Clinical Research Article

Paraneoplastic Secretion of Multiple Phosphatonin From a Deep Fibrous Histiocytoma Causing Oncogenic Osteomalacia

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Abstract

Context: Literature suggests that oncogenic osteomalacia is usually caused by a benign mesenchymal tumor secreting fibroblast growth factor subtype-23 (FGF-23), but the involvement of other phosphatolins has only been scarcely reported. We have previously published a seemingly typical case of oncogenic osteomalacia. Following curative neoplasm resection, we now report unique molecular characteristics and biology of this tumor.

Case Description: A 25-year-old man had been diagnosed with severe oncogenic osteomalacia that gradually crippled him over 6 years. 68Ga-DOTA-TATE positron emission tomography/computed tomography scan localized the culprit tumor to his left sole, which on resection revealed a deep fibrous histiocytoma displaying a proliferation of spindle cells with storiform pattern associated with multinucleated giant cells resembling osteoclasts. Circulating FGF-23, which was elevated more than 2-fold, declined to undetectable levels 24 h after surgery. Microarray analysis revealed increased tumor gene expression of the phosphatolins FGF-23, matrix extracellular phosphoglycoprotein (MEPE) and secreted frizzled-related protein subtype 4, with elevated levels of all 3 proteins confirmed through immunoblot analysis. Differential expression of genes involved in bone formation and bone mineralization were further identified. The patient made an astonishing recovery from being wheelchair bound to fully self-ambulant 2 months postoperatively.
Conclusion: This report describes oncogenic osteomalacia due to a deep fibrous histiocytoma, which coincidentally has been found to induce profound muscle weakness via the overexpression of 3 phosphatonin, which resolved fully upon radical resection of the tumor. Additionally, genes involved in bone formation and bone remodeling contribute to the molecular signature of oncogenic osteomalacia.

Key Words: phosphatonin, oncogenic osteomalacia, hypophosphatemia, FGF-23, deep fibrous histiocytoma

Oncogenic osteomalacia is a very rare endocrine paraneoplastic syndrome typified by chronic eucalcemic hypophosphatemia, phosphaturia, and normal parathyroid hormone (PTH) due to circulating phosphatonin secreted by a tumor (1). The majority of such cases are due to paraneoplastic secretion of fibroblast growth factor subtype-23 (FGF-23) by benign mesenchymal tumors, although malignant tumors have also been described (1,2). In some instances, PTH may be elevated due to secondary hyperparathyroidism as a compensatory response to overcome the inhibitory effects of FGF-23 on 1,25-dihydroxycholecalciferol.

We have previously reported the case of a young man who was diagnosed at age 25 with oncogenic osteomalacia incapacitated and wheelchair bound by the time he presented to us (3). The tumor in the sole of his left foot, which was responsible for his severe muscle weakness, was resected, whereupon he made an astounding recovery. This is a follow-up report that describes the interesting and unique molecular and cellular features of the tumor, which corroborates with the limited data in the extant literature.

