Primary osteoblast-like cells from patients with end-stage kidney disease reflect gene expression, proliferation, and mineralization characteristics *ex vivo*

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Osteocytes regulate bone turnover and mineralization in chronic kidney disease. As osteocytes are derived from osteoblasts, alterations in osteoblast function may regulate osteoblast maturation, osteocytic transition, bone turnover, and skeletal mineralization. Thus, primary osteoblast-like cells were cultured from bone chips obtained from 24 pediatric ESKD patients. RNA expression in cultured cells was compared with RNA expression in cells from healthy individuals, to RNA expression in the bone core itself, and to parameters of bone histomorphometry. Proliferation and mineralization rates of patient cells were compared with rates in healthy control cells. Associations were observed between bone osteoid accumulation, as assessed by bone histomorphometry, and bone core RNA expression of osterix, matrix gla protein, parathyroid hormone receptor 1, and RANKL. Gene expression of osteoblast markers was increased in cells from ESKD patients and signaling genes including *Cyp24A1*, *Cyp27B1*, *VDR*, and *NHERF1* correlated between cells and bone cores. Cells from patients with high turnover renal osteodystrophy proliferated more rapidly and mineralized more slowly than did cells from healthy controls. Thus, primary osteoblasts obtained from patients with ESKD retain changes in gene expression *ex vivo* that are also observed in bone core specimens. Evaluation of these cells *in vitro* may provide further insights into the abnormal bone biology that persists, despite current therapies, in patients with ESKD.

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Recent discoveries in the field of chronic kidney disease (CKD) mineral and bone disorder have led to a major shift in the way that bone metabolism and bone cell biology are viewed in the context of systemic disease.[1–5] Indeed, osteocytes, the predominant cell type in mineralized bone, were long thought to be relatively quiescent cells, with a function limited to transduction of signals related to mechanical stress.[6] These cells, which are derived from osteoblasts, are now known to secrete hormones that regulate mineral metabolism and control skeletal mineralization. CKD is associated with altered osteocytic protein expression[7] and abnormally elevated circulating levels of at least one osteocytic hormone—fibroblast growth factor 23 (FGF23)—have been linked to systemic disease, including cardiovascular disease,[5] in CKD.

Much current interest is focused on the role of osteocytes in the control of CKD-mineral and bone disorder; however, the study of these human cells, both in healthy individuals and in the context of kidney disease, is limited by the fact that osteocytes are terminally differentiated cells that exist in a mineralized milieu, limiting their potential to be isolated and propagated in cell culture. However, osteocytes are differentiated from osteoblasts and primary human osteoblast-like cells have been isolated from humans with CKD and other bone disorders and have been evaluated in culture since the 1980s.[8,9] These cells may be induced to transition to an osteocytic phenotype in culture under mineralizing conditions,[8,9] thus allowing for the study of osteoblast maturation and osteocyte transition in both healthy individuals and in patients with CKD. Previous *in vivo* and *in vitro* studies have demonstrated that both circulating factors and those intrinsic to osteoblasts themselves contribute to differences in osteoblast characteristics between patients with different types of renal osteodystrophy; indeed, primary osteoblasts obtained from patients with end-stage kidney disease (ESKD) have been shown to exhibit differences in proliferative characteristics *ex vivo*.[10–12] However, the mechanisms

underlying the changes in bone cell phenotype observed in CKD patients remain poorly understood. Therefore, we created and characterized a model system that may be used to study this abnormal phenotype, performing analysis of gene expression in whole bone biopsy samples and in cultures of osteoblast precursors derived from the same patients.

RESULTS

Study subjects

Twenty-four pediatric patients (14 males, 10 females) with ESKD treated with maintenance dialysis were included in the study. Subjects were 17.1 ± 0.7 years of age, were primarily Hispanic (83%), and had varying types of renal osteodystrophy (Figure 1). Eight subjects were treated with active vitamin D sterols (doxercalciferol or calcitriol) at the time of biopsy; 16 subjects were not on active vitamin D sterols for at least 4 weeks prior to the biopsy. Biochemical parameters of the subjects at the time of the biopsy are displayed in Table 1, and bone histologic variables are displayed in Table 2.

Bone core RNA, biochemical values, and bone histology

Consistent with the heterogeneous cellular composition of bone, both osteoblastic and osteocytic marker gene expression were readily detectable in bone cores from patients with ESKD, and, for the most part, the expression of these markers was increased compared with the healthy controls. These data suggest that the gene expression reflects abnormalities in osteoblast function that can be observed histologically. Differences in expression of osteoblast and osteocyte marker genes were not evident between patients with different types of bone histology (adynamic or normal bone turnover vs. high bone turnover) or based on the presence or absence of active vitamin D sterol treatment and the tight range of ages precluded any assessment of the effect of age on gene expression. Therefore, data from the different patient groups were pooled for subsequent analysis.

