Genetic Abnormalities in Parathyroid Nodules of Uremic Patients

Jerzy Chudek, Eberhard Ritz, and Gyula Kovacs
Laboratory for Molecular Oncology, Department of Urology [J. C., G. K.], and Department of Internal Medicine [J. C., E. R.], Ruprecht-Karls-University, D-69120 Heidelberg, Germany

ABSTRACT

The molecular pathway of autonomous growth of the parathyroid glands in uremic patients is poorly understood. We have analyzed 71 parathyroid lesions from 24 patients with refractory hyperparathyroidism for allelic loss at chromosomes 1, 3, 6, 9, 11, 12, 13, 15, and 17 and at the X chromosome. Microsatellite analysis was performed using 24 highly polymorphic markers. Deletions at chromosomes 1, 3, 6, 11, 12, and 13 and at the X chromosome were detected in only 10 of 67 nodules (15%). No allelic loss of the p16 and p53 tumor suppressor genes or the extracellular calcium receptor gene was found. The X-chromosome inactivation assay revealed a monoclonal pattern in 58% of hyperplastic nodules in females. Our results indicate monoclonal growth in the majority of hyperplastic nodules and suggest that some of these lesions might be considered precursors for adenoma development.

INTRODUCTION

Secondary hyperparathyroidism is a long-term complication that is associated with diffuse or nodular hyperplasia of the parathyroid glands in patients with chronic renal failure. The genesis of hyperactivity and proliferation of parathyroid cells in uremia is complex, involving low concentrations of calcitriol, ionized calcium, and high concentrations of phosphate (1, 2). Supplementation of calcium, normalization of serum phosphate, and administration of calcitriol usually lead to a decrease of ionized calcium, and high concentrations of phosphate (1, 2). Treatment of uremia is complex, involving bow concentrations of calcitriol, suppression of calcium, normalization of serum phosphate, and administration of calcitriol usually lead to a decrease of PTH concentrations (3–6) and to regression of parathyroid hyperplasia (7). The size of parathyroid gland seems to be one important factor limiting the effectiveness of such treatment, particularly in the presence of nodular hyperplasia (8, 9). The CaR gene (mapped to chromosome 3p1.q–q21) and VDR gene (mapped to chromosome 12q13–14) are involved in the regulation of the PTH synthesis and secretion. It has been suggested that altered expression of these genes results in uncontrolled PTH secretion and overgrowth of parathyroid cells. No mutation of the CaR gene, however, was found in parathyroid glands of 12 patients with secondary and tertiary hyperparathyroidism and in 23 primary adenomas (10). There is no information available concerning mutations of the VDR gene in the parathyroid glands.

Little is known about genetic alterations in different forms of proliferation of the parathyroid cells, such as diffuse or nodular hyperplasia or adenoma. On the basis of X-chromosome inactivation pattern, Arnold et al. (11) showed that, in 64% of hemodialyzed patients with refractory secondary hyperparathyroidism, at least one parathyroid lesion exhibits monoclonal growth. Incidentally, LOH at the X chromosome and at chromosomes 1 and 3q were found in hyperplastic parathyroid tissues (11–13). LOH at chromosomes 1p, 3q, 6q, 11p, 11q, and 15q was observed on more than 60% of parathyroid adenomas, and the cyclin D1 (PRAD1) gene was showed to be rearranged in some cases (14–18).

We examined parathyroid tissues from hemodialyzed patients with advanced hyperparathyroidism using a microsatellite assay to detect allelic loss at chromosomal regions and gene loci, which are implicated in the genetics of primary and secondary hyperparathyroidism. In addition, we analyzed the X-chromosome inactivation pattern in lesions from female patients.

PATIENTS AND METHODS

Patients and Tissue Specimens. Parathyroid tissues and peripheral blood were obtained at surgery from 24 unselected patients (12 women and 12 men) with advanced secondary or tertiary hyperparathyroidism. All patients were refractory to medical treatment. One part of each specimen was fixed and embedded for histology, and the remaining tissue was snap-frozen in liquid nitrogen at −80°C. Histological diagnosis was established according to Harach and Jasani (19). Nodular hyperplasia was diagnosed in 67 specimens, whereas a diffuse hyperplasia was diagnosed in only 4 specimens. DNA was isolated, after protease K digestion followed by phenol-chloroform extraction, from both leukocytes and parathyroid specimens. A single nodule from each nodular hyperplastic gland was used for each DNA extraction procedure.

