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Nuclear Chromatin-concentrated Osteoblasts in Renal Bone Diseases

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Abstract: The morphological appearance of an osteoblast largely alters with its differentiation and maturation, along with the change of cell function. We quantitatively observed the osteoblast morphology and compared it with bone metabolism. Biopsied iliac bone samples obtained from 77 dialysis patients (14 mild change, 37 osteitis fibrosa, 2 osteomalacia, 8 mixed, and 16 adynamic bone) were included in the study. Osteoblast appearances were classified into three groups: (i) type II and III osteoblasts, namely, active osteoblasts characterized by cuboidal or columnar shapes with or without a nuclear clear zone; (ii) type IV osteoblasts, lining osteoblasts characterized by extremely thin cytoplasm; and (iii) type V osteoblasts, apoptotic osteoblasts characterized by nuclear chromatin concentration. The results were quantitatively expressed as the length of bone surface covered by each type of osteoblasts. The type II and III osteoblasts were predominant in osteitis fibrosa, mixed, and mild change. The type IV osteoblasts were overwhelmingly predominant in adynamic bone. The type V osteoblasts appeared most frequently in osteitis fibrosa, followed by mixed and mild change. Both absolute and relative lengths of bone surface covered by the type V osteoblasts were significantly higher in the high-turnover bone group (osteitis fibrosa and mixed) than the low-turnover bone group (adynamic bone and osteomalacia). The type V osteoblasts were slightly correlated with serum intact parathyroid hormone levels. In conclusion, a high bone-turnover condition seems to be associated with the promotion of osteoblastic apoptosis in dialysis patients. This finding may explain the fact that osteopenia develops faster in CKD patients with high turnover of bone. **Key Words:** Apoptosis, Bone morphometry, Chronic kidney disease mineral and bone disorder, Osteoblast.

Bone diseases associated with chronic kidney disease mineral and bone disorders (CKD–MBD) are evaluated with tetracycline-labeling-dependent histological findings in biopsied iliac bone samples (1). Conventionally, the findings have been classified into five categories according to the two assessing axes, bone cell activity and bone mineralization (1). Recently, the principle of a new assessment system called the Turnover-Mineralization-Volume (TMV) system, which uses cancellous bone volume as another major assessing axis, has been advocated (2). However, since a concrete method to perform histomorphometry with the TMV system has not yet been established, bone assessment is still assessed by the conventional classification with five histological categories (3).

The conventional bone histological criteria do not have a significant relationship with bone mass (4). Moreover, it is difficult to tell future bone mass increases/decreases from the histological findings; however, osteoblastic apoptosis is believed to play an important role in the development of osteopenia in osteoporosis (5). If that is true, quantitative analyses of apoptotic osteoblasts in a bone specimen may serve as a predictor of future osteopenia.

The morphological appearance of osteoblasts varies in patients with CKD. Villanueva et al. classified it into four categories according to morphological characteristics (Table 1, Fig. 1) (6). These change greatly with differentiation and maturation, along with the change of osteoblastic function; therefore, these morphological categories would accord with the functional characteristics of osteoblasts in each

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TABLE 1. *Classification of osteoblasts by cell morphology*

Type I	Preosteoblast: cytoplasm rarely seen; the presence of these cells apparently adjacent to the osteoid seam is probably an artefact
Type II	Active osteoblast: cuboidal or columnar shape with adjacent nuclear clear zone
Type III	Active osteoblast: cuboidal or columnar shape without adjacent nuclear clear zone; usually smaller than type II cells
Type IV	Lining osteoblast: flat nucleus and extremely thin cytoplasm
Type V	Apoptotic osteoblast: cuboidal or columnar shape with evident nuclear chromatin concentration

differentiation grade. However, we found that a group of osteoblasts do not belong to any of the above four morphological categories (6). Those osteoblasts, termed the type V osteoblasts, are distinguishable from other types of osteoblasts by a high nuclear chromatin concentration, which indicates apoptotic osteoblasts (Fig. 1C); however, the relationship between these osteoblast morphological criteria and conventional tetracycline labeling-dependent bone histological classification remains obscure in patients with CKD. Thus, we attempted a clinicopathological study that compared osteoblastic morphology and bone metabolic condition in biopsied bone samples obtained from CKD stage 5 dialysis (CKD5D) patients.