Case Report

A 25-year-old Indonesian Malay man without any medical history developed a progressive debilitating illness 6 years prior to presentation at our hospital. He had seen many medical specialists in his home country to no avail. By the time he traveled by plane to seek an expert opinion from our medical team, he was confined to a wheelchair. At age 19, he was still a very athletic undergraduate. He developed an excruciating arthralgia associated with proximal myopathy, kyphosis, and pectus excavatum. Past history was negative for malabsorption, muscular dystrophy, tuberculosis, connective tissue disease, and glucocorticoid use. Extensive laboratory investigations were remarkable for a severe persistent refractory hypophosphatemia coupled with renal phosphate wasting associated with elevated serum alkaline phosphatase and osteoporotic-range bone mineral density with very low Z-scores as shown by dual-energy X-ray absorptiometry scan (T-score −5.5, Z-score −5.4) suggestive of a secondary cause of bone loss. Serum calcium, intact PTH, and 25-hydroxyvitamin D levels were all normal. Bone biopsy also revealed normal osteocytes and lamellar arrangement without any other diagnostic findings. Nerve conduction tests were normal while electromyography displayed small amplitude polyphasic discharges consistent with an irritable nonspecific myopathy. Despite severe muscle weakness, serum creatine phosphokinase, serum aldolase, and vastus lateralis muscle biopsy were normal. Given the negative family history, the late onset, normal dentition, and the biochemical picture of hypophosphatemic osteomalacia, the diagnosis of oncogenic osteomalacia was made and a tumor search was performed according to a diagnostic algorithm reflective of the acceptable standard of care, as described by other groups (4,5). Notably, an initial attempt with a whole body 18F-FDG positron emission tomography (PET) scan from head to mid-thighs done in the patient’s own country before he consulted our hospital was negative. A thorough clinical examination by a senior endocrinologist (MKSL) in the team led to the discovery of a small soft tissue mass lesion over the sole of the left foot. This was ultimately proven by 68Ga-DOTA-TATE PET-computed tomography (CT) scan to be solely responsible for a high uptake “hot” spot (Fig. 1A-C). The tumor was excised en bloc with a good margin of normal tissue. Part of it was formalin fixed and paraffin embedded while the rest was snapped frozen. Elevated preoperative serum FGF-23 (378 RU/mL; normal < 180) rapidly declined to 99 RU/mL 4 h later and became undetectable 24 h postoperatively (3). This was accompanied by impressive resolution of the hypophosphatemia and rapid recovery in muscle power. The patient was self-ambulant 2 months postoperatively.

On light microscopy, there was a nodular tumor with irregular borders lying within the subcutaneous fat (Fig. 2A) composed of spindle cells arranged in fascicles with a storiform appearance in some areas (Fig. 2B). A hemangiopericytoma-like appearance of the blood vessels with branching, staghorn blood vessels were identified, but a prominent vascular network was not present. A significant population of osteoclast-like multinucleated giant cells was featured. Areas of hemorrhage with hemosiderin-laden macrophages were noted. Adipocytic component within the tumor, hyalinized to smudgy appearing matrix and grungy calcifications, were not identified on immunohistochemistry.
common genes in the combined up- and downregulated genes. Overall, there were 400 genes in the tumor vs skin, with 110 common genes and 1837 genes in tumor vs skin, with 290 common genes. Similarly, there were 623 significantly upregulated genes in tumor vs adjacent and 1522 genes in tumor vs skin, with 110 common genes downregulated between the 2. Overall, there were 400 common genes in the combined up- and downregulated list, which were used for pathway analysis. The complete raw data pertaining to this microarray analysis can be found at the Gene Expression Omnibus data repository (accession no. GSE160445) (6). Subsequent pathway analysis revealed that processes, such as cell proliferation and differentiation, cell adhesion/migration, and epithelial-mesenchymal transition were significantly represented. In particular, Hedgehog and PTH signaling pathways in bone and cartilage development were significantly positively enriched (P-value = 4.084 x 10^-4 with a false discovery rate (FDR) of 0.02275) (Table 1), which is highly relevant in the context of a soft tissue tumor that influences muscle and bone.

Importantly, microarray analysis revealed that the expression of phosphatonin, previously linked to oncogenic osteomalacia (7), were significantly upregulated in tumor tissue, when compared to adjacent tissue, with an ~360-fold increase in FGF-23, ~206-fold increase in matrix extracellular phosphoglycoprotein (MEPE), and an ~150-fold increase in secreted frizzled-related protein subtype 4 (sFRP-4) gene expression noted (Table 2). A significant increase in the expression of FGF-23 (~3700-fold) and sFRP-4 (~3400-fold) was also noted in tumor tissue, when compared to skin tissue (Table 2) and although not significant (FDR P = 0.053), the expression of MEPE was also upregulated (~8300-fold) in tumor tissue, when compared to skin tissue (Table 2). Subsequent immunoblot analyses confirmed increased levels of the phosphatoinoms FGF-23, MEPE, and sFRP-4 (Fig. 3) in tumor tissue, when compared to adjacent tissue. Microarray analysis further revealed increased expression of DMP1 (a gene that encodes for dentin matrix protein-1), which is implicated in regulating bone mineralization (8), in tumor tissue, when compared to adjacent tissue (~30-fold) and skin tissue (~45-fold), although the increased expression was not significant (FDR P = 0.12 and P = 0.131 in tumor tissue vs adjacent skin tissue, respectively) (Table 2). However, in contrast to the microarray data, immunoblot analysis revealed a reduction in dentin matrix protein-1 levels in tumor tissue (Fig. 3).