Microsatellite Analysis. We used 22 DNA polymorphic markers, mapping to chromosomes 1p (D1S162), 1q (D1S1656), 3p (D3S1766 and D3S1289), 3q (D3S1269, D3S1744, and D3S1764), 6q (D6S1040 and D6S264), 9p (D9S171), 11p (D11S861), 11q (D11S987, D11S1314, and D11S614), 12q (D12S1064 and D12S375), 13q (D13S218 and D13S317), 15q (D15S97, D15S165, and D15S100), and Xp (DXS1060). All primers were supplied by Research Genetics, Inc. (Huntsville, AL). The oligonucleotides for DNA repeat polymorphisms at the p53 tumor suppressor gene (1p13.1 and DXAR (Xp) were synthesized according to the published sequences (20, 21). DNA amplification was carried out in 96-well polycarbonate plates using a MJ Research PTC 200 thermocycler. The reaction was carried out in 20-μl volume containing 100 ng of DNA, 50 mm...
KCl, 10 mM Tris (pH 7.0), 1.5 mM MgCl₂, 200 μM dNTPs, and 2 pm each primer, one of them labeled with 2 μCi of [γ-³²P]ATP. The samples were covered with mineral oil and subjected to the following amplification program with hot start: 10 cycles of touchdown (−1°C per cycle), starting at 65°C annealing temperature, and an additional 15 cycles of 30 s at 94°C, 20 s at 55°C, and 20 s at 72°C. After 10 μl of stop solution were added and the samples were heated for 2 min to 80°C, 7 μl of the product were separated on a 5% denaturing polyacrylamide gel at 90 W for 90 min. The signals were visualized by autoradiography after 1 or 2 days exposure. For informative cases, LOH was read when the autoradiographic signal was absent for one of the alleles in the tumor DNA.

### X-Chromosome Inactivation Analysis
DNA (200 ng) from each sample was digested with AluI (Boehringer Mannheim) at 37°C for 3 h and, after precipitation, digested a second time with HpaII (Boehringer Mannheim) at 37°C for 3 h. The digested samples were used after precipitation as templates for amplification of a highly polymorphic fragment of the androgen receptor gene (DXAR). PCR was performed with one primer labeled with [γ-³²P]ATP in a 20-μl volume containing 50 mM KCl, 10 mM Tris (pH 7.0), 1.5 mM MgCl₂, 200 μM dNTPs, and 2 pm each primer (5'-GCT GTG AAG GTT GCT GTT CCT C and 5'-AGA GCC CGC GAG GCG AGC ACC TC; Ref. 20) for 30 cycles (30 s denaturation at 94°C, 40 s annealing at 63°C, and 60 s extension at 72°C). Products were separated on 5% denaturing polyacrylamide gel. Signals were visualized by autoradiography.

### BsmI Restriction Polymorphism of VDR Genotyping
DNA (200 ng) from each sample was used. PCR was performed in 20-μl volume containing 50 mM KCl, 10 mM Tris (pH 7.0), 1.5 mM MgCl₂, 200 μM dNTPs, and 2 pm of primers (5'-CAA CCA AGA GTA CAA CCG GTG CAG TGA and 5'-AAC CAG CCG GAA GAG GTC AGG GG; Ref. 22) for 30 cycles with 30 s denaturation at 94°C, 45 s annealing at 60°C, and 60 s extension at 72°C. PCR product was digested after precipitation with MvaI269I (MBI Fermentas) at 37°C for 3 h, and the products were separated on a 1.5% agarose gel. Alleles were visualized by ethidium bromide staining of the gel.

### RESULTS AND DISCUSSION
Microsatellite analysis with 24 highly polymorphic markers showed LOH in 10 of 67 nodular hyperplastic lesions (Table 1). No LOH was found in parathyroids with diffuse hyperplasia. Of interest, the combination of LOH at chromosome 1p and 12q was detected in two nodules (Fig. 1). In patient HD224A, LOH at 1p was combined with LOH at the chromosome 6q region. A LOH at the long arm of X chromosome was detected in three samples. In patient HD77, two nodules (A₁ and A₂) showing monoclonal growth were found in the same parathyroid gland. Nodule HD77A₁ showed LOH at all four loci at chromosome 11, whereas nodule HD77A₂ retained the constitutional heterozygosity at all loci examined. A monoclonal nodule HD77B in another gland showed LOH at the X chromosome (Fig. 2). A nodular lesion from the third parathyroid gland (patient HD77C) showed a polyclonal growth by assessment of X-inactivation assay. The loss of one allele at chromosome 3q21–22 region was detected in three hyperplastic nodules (HD79A, HD218C, and HD222A). The break point was localized between loci D3S1269 and D3S1764, distal to the locus of the CaR gene, in each case. No deletions at chromosome 9p and 17p have been found by the application of microsatellite markers flanking the p16 and p53 tumor suppressor genes. Together, 15% of the hyperplastic lesions showed LOH at different chromosomal regions, indicating a monoclonal growth of such lesions.

