDs ($P>0.05$). As shown in Table 2, the expression levels of positive markers CD105 and CD73 were >60% (CD105) and >70% (CD73), respectively, in both patients and HDs.

Both APs (9 ADPs and 12 TPs) and HDs expressed *RUNX2* gene. However, expression levels were different. In fact, the expression of *RUNX2* in APs was higher than that in HDs (Fig. 2). In particular, *RUNX2* expression was higher in TPs than that in ADPs (Fig. 2). Interestingly, a negative correlation between *RUNX2* expression levels and osteoblasts number evaluated by histomorphometry was found only in TPs patients (R^2 0.35, $P<0.05$). This last observation suggests that *RUNX2* overexpression prevents osteoblastic maturation.

Osteogenic gene expression in serum-treated hMSCs

In order to evaluate the effects of APs sera on the osteogenic differentiation process, we cultured hMSCs with sera obtained from patients with active disease (ADPs) or

Table 2 Cell phenotypes of healthy donors (HD) and acromegalic patients (APs).

Cluster differentiation transcript	Pre-negative selection		Post-negative selection		Significance (post-negative selection HDs vs APs)
	HDs (%)	APs (%)	HDs (%)	APs (%)	
CD3	12.6 (±1.5)	11.4 (±1.2)	0	0	NS
CD14	9.4 (±0.8)	10.1 (±0.6)	0.6 (±0.07)	0.7 (±0.05)	NS
CD19	3.2 (±0.4)	3.4 (±0.3)	0	0	NS
CD45	5.2 (±0.6)	4.7 (±0.4)	2.1 (±0.5)	1.9 (±0.6)	NS
CD34	Low level	Low level	Low level	Low level	NS
CD105	Low level	Low level	62 (±10%)	65 (±7%)	NS
CD73	Low level	Low level	71 (±20%)	74 (±10%)	NS

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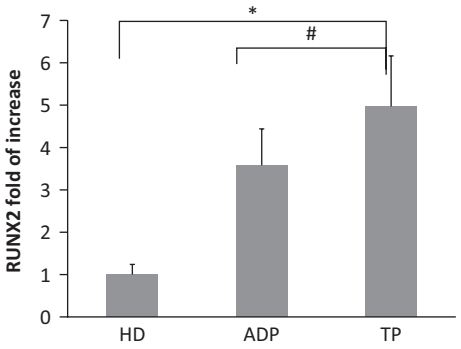

Figure 2
RUNX2 expression in cMSCs. Expression levels in ADPs and TPs were 3.5 (±0.8) and 4.6 (±0.9) fold higher than that in HDs (*$P<0.05$ ADPs and TPs vs HD; #$P<0.05$ ADPs vs TPs).

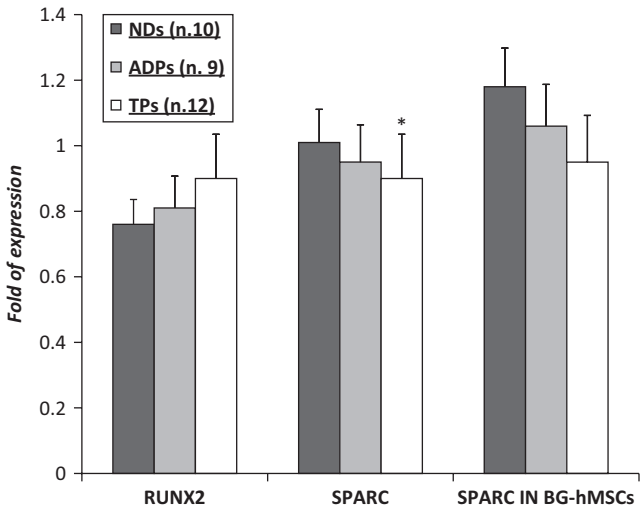

Figure 3
Gene expression levels in hMSC lines treated with sera of normal donors (NDs) or APs subgrouped in active disease (ADPs) and treated patients (TPs). An increased expression of the osteogenic commitment marker *RUNX2* was observed in APs sera-treated cells, although values did not reach statistical significance. Conversely, the expression of *SPARC* gene was lower in cells treated with APs sera and the difference reached a statistical significance in cells treated with TPs sera compared to cells treated with NDs sera (*$P<0.05$). The addition of beta glycerophosphate restored *SPARC* expression in cells treated with APs sera to levels comparable to those observed in cells treated with NDs sera.

under treatment (TPs), and we compared gene expression with that observed in cells cultured with serum from normal donors (NDs). hMSC lines treated APs sera showed an increased expression of the osteogenic commitment marker *RUNX2*, compared to cells treated with NDs sera (although values did not reach statistical significance). Conversely, the expression of *SPARC* gene, which codes for osteonectin, an osteoblast maturation marker, was lower in cells treated with APs sera; in particular, the reduction was statistically significant in cells treated with TPs sera. Furthermore, the addition of beta glycerophosphate-restored *SPARC* expression in cells treated with APs sera to levels comparable to those observed in cells treated with NDs sera (Fig. 3).