When examining genes involved in vitamin D metabolism, expression of Cyp27B1 and VDR was found to be increased in cores from patients with ESKD with respect to controls, whereas expression of Cyp24A1 was decreased. Expression of FGFR1, the receptor for the classical actions of FGF23, was increased in patient biopsies compared with controls as were genes critical to parathyroid hormone (PTH) signaling, including the PTH receptor (PTHR1) and Na^+/H^+ Exchange Regulatory CoFactor, a scaffold protein that interacts with the PTHR. Moreover, expression of osteocyte marker genes was significantly increased in patient samples (Table 3).

Consistent with the previously observed correlation between plasma concentrations of FGF23, bone protein FGF23, and bone mineralization,[13] bone core FGF23 RNA was inversely related to circulating alkaline phosphatase values ($r = -0.53$, $P = 0.01$), osteoid thickness ($r = -0.55$,

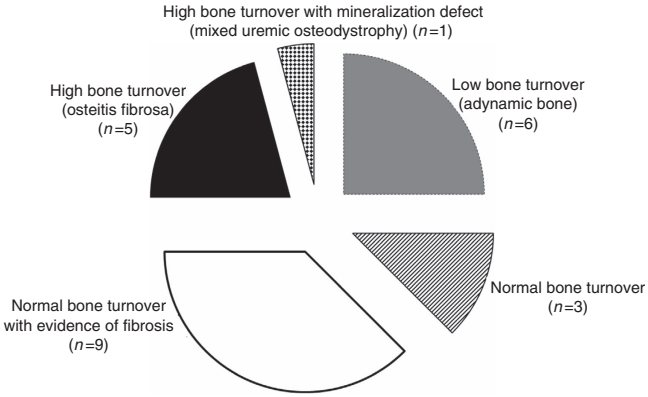

Figure 1 | Lesions of renal osteodystrophy on bone biopsy represented in the cohort of end-stage kidney disease patients evaluated for osteoblast-like cell and bone core RNA. Adynamic bone (low bone turnover; gray): $n = 6$; normal bone turnover (striped): $n = 3$; normal bone turnover with fibrosis (white): $n = 9$; osteitis fibrosa (high bone turnover; black): $n = 5$; mixed uremic osteodystrophy (high bone turnover with defective mineralization; checkered): $n = 1$.

Table 1 | Biochemical and growth parameters from 24 dialysis patients at the time of bone biopsy

Parameter	Patients
Calcium (mg/dl)	8.9 ± 0.2
Phosphorus (mg/dl)	6.5 ± 0.4
25(OH)vitamin D (ng/ml)	20.4 ± 2.0
Alkaline phosphatase (IU/l)[a]	160 (95, 283)
PTH (pg/ml)[a]	555 (357, 764)
FGF23 (RU/ml)[a]	1826 (425, 4825)
Height *Z*-score (SDS)[a]	−1.9 (−2.7, −0.2)
Weight *Z*-score (SDS)[a]	−0.6 (−1.8, 0.8)

Abbreviation: PTH, parathyroid hormone; SDS, standard deviation score.
[a]Indicates that the value is displayed as the median (interquartile range) because of non-normal distribution. All other values are displayed as mean ± s.e.m.

Table 2 | Bone histomorphometry in 24 dialysis patients undergoing bone biopsy

	Value	Normal range
Turnover		
Bone formation rate (BFR/BS) ($\mu m^3/\mu m^2$/year)[a]	25.6 (8.0, 63.9)	8.0–73.4
Eroded surface (ES/BS) (%)	9.1 ± 1.0	0.5–4.3
Mineralization		
Osteoid volume (OV/BV; %)	4.5 ± 0.7	0.2–5.8
Osteoid thickness (O.Th; μm)	8.9 ± 0.6	2.0–13.2
Osteoid surface (OS/BS; %)	31.0 ± 3.2	4.3–37.0
Osteoid maturation time (OMT; day)[a]	12.1 (8.9, 14.9)	1.2–11.5
Mineralization lag time (MLT; day)[a]	32.8 (20.4, 56.2)	2.3–63.8
Volume		
Bone volume (BV/TV; %)	32.0 ± 1.9	8.9–34.4
Trabecular number (Tb.N; no./mm^2)	2.1 ± 0.1	1.1–2.2
Trabecular separation (Tb.Sp; μm)	347 ± 34	351–737

[a]Indicates that the value is displayed as the median (interquartile range) because of non-normal distribution. All other values are displayed as mean ± s.e.m.