PATIENTS AND METHODS

Of those CKD5D patients who underwent iliac bone biopsy examination because of clinical reason at Niigata University Medical and Dental Hospital and related faculties, bone samples from 77 patients whose samples were in a good enough condition to discriminate the morphological characteristics of each osteoblast were included in the study. The methods for obtaining and preparing the samples have been described in detail elsewhere (7,8). In brief, bone samples were extracted from the iliac crest under local anesthesia after standard tetracycline double labeling. Extracted bone samples were fixed with 80% ethanol, stained with Villanueva's solution, and then embedded in methyl methacrylate resin.

First, a standard bone histomorphometry was performed on processed sections according to the method provided by the American Society for Bone and Mineral Research (9), and each sample was classified into one of the five conventional categories. Thereafter, osteoblasts on the bone surface were classified into five morphological categories according to the modified classification by Villanueva (Table 1). The results were quantitatively expressed as the length of bone surface covered by each type of osteoblasts; therefore, the type I osteoblasts, or preosteoblasts, were excluded from this analysis. Since the border line between type II and type III osteoblasts seemed obscure in both morphological and functional aspects, we combined these categories into one category—"active osteoblasts"—in this analysis.

All data were expressed as mean ± SD, and a P-value <0.05 was considered as significant. Written, informed consent was obtained from each patient before the sample was used for analysis. This study was performed as part of a clinical study project approved by the Niigata University Ethics Committee (No. 455).

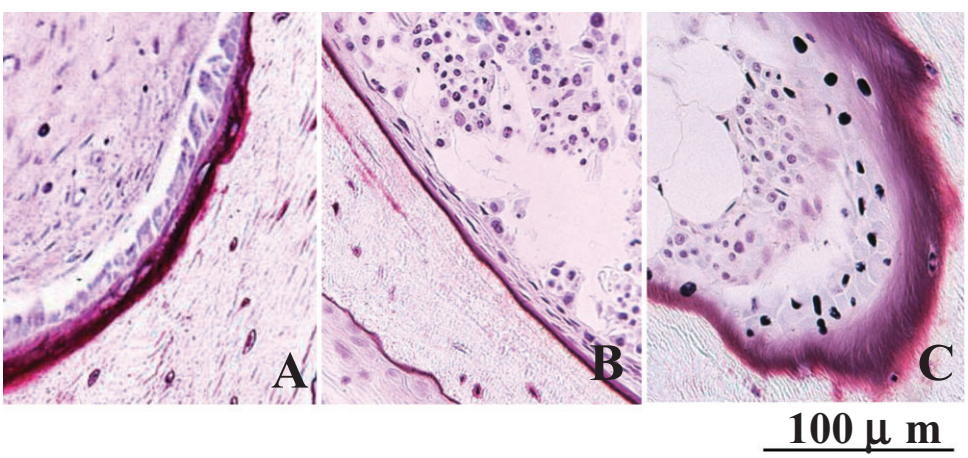

FIG. 1. (A) Typical appearance of the type II osteoblasts (active osteoblasts). (B) Typical appearance of the type IV osteoblasts (lining osteoblasts). (C) Typical appearance of the type V osteoblasts (apoptotic osteoblasts).

TABLE 2. *Clinical and bone histological features of the 77 chronic kidney disease stage 5 dialysis patients*