Discussion

The effects of acromegaly on MSCs differentiation are poorly understood. It has been previously reported that high levels of GH in APs inhibit the adipogenic differentiation process thus reducing adipose mass (Plockinger & Reuter 2008); data related to osteogenic differentiation in these patients are nevertheless incomplete. The aim of this study was to evaluate whether poor bone quality in APs could be ascribed to an impaired osteogenic differentiation resulting in an abnormal microarchitecture. We therefore monitored the expression of *RUNX2*, the master gene responsible for osteogenic commitment, in cMSCs. In previous studies, we had shown that cMSCs represent a useful tool to study bone physiology (Valenti *et al.* 2008) and that *RUNX2* expression variations can be associated to physiological and pathological changes in mineral density (Dalle Carbonare *et al.* 2009, Zanatta *et al.* 2012). Many authors have identified circulating MSC-like cells; inconsistent reports may be imputed to the different analysis approaches employed (Seebach *et al.* 2007, Alm *et al.* 2010). Depending on the surface markers used, mean values of 1–1000/10^6 cells have been identified as circulating MSCs (Marlicz *et al.* 2012, Blogowski *et al.* 2016, Ferensztajn-Rochowiak *et al.* 2017). Higher numbers of circulating MSC-like cells have been associated to muscle, damaged skin, fractures and hypoxia condition (Mansilla *et al.* 2006, Ramirez *et al.* 2006, Rochefort *et al.* 2006). It has been reported that an increased fat mass results from the recruitment of adipocyte progenitor cells (Hong *et al.* 2005); this may suggest that circulating progenitors derive from adipose tissue too. However, acromegalic active patients have been reported to show altered body composition, including water retention and reduction in body fat (Katznelson 2009). Thus, further studies might be necessary in order to evaluate actual levels of adipogenic circulating progenitors in these patients. The most remarkable finding of the present study is that in APs, the expression levels of *RUNX2* were significantly higher than those in HDs. It has been demonstrated that IGF-1/IGFR signaling stimulates *RUNX2* expression through the activation of MAPK/ERK pathways (D'Souza *et al.* 2009); this finding may justify the high levels of RUNX2 in APs. Furthermore, the bone impairment in APs, evaluated

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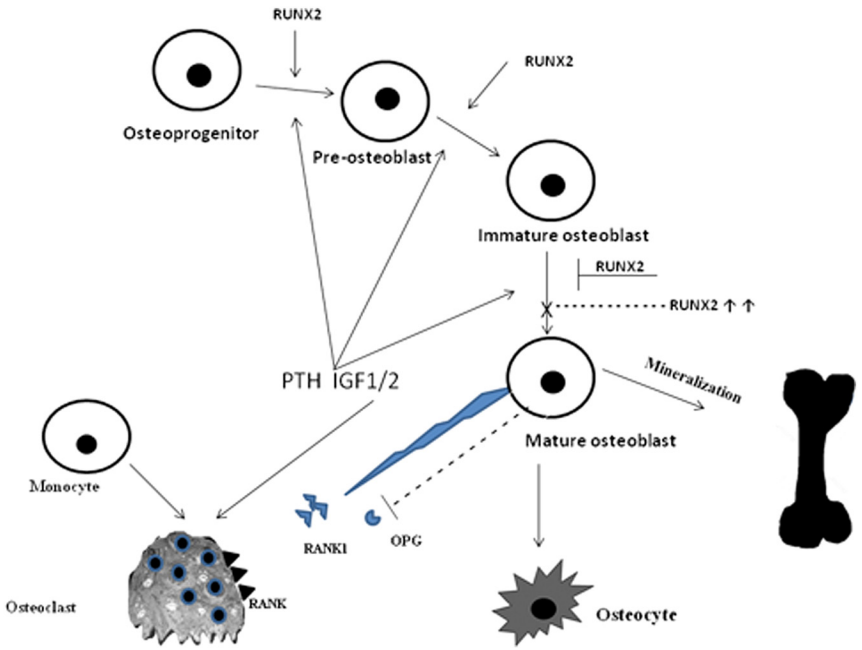

