Abnormal muscle and hematopoietic gene expression may be important for clinical morbidity in primary hyperparathyroidism

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THE PREVALENCE OF PRIMARY HYPERPARATHYROIDISM (PHPT) varies by age and sex, affecting 1 in 500 females and 1 in 2,000 males over the age of 40 yr (7), or ~1% of the adult population (19). The prevalence of PHPT increases with age in both males and females. PHPT is usually due to benign tumors or hyperplasia affecting one or several parathyroid glands, which therefore secrete constitutively too much parathyroid hormone (PTH), leading to increased levels of blood calcium. Loss of calcium from the skeleton leads to osteoporosis (OP) and osteomalacia (26). There is a growing recognition that symptoms rising early from organ systems other than bone and kidneys are important and add to the morbidity of patients with PHPT in addition to the more classical findings of nephrolithiasis and osteopenia (48). Many reports (14, 23, 24, 42, 43) emphasize the change in the clinical presenting picture from renal, bone-related, and gastrointestinal manifestations to psychological and psychiatric disorders as well as feelings of muscular weakness, apathy, and ill-defined mental symptoms. An increased cardiovascular morbidity has been reported even in mild disease (2), leading to overrepresentation of cardiac death in patients with symptomatic PHPT both before and after parathyroidectomy (PTX), suggesting development of non-reversible changes. The actions of PTH are mediated through three types of receptors: PTH/PTH-related peptide receptor (PTHR1), the NH2-terminal PTH receptor II (PTHR2), and the COOH-terminal PTH receptor (C-PTHR) (22, 38, 46, 47). In humans, PTHR1 mRNA is reported to be widely expressed in tissues like brain, striated muscle, heart muscle, kidney, and liver (38, 46). The PTHR2 mRNA shows a more restricted distribution and has lower affinity for PTH compared with PTHR1, but it is present in blood cells (39) and abundant in the brain and pancreas (47). The COOH-terminal part of PTH is essential for binding to the C-PTHR receptor, which has not yet been cloned but is highly expressed, e.g., in rat osteocytes (10).

Very little is known about the molecular pathology associated with a chronic excessive action exerted by PTH in different target tissues in patients with PHPT. The aim of the present study was to describe in detail changes in gene expression in biopsies from patients with well-defined and characterized early PHPT before and after surgery when normalization of bone and biochemical markers had occurred. We used each patient as his/her own control to describe the molecular pathology expressed as mRNA profiles and suggest that the results may have clinical consequences reflecting chronic PTH receptor stimulation.

METHODS

Patients and biopsies. Seven arbitrarily chosen patients from 53 to 75 yr of age (mean 60.3 yr), without signs of organ affection but with clinical biochemical evidence of mild PHPT, were included. Biochemical blood diagnostic and bone parameters for the patients have been described previously (35). Briefly, PTH, ionized calcium, and 1,25(OH)2D, as well as bone formation and resorption markers, and
were all significantly reduced 1 yr after surgery, whereas bone mineral
density levels in spine and hip increased significantly. The diagnosis
was established by elevated plasma PTH and ionized calcium, taking
care to rule out accessory conditions known to affect the PTH/calcium
balance. The patients were screened for interfering conditions such as
cardiovascular and renal disorders, endocrine disorders, and other
bone pathology. Vitamin D status [25(OH)D and 1,25(OH)2D] was
evaluated before inclusion. The patients had normal serum creatinine
levels. Following inclusion in this study, the patients underwent
successful PTX.

Biopsies containing bone, bone marrow, and remnants of skeletal
muscle tissue were taken from opposite but symmetrical places of os
ileum to avoid wounded bone from the first biopsy before and 1 yr after
surgery. Biopsies were immediately frozen in liquid nitrogen and
stored at −70°C for RNA extraction.

The distribution of PTH1R and PTHR2 in normal tissues and blood
cells, was studied in biopsies/blood samples obtained from other
patients taken during surgery. The receptors in central nervous system
(CNS) were analyzed in post-mortem tissue from non-demented
humans (obtained from Netherlands Brain Bank, Amsterdam, The
Netherlands). The study was performed according to Title 45, US
Code of Federal Regulations, Part 46, “Protection of Human Sub-
jects,” and to the declaration of Helsinki II and approved by the Local
Ethics Committee (Ethics ref no. 19980012). Informed written con-
sent was obtained from each PHPT participant and other healthy
persons before entry. Also the examination of post-mortem specimens
fulfilled all formal requirements.