Interestingly, microarray analysis further revealed significantly increased expression of genes associated with osteopenia (Gremlin1; GREM-1) (9), bone mineralization and osteoblast differentiation (secreted phosphoprotein 1; SPP1 [osteopontin; OPN] and tenasin C; TNC) (10, 11) and significantly reduced expression of a gene involved in bone formation (FosB proto-oncogene, AP-1 transcription factor subunit; FOBS) (12), in tumor tissue, when compared to both adjacent and skin tissue (Table 2). Subsequent quantitative polymerase chain reaction (qPCR) analysis was performed to confirm GREM1 (3 transcript variants), SPP1 (2 transcript variants), TNC (1 transcript variant) and FOBS (2 transcript variants) gene expression.
changes across the tissue samples. Quantitative PCR data were normalized to both GAPDH (Fig. 4A) and HPRT (Fig. 4B), with comparable gene expression changes noted. In contrast to microarray results, the expression of GREM-1 was reduced in tumor tissue samples, when compared to adjacent stroma tissue (Fig. 4A and B). However, consistent with microarray data, increased expression of SPP1 and TNC and reduced expression of FOSB was confirmed in tumor tissue, when compared to adjacent and skin tissues (Fig. 4A and B).

Table 1. Significantly enriched pathways in tumor tissue

| No | Pathway                                                                 | P-value | FDR P-value |
|----|------------------------------------------------------------------------|---------|-------------|
| 1  | Cell adhesion_ECM remodeling                                           | 2.76E-07| 0.0001247   |
| 2  | Cell adhesion_Chemokines and adhesion                                  | 1.06E-05| 0.0024      |
| 3  | Immune response_Antigen presentation by MHC class II                   | 4.31E-05| 0.006169    |
| 4  | Cell adhesion_Endothelial cell contacts by non-junctional mechanisms   | 5.47E-05| 0.006169    |
| 5  | Cell adhesion_Integrin-mediated cell adhesion and migration            | 1.93E-04| 0.01743     |
| 6  | LRRK2 in neurons in Parkinson’s disease                                | 2.68E-04| 0.02018     |
| 7  | Cell adhesion_Plasmin signaling                                       | 3.57E-04| 0.02275     |
| 8  | Development_Hedgehog and PTH signaling pathways in bone and cartilage development | 4.08E-04| 0.02275     |
| 9  | Cytoskeleton remodeling_Cytoskeleton remodeling                       | 4.54E-04| 0.02275     |
| 10 | Transport_Macropinoscytosis regulation by growth factors               | 8.59E-04| 0.03225     |
| 11 | Development_Leptin signaling via JAK/STAT and MAPK cascades           | 9.20E-04| 0.03225     |
| 12 | Development_Regulation of epithelial-to-mesenchymal transition (EMT)   | 9.34E-04| 0.03225     |
| 13 | Cell adhesion_Cadherin-mediated cell adhesion                          | 1.07E-03| 0.03225     |
| 14 | Immune response_IL-10 signaling pathway                               | 1.07E-03| 0.03225     |
| 15 | Development_S1P2 and S1P3 receptors in cell proliferation and differentiatation | 1.07E-03| 0.03225     |
| 16 | Regulation of degradation of deltaF508 CFTR in CF                      | 1.24E-03| 0.035       |
| 17 | Development_Slit-Robo signaling                                       | 1.86E-03| 0.04935     |

