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Gene expression profiles give insight into the molecular pathology of bone in primary hyperparathyroidism ☆

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Abstract

Global gene expression profiling has been used to study the molecular mechanisms of increased bone remodeling caused by PHPT. This disease is a model for chronic over-stimulation of target organs by PTH due to an inappropriate overproduction of the hormone. Hyperactivity of osteoblasts and osteoclasts lead to increased calcium and phosphate mobilization from the skeleton and hypercalcaemia. The ensemble of genes that alter expression and thus is responsible for the effects of chronic PTH stimulation is today largely unknown. The differentiated gene expression profiles revealed characteristic molecular disease modalities which define the bone remodeling abnormalities occurring in PTH dependent osteodystrophy. We analyzed mRNAs in transiliacal bone biopsies from 7 patients with PHPT using Affymetrix HG-U133A Gene Chips containing more than 22000 different probe sets. Similar analyses of the global transcriptional activity were repeated in a second bone biopsy from the same patient taken one year after surgery and reversal of disease parameters. Real time PCR was carried out on many genes for corroboration of the results. Out of more than 14500 different genes examined, 99 which were related to bone and extra-cellular matrix, showed altered expression. Of these were 85 up- and 14 down-regulated before operation. The majority of regulated genes represented structural and adhesion proteins, but included also proteases and protease regulators which promote resorption. Increased expressions of collagen type 1 and osteocalcin mRNAs in disease reflecting the PTH anabolic action were paralleled by increased concentrations of these proteins in serum. In addition, genes encoding transcriptional factors and their regulators as well as cellular signal molecules were up-regulated during disease. The identified genetic signature represents the first extensive description of the ensemble of bone and matrix related mRNAs, which are regulated by chronic PTH action. These results identify the molecular basis for this skeletal disease, and provide new insight into this clinical condition with potential bearing on future treatment.

Keywords: Bone remodeling; Gene expression; PTH; PTH/RP

Introduction

The calcium level in the human extracellular fluid is regulated within very narrow limits, primarily by a finely tuned release of parathyroid hormone (PTH) from the parathyroid glands. In bone, sustained increase in PTH serum levels leads to release of calcium from bones, mainly through degradation of bone matrix by the osteoclasts. This is known to be an indirect effect which requires prior PTH activation of bone-forming cells (osteoblasts) [1]. The molecular mechanisms and especially the ensemble of genes that alter expression and are responsible for the effects of chronic PTH stimulation are today largely unknown, making our

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understanding of the causes and pathophysiology of PTH-dependent osteodystrophia markedly insufficient. Despite the fact that net stimulation of bone resorption occurs during sustained PTH excess, intermittent injection of PTH (1–34) leads to overall anabolic effect and large increases in BMD [2]. The molecular mechanisms behind the dual and apparently opposite modes of PTH have not been accounted for. Many studies using cell cultures and animal models have examined the molecular effects of PTH on bone metabolism, but only few studies [3–6] have addressed the pathophysiological mechanisms in humans, which are of importance for the clinical condition.

Primary hyperparathyroidism (PHPT) is characterized by aberrant and increased secretion of PTH from the parathyroid glands in the absence of adequate hypocalcemic stimulation. The continued hyper-secretion which leads to enhanced bone resorption – and to a lesser extent formation – results in low bone mass [7,8] and increased risk of fractures [9]. When patients with PHPT are cured by surgical removal of parathyroid adenomas (PTX), bone metabolism and disease parameters return to normal [7], making PHPT a suitable model for studying skeletal PTH effects in vivo.

To our knowledge, no comprehensive study of the ensemble of bone-related mRNAs which are affected in PHPT has been performed. Our results show that several regulatory and structural genes related to bone and extracellular matrix are affected in disease, involving genes encoding proteins of distinct functional entities offering an explanation for the composite action of PTH.

Methods

Selection of patients and inclusion criteria

Seven patients (6 women and one man) aged 54–75 years were included in this study (Table 1A). The diagnosis of PHPT was established by elevated plasma PTH and ionized calcium by three repeated measurements. None of the patients had chronic illnesses or diseases known to affect bone metabolism. None received bisphosphonates, glucocorticoids or fluoride. The patients had normal serum creatinine levels. Following inclusion, all the patients underwent successful PTX leading to decrease of plasma PTH, serum ionized calcium and 1,25(OH)2D. Enlarged PTH glands were confirmed at operation and the diagnosis of PTH adenomas verified by histological examination. The study was performed according to the declaration of Helsinki II and approved by the Local Ethical Committee (Ethics ref no: 19980012). Informed written consent was obtained from each participant before entry.