**Purification of RNA.** RNA from biopsies and autopsies was purified
with the aid of the TRIzol (Life Technologies, Gaithersburg, MD) and
RNeasy (Qiagen), as described (35). Isolation of lymphocytes and
monocytes was performed as described (31), and RNA from the
purified cell fractions was isolated with the aid of TRIzol. The RNA
quality was controlled by gel electrophoreses.

**Microarray analysis.** Double-stranded cDNA and biotin-labeled
cRNA probes were made from 5 μg of total RNA by use of the
Superscript Choice system (Invitrogen) and the Enzo Bioarray, re-
spectively, according to recommendations from Affymetrix. This
cRNA was hybridized to HG-133A chips (Affymetrix) followed by
washing and staining on the GeneChips Fluidics Station 450
(Affymetrix). The chips were scanned on the Affymetrix GeneArray
2500 scanner. The quality of the RNA and probe was controlled by an
Affymetrix-based test measuring the ratio between 5’ and 3’ mRNAs
for β-actin and GAPDH and found to be highly satisfactory. The
datasets originating from the 14 bone biopsies were processed by the
Affymetrix Mas 5.0 software according to manufacturer’s instruc-
tions.

The patients were coded, and all analyses were carried out blindly.
The samples from each patient were analyzed using the same kit for
cRNA probe synthesis and hybridized to chips from the same batch.
Intrabatch chip variation was found to be negligible. One patient,
patient 7, showed different overall mRNA profiles for genes related to
muscle, hematological, and bone matrix and was analyzed on two
chips from different batches to rule out technical causes. In general,
the differences in expression levels before/after operation were less
for patient 7. Of the 175 mRNAs related to muscle, 123 were on
average more than twofold changed in patients 1–6 compared with
only 24 in patient 7. Of the 169 mRNAs related to hematopoiesis, 46
were on average more than twofold changes in patients 1–6 compared
with only 15 in patient 7. Furthermore, 96 and 80% of muscle- and
hematopoiesis-related mRNAs increased or decreased, respectively,
on average in patients 1–6, whereas the corresponding numbers in
patient 7 were 14 and 44%, respectively.

**Filtering and statistical analysis of Affymetrix data.** First, the genes
were sorted by criteria based on uniformity of expression among
patients 1–6. Second, the average acceptable signal level before or
after PTX was set to be above 50 to include the probe set. For,
inclusion in the statistical analysis, changes reflecting a mean increase
or decrease of at least 40% (representing a log2 value of more than 0.5
or less than −0.5) were required. Patient 7 was treated as a special
case because she was considered to represent a different entity (see
RESULTS). Two statistical criteria were employed. For **criterion 1**, the
ratio of mRNA values between “sick” and “cured” for each probe (trans-
script) was given a P value by the Affymetrix Mas 5.0 program.
The median P value for one transcript from six patients was required
to be 0.05 or better according to the 0-hypothesis. For **criterion 2**, the
ratio of mRNA values between “sick” and “cured” were given a
P value by the Affymetrix Mas 5.0 program for each gene, and values
were then used in the empirical Bayes method for evaluation of the
significance of regulation (33, 41, 49).

**Data evaluation using the Ingenuity Pathways Analysis program.**
The canonical pathways that were identified using the Ingenuity
Pathways Analysis (IPA) program were evaluated employing the
right-tailed Fisher’s exact test to calculate levels of significance. The
P value for each pathway was calculated by comparing the number of
user-specified genes of interest (i.e., the regulated genes) that partic-
ipated in a given function or pathway relative to the total number of
occurrences of these genes in all functional/pathway annotations
stored in the Ingenuity Pathways knowledge base. Only annotations
that have more functions/canonical pathways analysis genes than
expected by chance (“right-tailed” annotations) were used. Although
the number of genes associated with a given function/pathway is an
important measure when calculating the P value in global analyses,
the P value is not simply proportional to this number but takes into
account how much information for these genes can be found in the
Ingenuity functional/pathway annotations. The probe sets of the Af-
nymetrix chips that were used covered more than 80% of the genes in
106 of 136 metabolic pathways covered by Ingenuity.