Table shows significantly enriched pathways (FDR P-value < 0.05) in tumor tissue based on identified differentially expressed genes. Common up- and downregulated genes were identified upon comparing tumor tissue to both adjacent and skin tissue. Pathway names, raw P-value, and FDR P-value are given.
Institutional Review Board Approval

Ethical approval for the conduct of this research on the biobanked tissue samples of this patient was formally obtained from the Institutional Review Board of the National Healthcare Group (NHG) of Singapore. The ethics code is C/2013/00206. This study was done in accordance with the principles set out in the Declaration of Helsinki.

Materials and Methods

68Ga-DOTA-TATE PET-CT fusion scan

Peptide labeling with 68Ga in the Department of Nuclear Medicine and Molecular Imaging, Singapore General Hospital was performed manually. In brief, 250 µg HA-DOTA-[Tyr3] octreotate precursor was radiolabeled with gallium-68 eluted from 68Ge/68Ga generator (iThemba, South Africa). The 68Ge/68Ga generator was eluted with 10 mL of 0.6 N hydrochloric acid. Two milliliters of the 68Ga eluent was collected into a vented preheated vial. Two milliliters of Hepes buffer (280 mg/mL) was added, followed by 250 µg of HA-dotatate. The vial was heated for 15 min at 100°C. One milliliter of Hepes buffer (1060 mg/ml) and 0.8 mL of 2 N NaOH were then added. The final product was filtered through a 0.22 µm Millex filter. Radiochemical purity was established by thin layer chromatography and the purity exceeded 90%. Whole-body 68Ga HA-DOTATATE PET/CT imaging was performed using a dedicated General Electric PET/CT system (GE Discovery 690 VCT, GE Medical Systems, T able 2. Selected subset of differentially expressed genes

| Symbol | Tumor Tissue vs Adjacent Tissue | Tumor Tissue vs Skin Tissue |
|--------|---------------------------------|----------------------------|
|        | Fold change | P-value | FDR P-value | Fold change | P-value | FDR P-value |
| FGF23  | 361.36       | 0.0012  | 0.0425     | 3726.29     | 0.0001  | 0.0104     |
| MEPE   | 205.88       | 0.0005  | 0.0326     | 8308.04     | 0.0055  | 0.053      |
| SFRP4  | 155.13       | 0.0007  | 0.0354     | 3453.5      | 0.0003  | 0.0145     |
| DMP1   | 31.13        | 0.0151  | 0.12       | 44.46       | 0.0312  | 0.131      |
| GREM1  | 57.8         | 0.0009  | 0.0387     | 10.1        | 0.0047  | 0.0493     |
| SPP1   | 141.81       | 4.75×10^-5 | 0.0147   | 4805.01     | 0.003   | 0.0403     |
| TNC    | 90.5         | 9.13×10^-5 | 0.0191   | 215.67      | 0.0003  | 0.0149     |
| FOSB   | -37.43       | 0.0004  | 0.0305     | -368.83     | 7.87×10^-6 | 0.0033     |

Table lists a selected subset of differentially expressed genes, identified through microarray analysis comparing (i) tumor tissue and adjacent tissue and (ii) tumor tissue vs skin tissue. Gene symbols, fold changes, raw P-value and FDR P-value are given for each comparison. Negative numbers reflect fold repression of gene expression.