Eleven females showed heterozygosity at the androgen receptor locus by analysis of DNA from peripheral blood with marker XαR. Eighteen of the 31 samples from these patients (58%) showed a X-chromosome inactivation pattern corre-

### Table 1
Results of microsatellite analysis in parathyroid nodules that showed LOH

<table>
<thead>
<tr>
<th>Chromosome arm</th>
<th>1p</th>
<th>1q</th>
<th>3p</th>
<th>3q</th>
<th>6q</th>
<th>9p</th>
<th>11p</th>
<th>11q</th>
<th>12q</th>
<th>13q</th>
<th>15q</th>
<th>17p</th>
<th>Xp</th>
<th>Xq</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD68</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD77</td>
<td>A₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD77</td>
<td>A₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD79</td>
<td>A</td>
<td>NI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD218</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD222</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD224</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD227</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD240</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NI, not informative.
Fig. 2 Microsatellite analyses at chromosomes 11p and Xp in a patient with multiple hyperplastic nodules revealed LOH at chromosome 11 in one (A1) of the two nodules from the same gland. A LOH was detected at the X chromosome in hyperplastic nodule B.

The nodule of patient HD68 retained heterozygosity for both D12S375 and VDR loci and showed a LOH only at the distal locus D12S1064.

Fig. 1 Results of genetic analysis in nodules of patients HD68 and HD240. Microsatellite analysis showed deletion at chromosome 1p and retention of heterozygosity at chromosome 1q in both cases. Deletions at loci D12S375, VDR, and D12S1064 were detected in patient HD240. The nodule of patient HD68 retained heterozygosity for both D12S375 and VDR loci and showed a LOH only at the distal locus D12S1064.

Corresponding to monoclonal growth. Nine of 10 female patients with advanced renal hyperparathyroidism, refractory to medical treatment, have at least one hyperplastic parathyroid nodule with monoclonal growth. Our results are in agreement with those obtained by Arnold et al. (11) and Tominaga et al. (23) and support the hypothesis that nodular hyperplasia corresponds to clonal growth of parathyroid cells that have escaped from the normal growth control via concentrations of calcitriol, calcium, and phosphate in the serum.

The CaR plays an important role in the regulation of PTH secretion and the growth of parathyroid cells. It has been suggested, therefore, that altered expression of the CaR gene is involved in neoplastic transformation (11). Mutation analysis of the CaR gene in parathyroid adenomas and hyperplastic lesions did not reveal any abnormalities (10), and only 2 of 35 parathyroid adenomas showed LOH around the CaR gene (24). We found deletion at chromosome 3q22–24 in only 3 of 71 hyperplastic lesions, each with breakpoint distal to the CaR gene.

It was suggested that the b allele of the VDR gene, which is marked by a BsmI restriction polymorphism, yields a lower expression of mRNA (22, 25). A bb genotype of the VDR gene, which occurs in 30–40% of the general population, was found in 60% of patients with primary hyperparathyroidism (26). A decreased density of VDR on parathyroid cells was demonstrated by immunohistochemistry in patients with advanced secondary hyperparathyroidism (8). In our series of 24 patients, 16 were heterozygous (Bb), and 8 were homozygous (bb or 2 BB). LOH at chromosome 12q occurred with a breakpoint distal to the VDR gene locus in patient HD68.

Our data and those from literature indicate a clonal expansion of parathyroid cells in a substantial number of secondary hyperplastic lesions. The molecular basis for this monoclonal growth is as yet unknown. The common uremic etiology of parathyroid nodules suggests some common genetic alterations in these lesions. LOH is a rare event in the hyperplastic nodules and it occurs at different genomic sites; probably, they do not play a role in the development of nodular hyperplasia in uremic patients. However, LOH at chromosomes 1p, 3q, 6q, 11q, and 15q occurs frequently in primary parathyroid adenomas (12–14), as do deletions of one allele of the Rb and p53 tumor suppressor genes in parathyroid carcinomas (18, 27). Our findings in some of the hyperplastic nodules are very similar to those observed in primary adenomas and carcinomas. This suggests that some of the nodules might be on the way to developing adenomas that escape the host control on the proliferation of parathyroid cells. Confirmation of this hypothesis and establishing the molecular basis of this transition would be important for the clinical management of patients.

ACKNOWLEDGMENTS

We thank Prof. Dr. F. Spelsberg and Dr. H. P. Mühlig, Department of Surgery, Martha Maria Hospital (Munich, Germany), for kindly providing parathyroid specimens and clinical data.
REFERENCES


Genetic abnormalities in parathyroid nodules of uremic patients.

J Chudek, E Ritz and G Kovacs


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/4/1/211

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.