**Real-time RT-PCR analysis.** cDNA was synthesized from the same
RNA as was used for Affymetrix analysis using the High Capacity
cDNA Archive Kit (Applied Biosystems, Stockholm, Sweden) ac-
cording to the manufacturer’s specifications. Real-time RT-PCR was
performed using the ABI PRISM 7900HT Sequence Detection Sys-
tem and the 7900HT Micro Fluidic Card containing eight ports (PE
Applied Biosystems), using probes labeled with the fluorescent dye
FAM. TATA-binding protein (TBP) mRNA was included in the
reactions and used as internal standard since the variation between
signal values in each patient pre- and postsurgery was found to be
small [average 2.8% (SD 16.4%)]. Predesigned primers and a probe
labeled with the reporter fluorescent dye VIC, specific for TBP, were
used. The cDNA was amplified under the following conditions: 50°C
for 2 min, 94.5°C for 10 min, followed by cycles at 97°C for 30 s and
59.7°C for 1 min. The relative amount of mRNA for each gene was
calculated using the comparative Ct method “Separate Tubes” ac-
cording to the manufacturer’s instructions and adjusted and calculated
relative to the expression of TBP mRNA.

The genes and assay IDs selected for analysis are included in Fig.
4, A and B. The distributions of PTH1R and PTHR2 were analyzed by
real-time RT-PCR employing the LightCycler and the Fast Start
Master SYBR Green kit (cat. no. 2239264, Roche Diagnostics)
according to the manufacturer’s instructions. One sample from each
organ/region from three different persons was analyzed in triplicate.
Cycling profile was 94°C for 5 min, then 40 cycles of 60°C for 30 s,
72°C for 30 s, and 95°C for 30 s and 3 min at 72°C. Gene expression
was normalized to β-actin. Primers were as follows: β-actin, forward
GCTACGCGTACCACCAAA, reverse GCCATCTCTGTGTC-
GAAGTC; PTHR1, forward GTCCCCAGACCTCGGTTGA, re-
verse AGTACCGGAAGTGCTTACAA; PTHR2, forward ATAGT-
GGAGCCAGGGAGAT, reverse TGGGCATCTCCTAGTGTCG.

**RESULTS**

**Individual mRNA expression profiles in patients with PHPT before and after PTX.** The mRNA levels were analyzed in biopsies from seven patients by use of the Affymetrix HG-
U133A array containing more than 22,000 probe sets for 14,500 different genes. Approximately 10,000 genes were found to be expressed in all the samples according to the threshold of acceptance described in Methods. The mRNAs were categorized as tissue characteristic, but not necessarily tissue/cell specific, by annotations from the NetAffx analysis center. Figure 1A summarizes the overall number of muscle (175) and hematopoietic tissue (169)-associated mRNAs, leaving a large group of tissue-uncharacterized mRNAs or unknown species. A total of 99 mRNAs were characteristic of bone and extracellular matrix, as described previously (35).

Employing both criteria 1 and 2 for filtering and statistical analysis (Methods), 139 individual muscle- and 88 hematopoiesis-related mRNAs were detected. For patients 1–6, a highly corresponding profile was found, in which the majority of mRNAs were increased in muscle (Fig. 1B, a) and decreased in hematopoietic tissue (Fig. 1B, b). Many of the muscle and hematopoietic transcripts were more than fourfold increased and 50% decreased, respectively, due to PHPT (Fig. 1B, a and b).

Patient 7, however, showed a different mRNA expression pattern of both hematopoiesis- and muscle-related genes compared with patients 1–6 (see Methods for detailed assessment) and was therefore regarded as an outlier. The different data set for patient 7 was not due to chip variations, as they are all from the same batch as are the kits used in the other processes, nor was it due to differences in mRNA quality. Patient 7 probably represents a biological variant or possibly a different disease stage; she was also much older than the other patients (75 yr old compared with a mean age of 57.9 yr for the remaining 6 patients).