	Mild change	Osteitis fibrosa	Osteomalacia	Mixed	Adynamic bone
N	14	37	2	8	16
Age (years)	53.5 ± 13.4	52.0 ± 11.1	41.5 ± 17.7	43.3 ± 9.8	53.0 ± 14.7
Gender (male : female)	4:10	14:23	0:2	4:4	9:7
Diabetes	1	0	1	0	5
Dialysis vintage (months)	132.2 ± 86.3	133.9 ± 69.7	3.5 ± 0.7	4.6 ± 9.1	61.9 ± 82.2
Ca (mg/dL)	10.5 ± 1.4	10.2 ± 0.9	7.9 ± 0.4	9.1 ± 1.5	9.8 ± 1.0
P (mg/dL)	5.3 ± 1.6	6.6 ± 1.6	5.2 ± 0.3	5.5 ± 1.8	5.2 ± 1.4
Intact PTH (pg/mL)	388.1 ± 186.3	949.3 ± 534.5	313.0 ± 140.0	1030.1 ± 534.5	189.7 ± 220.1
Fb.V/TV (%)	0.3 ± 0.2	4.7 ± 7.1	0.2 ± 0.1	10.1 ± 16.0	0.0 ± 0.1
OV/BV (%)	6.6 ± 3.8	8.2 ± 3.1	22.1 ± 4.7	18.6 ± 3.4	2.8 ± 3.9
BFR/BV (%/year)	49.8 ± 55.9	87.4 ± 45.7	11.2 ± 15.8	88.8 ± 88.6	1.9 ± 3.4
Type II and III/BS (%)	12.0 ± 8.5	19.4 ± 14.3	21.1 ± 27.4	29.0 ± 24.0	0.8 ± 2.0
Type II and III/Ob.S (%)	49.0 ± 25.1	51.8 ± 29.3	46.7 ± 55.4	57.9 ± 38.4	10.4 ± 19.6
Type IV/BS (%)	7.9 ± 5.0	6.8 ± 4.4	15.4 ± 12.3	8.3 ± 9.4	3.4 ± 3.6
Type IV/Ob.S (%)	37.2 ± 22.7	23.6 ± 19.2	53.3 ± 55.4	30.8 ± 39.1	84.0 ± 25.9
Type V/BS (%)	3.6 ± 4.8	8.5 ± 10.7	0	4.7 ± 7.2	0.1 ± 0.4
Type V/Ob.S (%)	13.9 ± 18.6	24.6 ± 28.0	0	11.3 ± 18.9	5.6 ± 20.2

BFR, bone formation rate; BS, bone surface; BV, bone volume; Fb.V, bone marrow fibrosis volume; Ob.S, osteoblast surface; OV, osteoid volume; PTH, parathyroid hormone; TV, tissue volume.

RESULTS

The clinical and bone histological profiles of the 77 participants are shown in Table 2. The type II and type III osteoblasts were predominant in terms of both absolute and relative covering length in the osteitis fibrosa group, the mixed type group, and the minimal change group. In contrast, the relative bone surface length covered by the type IV osteoblasts was overwhelmingly predominant in the adynamic bone group.

The type V osteoblasts appeared most frequently in osteitis fibrosa, followed by mixed and mild change. When the five histomorphometric categories were classified into three groups, namely the high-turnover bone group (osteitis fibrosa and mixed), mild change, and the low-turnover group (adynamic bone and osteomalacia), both the absolute and relative bone surface lengths covered by the type V osteoblasts were significantly higher in the high-turnover bone group (Fig. 2). The bone surface length covered by the type V osteoblasts was slightly correlated with serum intact parathyroid hormone (iPTH) levels (Fig. 3), while the correlation between the type V osteoblasts and serum phosphate level did not reach statistical significance.

DISCUSSION

This study revealed that the type V osteoblasts appeared more in high-turnover bone in CKD5D patients. The type V osteoblasts are defined as those osteoblasts with nuclear chromatin aggregation, which represent morphological apoptotic cell characteristics. Thus, it was suggested that osteoblastic apoptosis is promoted in the high-turnover bone condition. In contrast, osteoblastic activity was severely suppressed, which was indicated by the increased bone surface length covered by the type IV osteoblasts, while osteoblastic apoptosis was rarely seen in the adynamic bone. These findings suggested that the bone metabolism is simply suppressed, but not injured, in the adynamic bone.

Osteoblasts differentiate into osteocytes. Nevertheless, not all osteoblasts become osteocytes; many

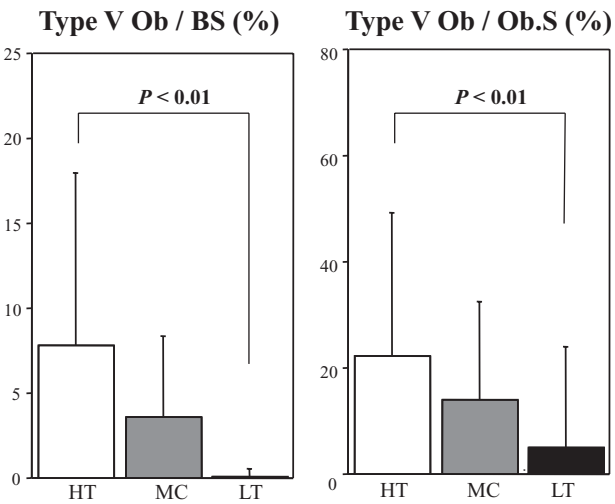

FIG. 2. The relationship between the length covered by the type V osteoblasts and bone turnover. The high-turnover bone group (HT; osteitis fibrosa and mixed) showed significantly more of both the absolute length covered by the type V osteoblasts (type V Ob/BS) and the relative length covered by the type V osteoblasts (type V Ob/Ob.S) than the low-turnover bone group (LT; adynamic bone and osteomalacia) did. BS, bone surface; MC, mild change; Ob, osteoblast; Ob.S, osteoblast surface.