Figure 3. Immunoblot analysis. (A) Fibroblast growth factor subtype 23 (FGF-23), (B) matrix extracellular phosphoglycoprotein (MEPE), (C) secreted frizzled-related protein subtype 4 (sFRP4) and (D) Dentin matrix protein 1 (DMP1) protein levels within tumor tissue (tumor) and adjacent normal tissue (control). Ponceau S staining was performed as a loading control. The size (in KDa) of relevant molecular weight marker proteins are indicated on the left of each image.
LLC, Waukesha, WI, USA). The scan was acquired from the skull vertex to feet. Computed tomography was performed without intravenous contrast media application for the purpose of attenuation correction as follows: 100 kV; “GE smart mA dose modulation” helical thickness, 3.75 mm; table speed, 0.984 mm; rotation time, 0.8 s. Attenuation-corrected whole-body (vertex of skull to feet) scan was acquired in 3-dimensional mode (2-min emission time per bed position). Depending on body length, 16 bed positions were used with a field of view of 70 cm. For iterative reconstruction of the time of flight data, 3 iterations and 24 subsets with a filter cutoff of 7.0 were used. The patient received an intravenous injection of 203 MBq of 68Ga HA-DOTATATE and the acquisition was started after an uptake time of 60 min. Multiplanar reconstruction was performed for image interpretation. Images were interpreted by an experienced nuclear medicine physician. A semiquantitative measurement of intensity of tracer uptake was performed using the maximum standardized uptake value. For maximum standardized uptake value calculations, volumes of interest of the lesion was drawn automatically and adjusted manually to lesion size, applying a commercially available software provided by the vendor of the PET/CT-scanner (GE AWS).

**Histology and immunohistochemistry staining**

The excision specimen from the left foot comprising skin and subcutaneous tissue was received in buffered neutral formalin. The tumor in the specimen was extensively sampled and processed for routine hematoxylin and eosin–stained sections. Five-μm sections from formalin-fixed, paraffin-embedded tissues were stained with antibodies against CD31 (Neomarker), CD34 (DAKO), ERG (Cell Marque), STAT6 (Cell Marque), Factor XIIa (Cell Marque), CD163 (Cell Marque), desmin (DAKO), and SMA (DAKO) using the Ventana Optiview detection kit on the Roche Ventana BenchMark ULTRA slide stainer (Roche Diagnostics) after antigen retrieval, if required. For CD34, STAT6, Factor XIIa, CD163, and desmin stains, the ULTRA cell conditioning method on the Roche Ventana BenchMark ULTRA slide stainer was used for antigen retrieval. For ERG and CD31 stains, the pressure cooker with citrate buffer at pH6 method was employed. For SMA, no antigen retrieval treatment was required.

**Ribonucleic acid isolation**

Tumor tissue, adjacent normal tissue and resected overlying skin tissue biopsies were stored in RNAlater (Ambion) prior to ribonucleic acid (RNA) extraction. Total RNA was isolated from each tissue using QIAzol lysis reagent (Qiagen), as per the manufacturer’s instructions. The integrity of isolated RNA was evaluated using the Agilent 2100 Bioanalyzer system and the RNA 6000 Nano Kit (Agilent). All samples were stored at −80°C prior to further use.

**Microarray and pathway analysis**

Purified RNA was subjected to microarray analysis using the Illumina human HT-12 beadchip and in-house hybridization, quantification and analysis protocols (OCI Genomics Centre, Toronto, Canada). Microarray raw data were submitted to Gene Expression Omnibus (Accession No. GSE160445) (6). From the microarray data, 2 comparisons were made—tumor vs adjacent tissue and tumor vs skin. A 1-way analysis of variance and Benjamini-Hochberg multiple testing correction procedure were used to identify statistically significant changes in gene expression (defined as adjusted P-value < 0.05) using the software ArrayStudio (version 6.0) from Omicsoft. MetaCore (version 6.8, build 29806; GeneGo Inc.) was used to identify significantly
enriched pathways based on the differentially expressed genes. For this purpose, the common up and down genes (significant at FDR P-value < 0.05) were identified from the 2 comparisons mentioned earlier. To calculate the FDR, we have used the method by Benjamini and Hochberg, as is standard in gene expression analysis studies (13).