Expression levels of PTHR1 and PTHR2 mRNAs in bone biopsies from patients with PHPT and their distribution in human tissues of unrelated persons. Real-time RT-PCR of PTHR1 mRNA showed a 2.0 ± 0.9-fold increase in PHPT, whereas PTHR2 mRNA expression was unaltered, confirming the Affymetrix data (35). The discovery of differential PTHR1 mRNA expression and the knowledge that PHPT precipitates symptoms from various organs prompted us to study receptor mRNA distribution in CNS, muscle, and blood cells. PTHR1

![Fig. 1. A: overview of tissue-related genes differentially expressed pre- and postoperatively in primary hyperparathyroidism (PHPT) and fulfilling statistical criterion 1 or 2 for filtering. Most muscle tissue-related mRNAs are increased with disease, in contrast to hematopoietic tissue mRNAs. B: individual expression of mRNAs characteristic of muscle (119) (a) and hematopoietic cells (88) (b) in each of 7 patients, representing the most significantly regulated genes (1,388). Each gene is displayed as a bar and expressed as a ratio in log2 scale between pre- and postoperative mRNA values for each individual patient. Statistical criteria 1 and 2 for filtering were fulfilled (see Methods).](http://ajpendo.physiology.org/)

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and PTHR2 mRNAs were both present in all human tissues and cells tested, except for the small intestine, where only PTHR1 mRNA was detected (Fig. 2). The abundant and uniform representation of the two receptor mRNAs in the CNS, especially in the cerebellum, amygdala, hippocampus, superior frontal gyrus, superior parietal gyrus, thalamus, and hypothalamus is noteworthy, reaching almost the level of actin (Fig. 2). Cardiac and skeletal muscle contain, on average, about one-tenth of the mRNA amounts present in the most enriched regions of the brain. Thus, the prerequisite for PTH to exert a direct action on muscle and hematopoietic tissue/cells is present.

Differential expression of muscle- and hematopoiesis-related mRNAs in PHPT. Functional groups of genes were identified using annotations in the NetAffx analysis center and from Eli Lilly as a second validation. The results were highly comparable. The number of differentially displayed genes in each functional category is presented in Fig. 3. Among muscle-related transcripts there was an overrepresentation in the groups “actin binding” and “protein binding.” Also, mRNAs coding for ion binding and transport proteins were prominent. Alterations in immune response and immune receptor genes were the most striking features among the hematopoiesis-related genes. The number of affected mRNAs for transcription factors and transferases seemed to be similar in hematopoiesis and muscle-characteristic genes (Fig. 3). The mRNAs defining muscle proteins were related especially to energy processes (e.g., creatine transferases) and to proteins participating in cellular contractility and ion binding and transport (Figs. 3 and 4).

Individual changes in each patient describing 50 of the statistically most affected genes in each functional category characteristic of the two tissues are depicted in Fig. 4. By compiling the data, reproducibility and consistency of the gene expression results during disease in patients 1–6 were visualized. During disease it was characteristic that mRNAs related to the contractile and energy providing systems were most significant.
increased in muscle (Fig. 4A). Also, mRNAs for ion binding/transport and transcription factors were highly regulated in the patient biopsies before compared with after PTX. Some muscle-related mRNAs were increased up to 10-fold (e.g., myozennin) during PHPT, whereas the majority were two- to fourfold increased (Fig. 4A) (see http://www.med.uio.no/imb/medbiokj/kgautvik/index.html for the genes affected in this and the other affected canonical pathways). Of the 50 muscle-related mRNAs depicted in Fig. 4A, only plakophilin-4 was reduced (almost 3-fold), whereas several other muscle-related mRNAs also were significantly reduced in PHPT (chondroitin sulfate, GalNACT-2, ganglioside-induced differentiation-associated protein-1, syntrophin, β1, glycerol-3-phosphate dehydrogenase-2, calcitonin receptor-like; Fig. 1B, a, but not shown in Fig. 4A). These do not represent members of any obvious particular group or pathway, and their clinical translation appears unclear.

During PHPT, some mRNAs related to hematopoietic functions were increased, e.g., hepatic leukemia factor (HLF), chemokines, and some mRNAs related to DNA binding and transcription factors (Fig. 4B). The median degree of hematopoietic mRNA alteration (up/down) was ~45%, whereas most of the downregulated mRNAs were in fact reduced by ~75%, e.g., immunoglobulin heavy constant-γ (IGHG1; Fig. 4B). In addition, receptor-signal transduction, certain chemokines, e.g., IL-4, and transcription factors represented major mRNA groups that were highly affected before and after curative surgery.

To validate the findings of transcript measurements by the microarray approach, we repeated the measurements of selected mRNA levels using real-time RT-PCR on RNA from patients 1, 2, 4, 5, and 6 (patient 3 was omitted due to limited amounts of RNA). The results demonstrated a very good consistency with the findings obtained using microchip analysis and in general showed an even more pronounced change in expression (Fig. 4).