Figure 4
A schematic representation of the osteogenic process in APs. Note the osteogenic maturation hindrance due to high RUNX2 expression.
A full color version of this figure is available at https://doi.org/10.1530/ERC-17-0523.

by histomorphometric analysis, is associated to the upregulation of RUNX2 levels that were higher in both ADPs and TPs compared to HD. However, this finding is more evident in the TPs group, where a negative correlation between RUNX2 levels and osteoblast number was observed. This seemingly contradictory observation may, however, be explained by considering that *RUNX2* expression needs to be suppressed in order to allow osteoblast maturation, as demonstrated by studies on murine models. Transgenic mice overexpressing *Runx2* in osteoblasts showed osteopenia with multiple fractures (Liu *et al*. 2001, Geoffroy *et al*. 2002). Bone formation was increased, inducing high turnover rates; the number of osteoblasts was increased at an early developmental stage, but their transition to mature osteoblasts was blocked, thus impairing both matrix production and mineralization. Likewise, by comparing histomorphometric data with *RUNX2* expression in cMSCs, we inferred that in APs, osteoblasts linger in an immature state. In order to confirm this hypothesis, we cultured hMSCs with sera obtained from patients (ADPs and TPs). We observed levels of *RUNX2* expression higher than those found in cells cultured with NDs sera. On the contrary, the expression levels of SPARC, an osteoblast maturation marker, were reduced in cell cultured with APs sera. Interestingly, beta glycerophosphate, an osteogenic inducer, did restore osteoblastic maturation when added to APs sera-treated cell cultures.

It has also been reported that *RANKL* gene, encoding a cytokine involved in osteoclastogenesis, is a RUNX2 target (Geoffroy *et al*. 2002). This finding is supported by the fact that *RANKL* promoter contains a putative binding site for RUNX2 (Kitazawa *et al*. 1999). Accordingly, in transgenic mice overexpressing *Runx2*, bone resorption rate exceeded bone formation rate (Geoffroy *et al*. 2002). Histomorphometry studies on giant transgenic mice overexpressing bovine GH (bGH) revealed altered cortical and trabecular bone architecture (Lim *et al*. 2015). In humans, a duplication in *RUNX2* gene, leading to a gain of function, causes a rare autosomal dominant condition, metaphyseal dysplasia with maxillary hypoplasia and brachydactily (MDMHB, MIM#156510) (Moffatt *et al*. 2013). Affected individuals from a French Canadian family had low lumbar spine BMD; histomorphometric analysis of iliac bone biopsy samples showed thin cortices and low amount of trabecular bone; cortical osteocyte density resulted to be 32% lower than that in controls; osteoclastogenesis however was not increased.

In our controlled APs (TPs), bone turnover markers were within the normal range. Osteoclastic function and activity may yet be directly stimulated by GH/IGF-1 (Ueland 2005). Moreover, parameters of osteoblastic activity were significantly reduced in TPs compared to ADPs. In TPs, *RUNX2* expression levels were higher than those in ADPs. We speculate that *RUNX2* upregulation, which inhibits osteoblast maturation, added to vestiges of the osteoclastic activity stimulated by GH overproduction during the active disease period, may concur to the altered bone microarchitecture and cortical bone porosity phenotypes in acromegaly, despite normal BMD values.

A schematic representation of the osteogenic process in APs is suggested in Fig. 4.

On the basis of our findings, we believe that a comprehensive treatment of the acromegalic disease should aim not only at the reduction of GH production but also at the improvement of bone quality. The aim of reducing bone fragility might be achieved by the employment of antiresorptive agents (e.g. bisphosphonates) in the active phase of the disease, followed by the administration of an anabolic agent (e.g. PTH), in order to stimulate osteoblastic maturation, in the controlled phase of the disease (i.e. after normalization of biochemical parameters).

Declaration of interest

All authors disclose no financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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Author contribution statement

M T V and L D C designed the experiments, analyzed data and wrote the manuscript; M M and G M carried out molecular analysis, analyzed data and wrote the manuscript; A D and A M carried out the experiments; M Z and M P analyzed the data.

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