Calcium metabolism and bone markers

Sampling was performed before and 1 year after PTX. Blood was collected after one night fast. Serum was immediately centrifuged and frozen at −80°C for later analyses of osteocalcin, propeptide of type 1 collagen C-propeptide (P1CP), N-propeptide (P1NP) and Cross-links. Serum PTH was measured as the intact hormone by a two-site chemiluminescent immunometric assay (Immulite, Los Angeles, USA) (intra-assay precision: CV: 5.9%, inter-assay precision, CV: 6.1%.); serum ionized calcium was measured by an ion-selective electrode (intra-assay precision: CV: 0.5%, day to day variation: CV: 2.0%). Serum inorganic phosphate was measured by the enzymatic colorimetric method (intra-assay precision: CV: 2.5% inter-assay precision, CV: 3.5%). Blood samples for serum PTH, ionized calcium and phosphate were taken at the time of biopsy and measured as part of the daily hospital routine. and mean values of 3 separate samples which showed a variation of less than 9% are shown in Table 1A. Serum levels of 1,25(OH)2D were measured using radioimmunoassay (RIA) (Nichols Institute Diagnostics, California, USA) (intra-assay precision, CV: 5.4–10.6%, inter-assay precision, CV:9.3–15.3%).

Bone formation markers

Serum total alkaline phosphatase was measured at the time of collection by methods described by the Scandinavian Society for Clinical Chemistry and Clinical Physiology [10] (intra-assay precision: 3.0%, inter-assay precision: CV: 4.0%). Osteocalcin (also called bone GLA protein, BGLAP) was measured using "N-MID® Osteocalcin" by Roche (intra-assay precision: CV: 1.4–4.0%, total precision: CV: 1.8–6.5%); P1CP by Orion Diagnostica (Espoo, Finland) (intra-assay precision: CV: 2.1–3.2%, inter-assay precision: CV: 4.1–6.6%); P1NP by Orion Diagnostica (Espoo, Finland) (intra-assay precision: CV: 4.8–13.7%, inter-assay precision: CV: 3.1–8.2%).

Bone resorption markers

Cross-links using "β-CrossLaps/serum" by Roche (intra-assay precision: CV: 1.0–4.6%, total precision: CV: 1.6–4.7%). Urine for amino-terminal telopeptides of type 1 collagen (NTX/kreatinin) was collected as second void morning urine and measured using the "NTx Reagent Pack" from OrtoClinical Diagnostic (Amersham, UK) (intra-assay precision: CV: 0.8–3.3%, total precision: CV: 2.4–8.5%).

Dual-energy X-ray absorptiometry (DEXA) and bone biopsy

Bone mineral content (BMC) and bone mineral density (BMD) were measured using a Hologic QDR-2000 densitometer at the spine, proximal femur and distal radius (non-dominant forearm) and analyzed in accordance with operator's manual.

All patients had one bone biopsy taken before and 1 year after surgery from opposite but symmetrical places of os ileum to avoid woven bone. As local anesthetics could influence the cytokine responses, all biopsies were taken with the same amount of local anesthesia (Lidocain). The biopsies were taken with Bordier's trephine and immediately frozen in liquid nitrogen and stored at −70°C for later RNA extraction.

Purification of RNA

The frozen bone cylinder biopsies were pulverized with mortar in liquid nitrogen with their content of marrow intact. The pulverized bone was added to TRIZOL reagent (Life Technologies, Gaithersburg, MD), homogenized and RNA purified according to the manufacturer's instructions. Routinely, 50–80 μg total RNA was obtained and the quality controlled by gel electrophoresis. RNA was further purified prior to cDNA synthesis using the RNeasy kit (Qiagen) to remove organic components.

Table 1A
Individual patient information before operation

Patient	1	2	3	4	5	6	7	Normal range
Age (years)	54.6	57.7	58.6	53.6	55.8	67.0	75.1	
Sex	Female	Male	Female	Female	Female	Female	Female	
PTH (pmol/l)	11.9	43.2	21.7	27.6	34.5	24.9	16.0	1.1–6.9
Ca^{++} (mmol/l)	1.43	1.74	1.48	1.63	1.63	1.82	1.37	1.19–1.29
1,25 (OH)2D pmol/l	121	202	249	168	198	134	112	50–145