CLINICAL STUDY

Chromosomal imbalances identified by comparative genomic hybridization in sporadic parathyroid adenomas

Juan Luis García, Juan C Tardío¹, Norma Carmen Gutiérrez, María Belén González, José Ramón Polo², Jesús María Hernández and Javier Menárguez³

Department of Haematology, Hospital Universitario de Salamanca and Centro de Investigación del Cáncer, Universidad de Salamanca-CSIC, Salamanca, Spain, ¹Department of Pathology, Hospital El Escorial, Madrid, Spain, and Departments of ²Surgery and ³Pathology, Hospital General Universitario Gregorio Marañón, Madrid, Spain

(Correspondence should be addressed to J Menárguez, Department of Pathology, Hospital General Universitario Gregorio Marañón, Dr Esquerdo 46, 28007 Madrid, Spain; Email: anatomiap@anat.hggm.es)

Abstract

Objective: To identify chromosomal gains and losses in sporadic parathyroid adenomas (PAs).

Methods: Fourteen sporadic PAs were studied by comparative genomic hybridization (CGH).

Results: The fourteen studied PAs showed chromosomal imbalances. All cases except one exhibited two or more abnormalities. Chromosomal gains were found in all cases, and three cases (21%) also presented chromosomal losses. Genomic amplification was not observed. Chromosome 9 was involved in ten cases. Recurrent genetic gain was found on 9p22–24 and on 9q34, each in 6 of 14 cases (43%). Other recurrent gains included Xq26 in 6 PAs (43%) and 4q21–28 and 8p22–23, each in 4 of 14 cases (29%). Regions of recurrent genetic loss involved whole chromosome 11 and 20q12–13, each in 2 of 14 cases (14%).

Conclusions: Our findings show chromosomal imbalances in all sporadic PAs studied by CGH, partly confirming previous reports, with the exception that we observed more chromosomal gains than losses. Several regions (9p22–24, 9q34, Xq26, 4q21–28, and 8p22–23) probably deserve further investigation in order to discard the presence of genes involved in parathyroid tumorigenesis.

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Introduction

Primary hyperparathyroidism is a common endocrine disorder characterized by an excessive production of parathyroid (PT) hormone (PTH). Sporadic PT adenomas (PAs) are currently the cause of primary hyperparathyroidism in the vast majority of the patients. PAs are benign clonal proliferations of PT chief cells (1).

Two genetic aberrations are known to be implicated in the pathogenesis of sporadic PAs: (i) cyclin D1 overexpression in a small subset of these tumors, as a result of rearrangement of the cyclin D1/PRAD1 oncogene, located on chromosome 11q13, with the PTH gene on 11p15 (2–4), and (ii) multiple endocrine neoplasia type 1 (MEN1) tumor suppressor gene (on 11q13) inactivation by allelic loss and somatic mutation of the remaining allele (5–9) in about 20–30% of cases. Lowered levels of calcium receptor messenger RNA detected by *in situ* hybridization (10), t(1;5) (11), and other genetic alterations, such as loss of heterozygosity (LOH) by microsatellite analysis, involving chromosomes 1p (8, 12–15), 3q (16), 6q (17), 9p (18), 11p (19) and 15q (17) have also been reported in a variable percentage of

PAs. Allelic losses of 13q involving the Rb and the BRCA2 genes have been reported in PAs with aggressive clinical and histopathological features (20). However, the genetic bases of the majority of PAs are unknown.

Comparative genomic hybridization (CGH) is a double color hybridization procedure which provides, in a single experiment, a general view of genomic imbalances, including partial or complete trisomies, monosomies or amplifications within the tumor genome (21). This technique can be used to identify previously unexpected genetic abnormalities. Several groups have studied chromosomal changes by CGH in different solid tumors, but limited information concerning sporadic PAs is presently available (22–25). We have applied CGH to identify genomic imbalances in 14 patients with PAs.

Materials and methods

Tumor specimens from 14 patients with sporadic PAs were included in the study. All samples were studied at diagnosis.

Table 1 Comparative genomic hybridization findings in sporadic parathyroid adenomas.