The data have been submitted to the European Bioinformatics Institute (EMBL-EBI) ArrayExpress repository (acc. no. E-MEXP-847).

Identification of affected canonical pathways at PHPT and clinical significance. A total of 1,388 genes that fulfilled statistical criterion 1 or 2 (see Fig. 1A) (Methods) were analyzed by canonical IPA (Ingenuity Systems, www.ingenuity.com).

The canonical pathways present in the IPA library and showing highest significance for our data set are displayed in Table 1.

Several pathways that were not assigned to a predominant tissue, but are of general biological importance, included energy metabolism represented by oxidative phosphorylation, citrate cycle, pyruvate metabolism, pentose phosphate pathway, and ubiquinone synthesis, all of which were strongly and highly significantly affected during PHPT, showing an overall increased expression pattern. It is of particular interest that the “calcium-signaling” pathway, known to be essential in muscle and blood cell functions, is by far the most affected system, in which 50 of 172 mRNAs (~30%) are altered. In agreement with the profound alterations in the mRNA profiles of cell receptors, cell signaling and chemokines as related to both muscle and hematopoietic tissue/cells (Fig. 4) and the molecular networks reflecting calcium and β-adrenergic signal transductions were statistically most, and quite remarkably, pre-dominantly increased during disease. Calcium plays essential roles in skeletal and cardiac signal-contraction coupling but is also of central importance for several hematological/immunological cellular functions. For instance, we find that several mRNAs within the calcium-signaling pathway (CRAC, MEF2, DSCR1, RyR) are affected in PHPT. Their corresponding proteins participate in immune cell activation as well as in B and T cell development via activation of the transcription factor NFATC (the pathway and affected genes are illustrated at http://www.med.uio.no/imb/medbiokj/kgautvik/canonical.html and reviewed in Ref. 9). The 11 genes affected and present in the β-adrenergic signaling respond to the catecholamines nor-epinephrine and epinephrine, providing coordinated control of contractility, metabolism, and gene regulation, and can be viewed at http://www.med.uio.no/imb/medbiokj/kgautvik/canonical.html (reviewed in Ref. 37). It is well known that an upset regulation of the β-adrenergic system, which appears also to be greatly overactivated in patients with PHPT, will cause erroneous cardiac muscle cell function and, if of longer duration, may lead to heart symptoms (reviewed in Ref. 25).

Other regulated pathways with long-term potential clinical consequences are phenylalanine, tyrosine, and tryptophan biosynthesis and Parkinson’s signaling [a signaling cascade that in brain leads to the death of dopaminergic neurons (reviewed in Ref. 21)]. It is noteworthy that seven of nine enzyme complexes in the oxidative phosphorylation chain were among the most significantly altered, causing a potentially important imbalance of muscle cell energy requirement (Table 1 and website http://www.med.uio.no/imb/medbiokj/kgautvik/canonical.html).

DISCUSSION

The results show that patients with asymptomatic, mild PHPT without known clinical organ involvement have a marked, disease-dependent change in expression of mRNAs characteristic of muscle- and hematopoiesis-related genes as previously described for bone related genes (35). This dysregulation of a large number of genes related to muscle and hematopoiesis during PHPT has not previously been recognized. Because the pathological biochemical parameters and loss of bone mineral density were reversible after the operation, it is reasonable to attribute the molecular changes to being caused by the disease. Also, changes in several bone-associated mRNAs (e.g., osteopontin, osteocalcin, fibronectin-1, and several collagens, including 1A1 and 1A2) previously shown to be affected by PTH overstimulation (35) may be argued to represent a validation of the results even if the number of patients is limited. All of the patients had high serum PTH while showing small or modest rises in serum Ca²⁺. Since the median changes in serum PTH and Ca²⁺ during disease were 5- and 1.25-fold, respectively, it is tempting to regard PTH, and not serum Ca²⁺, as a major cause of the molecular derangement. In support of this view, it may be argued that, since the local [Ca²⁺] in bone marrow adjacent to trabeculae can be very high irrespective of disease (40), a mere 25% increase in [Ca²⁺] would hardly have a significant effect on the hematopoietic stem cells that are closely associated with bone. Furthermore, the fourth Tromso Epidemiological Study, involving nearly 7,000 persons, demonstrated a clear relationship between left ventricular hypertrophy and serum PTH with no relation to serum calcium levels (36). As serum 1,25(OH)2D is
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### Diagram A