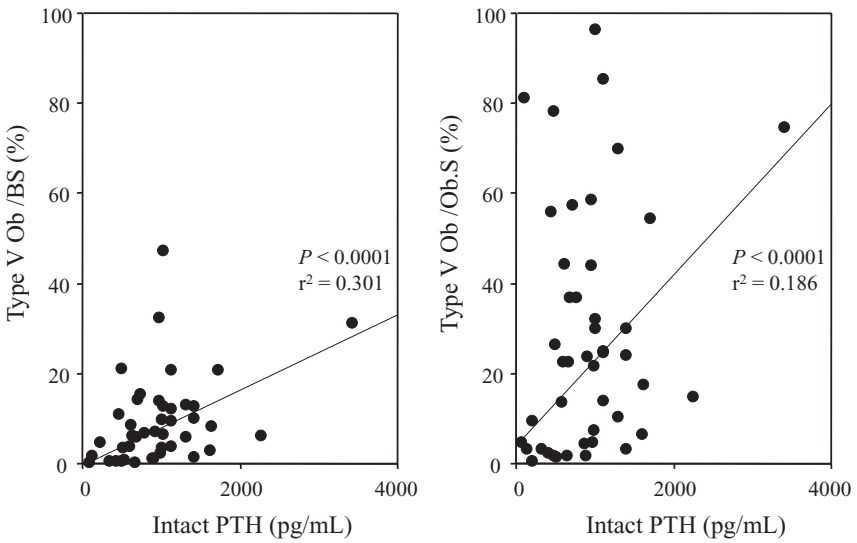

FIG. 3. Relationship between the length covered by the type V osteoblasts and serum levels of intact parathyroid hormone (PTH). Intact PTH levels showed a significant but weak correlation with the length covered by the type V osteoblasts. BS, bone surface; Ob, osteoblast; Ob.S, osteoblast surface.

of them apoptose before the differentiation. The frequency of osteoblastic apoptosis seems to depend on the number of active osteoblasts; therefore, the fact that more type V osteoblasts appeared in the high-turnover bone could be explained as a physiological consequence, at least in part. However, as Figure 1C demonstrates, the majority of one whole basic multicellular unit was often occupied by the type V osteoblasts, and it is difficult to explain this phenomenon by a known physiological mechanism. Some yet-unknown pathological reason might have induced osteoblastic apoptosis in such cases.

If apoptotic osteoblasts appear in the high-turnover bone condition, factors specific in such a condition may be promoting cell apoptosis in CKD patients. One such possible candidate is PTH. Although PTH is considered to be an inhibitor of osteoblastic apoptosis (10), such action appears only when PTH is intermittently administered; while it does not inhibit osteoblastic apoptosis when it is continuously administered (11). Continuous PTH-I receptor stimulation even has the potential to promote apoptosis (12). In fact, the bone surface length covered by the type V osteoblasts showed a significant correlation with serum iPTH levels in this study; however, the correlation seemed too weak to assume that PTH is the only factor that induces osteoblast apoptosis in this disease condition. Another possible candidate is phosphate. Phosphate promotes osteoblastic apoptosis in vitro (13). This study showed no significant correlation between the type V osteoblasts and serum phosphate levels; however, the osteoblastic metabolism is affected by the phosphate in the intraskeletal fluid, but not that in the circulating serum. Since phosphate is consistently supplied from bone to intraskeletal fluid in the high-turnover bone state, it may affect osteoblastic maturation and apoptosis.

In conclusion, more bone surface length was covered by the type V osteoblasts in high-turnover bone, which suggests that high bone turnover is associated with the promotion of osteoblastic apoptosis in CKD5D patients. Abnormal metabolic conditions associated with CKD have a potential effect on the development of this phenomenon. The promotion of osteoblastic apoptosis in high bone-turnover conditions may explain the fact that osteopenia develops in CKD patients with high-turnover bone (14–17).

Conflict of interests: The authors have no conflict of interests.

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