Reverse transcription and quantitative PCR
A total of 0.250 μg of RNA was subjected to reverse transcription using the iScript complementary deoxyribonucleic acid (DNA) synthesis kit (Bio Rad Cat No: 170–8891), as per the manufacturer’s protocol. Prior to qPCR analysis complementary DNA was diluted 1:10 in autoclaved MilliQ water. Quantitative PCR analysis was performed with 3 technical replicates using the SsoFast EvaGreen Supermix (Bio-Rad cat no: 7500001227) and the Bio-Rad CFX96 real-time polymerase chain reaction detection system. The qPCR conditions used were as follows: 95°C for 3 min, followed by 40 cycles of a 3-step reaction: 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s. These cycles were followed by a melt curve protocol: 95°C for 10 s, followed by 65°C to 95°C in 10 s increments of 0.5°C. Gene expression fold change was calculated using the 2^ΔΔCt method, with values normalized to GAPDH and HPRT.

The sequences of the primers used in this study are GREM1 (Var1)-F: 5’-ACT GAC AGT ATG AGC CGC AC-3’, GREM1 (Var1)-R: 5’-GCA CCT TGG GAC CCT TTC TT-3’, GREM1 (Var2)-F: 5’-AGA CAA GGC CCT GCA TGT G-3’, GREM1 (Var2)-R: 5’-GAA GCA GGT GTT GAT GGT GC-3’; GREM1 (Var3)-F: 5’-CGC GTT ACC GTCT CTT CCT GC-3’, GREM1 (Var3)-R: 5’-GAC ACT ATG AGC CGC AC-3’. The sequences of the primers used in this study are HPRT-F: 5’-TGA AGA AAT TCA TCC AAG AGA-3’, HPRT-R: 5’-TTC TT-3’; FOSB (Var1)-F: 5’-GAG CTG ACC GAG GCC TCC CAC-3’, FOSB (Var1)-R: 5’-CGG ATC AGT AGG TGC TCC ACA-3’; GAPDH (All)-F: 5’-TGT TGG AGG TGT GCC ATA C-3’, GAPDH (Var1)-R: 5’-CAA ATC TCT CAC CTC CGC-3’. The sequences of the primers used in this study are GREM (Var1)-F: 5’-ACT GAC AGT ATG AGC CGC AC-3’, GREM (Var1)-R: 5’-GCA CCT TGG GAC CCT TTC TT-3’, GREM (Var2)-F: 5’-AGA CAA GGC CCT GCA TGT G-3’, GREM (Var2)-R: 5’-GAA GCA GGT GTT GAT GGT GC-3’; GREM (Var3)-F: 5’-CGC GTT ACC GTCT CTT CCT GC-3’, GREM (Var3)-R: 5’-GAC ACT ATG AGC CGC AC-3’.

Protein extraction and immunoblot analysis
Total protein was extracted from tumor tissue and control (adjacent normal tissue) using radioimmunoprecipitation assay buffer containing: 50 mM Tris HCl pH7.5, 150 mM NaCl, 1.0 mM ethylenediamine tetra-acetate pH 8.0, 1.0% NP-40, 0.1% sodium dodecylsulphate, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 25 mM sodium fluoride, 1 mM sodium orthovanadate, 1x complete protease inhibitor cocktail (Roche Diagnostics) in phosphate-buffered saline. Bradford reagent (Bio-Rad) was used to quantify protein samples. For immunoblotting, 20 μg of total protein was separated on 4% to 12% NuPAGE Bis-Tris protein gels (Invitrogen), followed by transfer to nitrocellulose membrane using the Xcell Blot Module (Invitrogen). The primary antibodies used in this report were human anti-dentin matrix protein-1 (1:200, Santa Cruz, no. sc-73633), anti-MEPE (1:400, Santa Cruz, no. sc-377035), anti-FGF-23 (1:400, Abcam, no. ab56326), anti-sFRP4 (1:1000, Abcam, no. ab154167).

Secondary HRP-conjugated antibodies used in this report were antigoat (1:5000, Bio-Rad, no. 1721034), antirabbit (1:5000, Bio-Rad, no. 1706515) and antimouse (1:5000, Bio-Rad, no. 1706516). Protein bands were detected using Western Lightning Plus Chemiluminescent reagent (Perkin Elmer Life Sciences) and the ChemiDoc Imaging System (Bio-Rad). Ponceau S staining was conducted as a loading control.