[Image of gene expression data]

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression Level</th>
<th>Function/Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH</td>
<td>High</td>
<td>Muscle Contractility</td>
</tr>
<tr>
<td>PTPN11</td>
<td>Low</td>
<td>Tumor Suppression</td>
</tr>
<tr>
<td>AKT1</td>
<td>Variable</td>
<td>Metabolic Regulation</td>
</tr>
<tr>
<td>HSP90</td>
<td>Increased</td>
<td>Chaperone Activity</td>
</tr>
<tr>
<td>NOS3</td>
<td>Decreased</td>
<td>Nitric Oxide Pathway</td>
</tr>
</tbody>
</table>

### Diagram B

[Image of time course of gene expression]

<table>
<thead>
<tr>
<th>Time</th>
<th>Genes of Interest</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preop</td>
<td>MYH, PTPN11, AKT1</td>
<td>Baseline</td>
</tr>
<tr>
<td>Postop</td>
<td>HSP90, NOS3</td>
<td>Increased</td>
</tr>
<tr>
<td>Long-term</td>
<td>MYH, PTPN11, AKT1</td>
<td>Persistent</td>
</tr>
</tbody>
</table>

### Table

<table>
<thead>
<tr>
<th>Gene</th>
<th>Preop/Postop/Long-term</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH</td>
<td>High/High/High</td>
</tr>
<tr>
<td>PTPN11</td>
<td>Low/Low/Low</td>
</tr>
<tr>
<td>AKT1</td>
<td>Variable/Variable/Variable</td>
</tr>
<tr>
<td>HSP90</td>
<td>Increased/Increased/Increased</td>
</tr>
<tr>
<td>NOS3</td>
<td>Decreased/Decreased/Decreased</td>
</tr>
</tbody>
</table>

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also increased in PHPT (35), a contributing effect on gene expression is possible due to increased activation of the vitamin D3 receptors, since they are ubiquitously expressed (8, 12). Vitamin D has antiproliferative effects on hematopoietic cells mediated via induction of the cyclin-dependent kinase (CDK) inhibitor p27kip1 mRNA is present but not changed, whereas p21WAF1/CIP1 mRNA appears to be slightly increased. Al -

Pathways within the Ingenuity Pathways Knowledge Base that were most significantly regulated among the 1,388 genes fulfilling statistical criterion 1 or 2 (see METHODS). PHPT, primary hyperparathyroidism. Significance of the association between the data set and the canonical pathway were analyzed in 2 ways: 1) the number of genes in the dataset that map to the pathway divided by the total no. of genes that map to the canonical; 2) Fischer’s exact test, used to calculate a P value testing the hypothesis that the association between the genes in the dataset and the canonical pathway is explained by chance alone.

The present study therefore indicates that chronic PTH stimulation affects defined groups of mRNAs expressed in all target cells. It therefore became important to better define the human target cells/tissues for PTH in order to understand the observed molecular changes in relation to clinical and organ pathol -