Discussion
Oncogenic osteomalacia is a very rare paraneoplastic endocrinopathy with metabolic bone sequelae usually caused by benign mesenchymal tumors. In the literature, malignant tumors such as osteosarcoma and giant cell tumors have also been found to overexpress FGF-23 and resulted in oncogenic osteomalacia (1). However, our patient’s paraneoplastic phosphatonin-mediated hypophosphatemia due to a deep fibrous histiocytoma is much rarer by comparison to benign mesenchymal tumors (14); an extensive search only yielded a similar case reported nearly a decade ago (15). Notably, we have also demonstrated in our case of oncogenic osteomalacia not only elevated tumor production of FGF-23, but also simultaneous tumor co-expression of the additional phosphatonins MEPE and sFRP4. Only 1 other group had previously published the involvement of these phosphatonin in oncogenic osteomalacia nearly 2 decades ago and defined their role in the biology of bone and mineral metabolism (16). It is likely that these 3 phosphatonin jointly exert synergistic phosphate wasting actions and intensify the degree of muscle weakness and deterioration of bone micro-architecture. In agreement with De Beur et al (16), we found increased tumor expression of DMP1 messenger RNA via microarray analysis in our case of oncogenic osteomalacia. To our surprise, Western blots actually revealed reduced protein...
levels of DMP1 in tumor tissue relative to control adjacent tissue. Importantly, these data are consistent with previous work that suggests a role for reduced DMP1 in stimulating increased expression of FGF-23, hypophosphatemia, and osteomalacia (8,17), which is quite consistent with the increased FGF-23 and phenotype observed in this patient. It is interesting to surmise that the inverse relationship between DMP1 messenger RNA and DMP1 protein levels noted in this study may be due to post-transcriptional regulation processes or perhaps altered protein stability. However, further work will need to be undertaken to study DMP1 gene/protein regulation in more detail in cases of oncogenic osteomalacia to determine this.

Because a normal serum phosphate is necessary for calcium phosphate homeostasis in bones for skeletal health, chronic severe hypophosphatemia can cause osteomalacia. Fortuitously, based on the microarray analysis data, we found GREM1, SPP1, and TNC genes to be significantly upregulated while the FOSB was significantly downregulated. GREM1 (Gremlin-1) encodes for a secreted glycoprotein that antagonizes the function of bone morphogenetic proteins. Overexpression of Gremlin has been reported to lead to osteopenia (9); thus, it is interesting to surmise that elevated GREM1 may contribute to the bone loss and osteomalacia phenotype observed in this individual. However, it is important to highlight that the increased GREM1 expression identified by microarray analysis was not validated by subsequent qPCR and in fact, GREM1 expression was conversely found to be elevated in adjacent normal tissue, when compared to tumor tissue by qPCR (Fig. 4). Although microarray is a valid approach to assess gene expression it is important to note that due to the intrinsic variability present in microarray data there is a potential for identification of false positives (18). This may explain the disparity in GREM1 expression noted in the present study and further emphasizes the importance of validating microarray data using additional methodology such as qPCR. SPP1 (secreted phosphoprotein 1), otherwise called osteopontin, is a small integrin-binding ligand, N-linked glycoprotein that constitutes part of the extracellular matrix of bones and dentin (19). SPP1 has been shown to exhibit strong mineral binding properties and function to inhibit bone mineralization (10,20). Additionally, SPP1 is required for stimulating osteoclast-mediated bone resorption and as such has important roles in regulating osteoclast activity during bone remodeling (21,22). Overexpression of SPP1 has also been reported to inhibit bone mineralization in FGF-23-deficient mice and is suggested to contribute to the osteomalacia observed in these mice (23). Hence, the severe loss of bone mass encountered in this case is unlikely to be due to chronic hypophosphatemia alone. We postulate that SPP1 plays a role in contributing to the gross osteomalacia in this form of paraneoplastic metabolic bone disorder. If SPP1 is subsequently substantiated by others in future, it would constitute a novel pathway in the pathobiology of oncogenic osteomalacia. Tenascin-C, the protein product of the TNC gene, is a matricellular protein whose physiological function includes embryonic development, cell adhesion, cell migration, wound healing, and osteoblastic proliferation and differentiation (11,24). Notably, tenascin-C also plays a role in tissue injury, inflammation, pathological tissue remodeling and cancer invasion (25). It remains intriguing whether tenascin-C expression by this tumor contributed to patient disease manifestation or acted as a survival factor for the tumor itself. One plausible way to delineate the role of tenasin C in tumor survival as opposed to inducing oncogenic osteomalacia is to study its relative expression in similar tumors (i.e., deep fibrous histiocytomas), which lack any clinical association with osteomalacia. The other remarkable finding is the significant downregulation of the FOSB gene, one that deserves some comment and perhaps further research. FOSB is a protein encoded by the FBJ murine osteosarcoma viral oncogene homolog B gene. FOSB heterodimerizes with DNA-binding proteins, including the Jun-related family of proteins (c-Jun, JunB, and JunD) to form the dimeric transcription factor AP-1, which has a critical role in regulating downstream gene expression in response to numerous stimuli (26). In terms of bone physiology, there is evidence to suggest that AFOSB, a naturally occurring truncated variant of FOSB formed through alternative splicing of the FOSB gene (12), has important functions in bone formation. Specifically, it has been shown that overexpression of ΔFOSB leads to significantly increased bone formation in mice (12). Thus, it is interesting to surmise that reduced expression of FOSB, as noted in this case, may be a factor contributing to osteomalacia; however, further work will need to be performed to confirm this. Nevertheless, we acknowledge that we have not measured the levels of these proteins in the circulation, and hence the previously discussed endocrine effects mediated by SPP1, TNC, and FOSB in disordered bone turnover in this patient remains speculative at best.

The fact that muscle power was rapidly restored following successful surgical resection of the tumor implied that the myologic changes are, at least in part, relatively reversible. Unlike established severe primary osteoporosis, which typically requires a much longer duration of several years before any significant reversal in bone mass and quality following appropriate therapeutic intervention, the rate of recovery in bone density upon eradication of the underlying cause in secondary osteoporosis is much more rapid (27,28). Despite very severely reduced bone density, it is thus remarkable to find our patient's postoperative...
dual-energy X-ray absorptiometry performed 11 months later showing dramatic improvement in bone density to a T-score of −1.8.

In summary, the strengths of our report include an in-depth analysis of the gene expression profile of a culprit oncogenic osteomalacia-inducing tumor by microarray technique along with simultaneous immunoblot evidence of protein expression; we also highlight the plausible role of newer factors like SPP1, TNC, and FosB for the first time and elaborated on the pathway analysis. We acknowledge some weaknesses, one of which is the unavailability of a similar tumor tissue without osteomalacia as a control, which could then provide more insightful analysis and interpretation for the differentially expressed genes beyond what we can surmise by comparison against adjacent nontumoral tissue. Also, while we have found upregulation of SPP1 and TNC and downregulation of FosB in the tumor, we have not assayed the plasma for these proteins. Thus, we have not confirmed that such proteins are circulating and exert endocrine actions on the skeleton per se. However, given that these proteins play a role in the molecular pathways of bone turnover, it would be important for more research to be done to ascertain their role in bone biology and the pathogenesis of oncogenic osteomalacia.

Finally, a note should be emphasized about how standard-field 18F-FDG-PET imaging can totally miss the culprit tumors causing oncogenic osteomalacia (29,30). Our case illustrates this pitfall very elegantly and reiterates the need for clinicians to be especially thorough in the physical examination of patients being worked up for muscle weakness associated with skeletal disorders and for nuclear physicians to ensure that the PET imaging field actually covers the whole body in those suspected to suffer from this rare disorder.

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Additional Information

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