### Table 1. Canonical pathways affected in PHPT

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Number Up/Downregulated in PHPT</th>
<th>Significance (Fischer’s Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium signaling</td>
<td>50/112</td>
<td>0.000</td>
</tr>
<tr>
<td>Cardiac-β-adrenergic signaling</td>
<td>19/77</td>
<td>0.000</td>
</tr>
<tr>
<td>Oxidative phosphorylation</td>
<td>28/143</td>
<td>0.001</td>
</tr>
<tr>
<td>Citrate cycle</td>
<td>8/27</td>
<td>0.007</td>
</tr>
<tr>
<td>Phospholipase, tyrosine, and tryptophan biosynthesis</td>
<td>5/4</td>
<td>0.012</td>
</tr>
<tr>
<td>Pyruvate metabolism</td>
<td>14/70</td>
<td>0.016</td>
</tr>
<tr>
<td>Pentose phosphate pathway</td>
<td>8/33</td>
<td>0.028</td>
</tr>
<tr>
<td>Parkinson’s signaling</td>
<td>5/17</td>
<td>0.029</td>
</tr>
<tr>
<td>Ubiquinone biosynthesis</td>
<td>10/49</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Fig. 4. Cumulative changes of regulated known mRNAs shown as pre- and postoperative ratios and grouped into functional categories. In both panels, a selection of the 5 most regulated mRNAs in 10 different groups is displayed. Each bar shows the compiled change in 1 gene for each patient. Results from real-time RT-PCR are shown as an adjacent bar. Means (SD) of the 5 most regulated mRNAs in 10 different groups is displayed. Each bar shows the compiled change in 1 gene for each patient. Results from real-time RT-PCR are shown as an adjacent bar. Means (SD) of the 5 most regulated mRNAs in 10 different groups is displayed. Each bar shows the compiled change in 1 gene for each patient. Results from real-time RT-PCR are shown as an adjacent bar. Means (SD) of the 5 most regulated mRNAs in 10 different groups is displayed. Each bar shows the compiled change in 1 gene for each patient. Results from real-time RT-PCR are shown as an adjacent bar. Means (SD) of the 5 most regulated mRNAs in 10 different groups is displayed. Each bar shows the compiled change in 1 gene for each patient. Results from real-time RT-PCR are shown as an adjacent bar. Means (SD) of the 5 most regulated mRNAs in 10 different groups is displayed. Each bar shows the compiled change in 1 gene for each patient. Results from real-time RT-PCR are shown as an adjacent bar. Means (SD) of the 5 most regulated mRNAs in 10 different groups is displayed. Each bar shows the compiled change in 1 gene for each patient. Results from real-time RT-PCR are shown as an adjacent bar. Means (SD) of

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production of IL-6, sIL-6R, and TNF-α from cultured white blood cells is elevated in patients with PHPT before operation, showing a PTH-dependent biochemical abnormality in a hematological cell lineage (15–17, 34) (IL-4 was not analyzed in blood). These molecular and biochemical pathways point to a possible clinical relationship. In this respect, it is of interest that PTH-(1–34) treatment of mice caused expansion of hematopoietic stem cell precursors (4). Furthermore, we find mRNAs for immunoglobulins, immunoglobulin receptors, signal transduction mediators, and growth factors to be altered in PHPT. A possible reduced response to antigens may contribute to the increased susceptibility to cancer and infections.

Mesenchymal cells in the bone marrow are the source of osteogenic cell differentiation and osteoblastic cells, which together with immune cell precursors constitute the hematopoietic stem cell niche (4). The Wnt pathway is central in both osteoblast and hematopoietic differentiation and proliferation. Interestingly, several genes involved in Wnt signaling show significantly higher expression in PHPT [Wnt5A, Wnt3, frizzled 7, dissevelled 1, LRP16, and Dickkopf (Dkk) 2; Array Express Repository (E-MEXP-847)], suggesting a role for some of these factors in contributing to the pathological picture in PHPT. mRNAs encoding downstream mediators of the Wnt pathway (β-catenin, GSK-3β, LEF-1/T cell factor) were not significantly altered or were undetectable on the chip.

Because PTHR1, but not the PTHR2, was found to be significantly (2-fold) increased in disease, thereby providing a molecular basis for PTH target cell dysregulation, we examined in some detail their distribution in human CNS, muscle, and blood cells. By real-time RT-PCR analysis we showed the presence of both PTHR1 and PTHR2 mRNAs in human monocytes, lymphocytes, bone marrow, and heart muscle, and both receptor mRNAs were markedly expressed in several regions of the brain. These results, which add to and extend previous knowledge (39), offer a molecular understanding to explain the CNS and muscle pathophysiology in PHPT.

In gene-modulated mice, bone-specific expression of constitutively active PTHR1 led to perturbed hematopoiesis and a greatly reduced number of clonogenic stromal cells (27). Furthermore, other animal studies have shown that osteopontin reduces the hematopoietic stem cell pool (45). In PHPT we have observed activation of PTH1R as well as increased osteopontin mRNA levels, as shown previously (35). Thus, one interesting hypothesis warranting further research is that the number of hematopoietic stem cells may be reduced in PHPT as a consequence of chronic PTH stimulation, an effect opposite to the action of brief PTH infusion (4). The profound changes observed in specific molecular patterns analyzed by global gene expression as demonstrated in our study suggest a number of mechanisms that may translate to and well explain recognized clinical consequences of PHPT, providing strong directions for further research. The present results indicate that early diagnosis and treatment of these patients may avoid irreversible molecular pathology.

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