Case	Age (years)/Sex	Gains	Losses
1	72/F	3p24–p26, 9p22–p24	
2	60/F	6p11–p25	6q12–q27, 9p24–q33, 11, 15, 18q12–q22, 20
3	64/M	1q41–q43, 3q27–q29, 4q34–qter, 6q27–qter, 8p22–p23, 9p22–p24, 10p14–p15, 11q24–q25, 14q32–qter, 16q23–q24, 17q24–q25, 19q13, 20q13, 21q22, Xq26–q27	
4	75/F	2p24–p25, 3q21–q24, 4q21–q28, 7p15–p22, 7q35, 9q33–q34, 14q32–qter, 18q22–q23, X	
5	67/F	1p32–p36, 9q32–q34, 11q32, 12q24, 14q31–q32, 17q24–q25, 19p12–p13, Xq26–q28	
6	68/F	2q22–q31, 4q13–q31, 6q16–q22, 10q25–q26, 13q22–q32, 15q25–q26, 18q22–q23	
7	75/F	1q41–q43, 9q34–qter, Xq12–q26	
8	68/F	1q24–q31, 19q13, 20q12–q13, 22q, Xq12–q28	
9	52/F	2q22–q31, 4q13–q31, 5q12–q15, 13q22–q32, Xq13–q27	
10	78/F	9	
11	55/F	8, 9	
12	50/F	1, 6p21–p23, 7, 8, 9, 10, 12, 16, 20q13	11, 13, 19p12–p13
13	52/F	1p22–p32, 3q21–q26, 4, 6q12–q23, 8, 11q14–q22	
14	63/F	4q34, 5q33–q35, 9p22–p24, 10q25–q26, 12q23–q25, 13q33–q34, 18q22–q23	20q12–q13

Tumor DNA was isolated from PAs. Reference DNA was obtained from peripheral blood lymphocytes of healthy donors (same sex as patients). Phenol–chloroform was used for DNA extraction according to standard procedures (26). CGH analysis was performed according to the method described by Lichter and Ried (27). Briefly, tumor DNA (test DNA) was labeled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) and normal DNA (reference DNA) was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by a standard nick translation reaction. The size of the nick-translated fragments ranged from 300 to 1000 bp. Equal amounts (1 µg) of labeled tumor and normal DNAs, and 70 µg unlabeled human Cot-1 DNA (GIBCO/BRL, Gaithersburg, MD, USA) were cohybridized to slides with human metaphase chromosome spreads prepared from phytohemagglutinin-stimulated lymphocytes from normal individuals. After hybridization for 1 to 2 days in a moist chamber at 37 °C, post-hybridization washes were performed to a stringency of $0.1 \times \text{SSC}$ at 42 °C. Tumor and normal DNA were detected by avidin-fluorescein isothiocyanate (FITC) and rhodamine-conjugated anti-digoxigenin respectively. The slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted with an antifade solution. Image acquisition was performed with an epifluorescence microscope (Olympus BX60) equipped with a cooled charge-coupled device (CCD) camera. Calculation of the tumor DNA to normal DNA fluorescent ratios along the length of each chromosome was performed by means of an automated CGH software package (Cytovision, Applied Imaging, Sunderland, UK). Ratio values obtained from at least 10 metaphase

cells for each case were averaged. Ratio values above 1.25 and below 0.75 were considered to represent chromosomal gain and loss respectively. Over-representations were defined as high-level amplifications when the profiles exceeded the cut-off value of 1.5. Chromosomal gains exceeding 1.5 involving the whole chromosome or large areas of a chromosomal arm were not considered as high-level DNA amplification. Negative control experiments were performed using differentially labeled male versus male and female versus female DNA. Additional control experiments included the interchange of the digoxigenin-dUTP and biotin-dUTP labels between normal and tumor DNA.

Results

The patients were thirteen women and one man, and their ages ranged from 50 to 78 years (mean 64.2; median 65.5) (Table 1). Diagnosis of PAs relied upon clinical, surgical and pathological findings. Histologically, all cases were conventional PAs.

The fourteen studied PAs showed chromosomal imbalances, including losses or gains of whole or part of chromosomes. All cases except one exhibited two or more abnormalities (Table 1). Chromosomal gains were observed in all cases. Three of them (21%) presented chromosomal losses. No genomic amplification was observed. The summary of gains and losses is shown in Fig. 1.

Chromosome 9 was involved in ten cases. Recurrent genetic gain was found on 9p22–24 and on 9q34, each in 6 of 14 cases (43%). Other recurrent gains involved chromosome X in 6 PAs (43%) with a consensus region

on Xq26; 4q with a consensus region on 4q21–28 and chromosome 8 with a consensus region on 8p22–23, each in 4 of 14 cases (29%); as well as 1p with a consensus region on 1p32, 1q41–43, 4q34, 10q25–26, 12q with a consensus region on 12q24, 14q with a consensus region on 14q32, 18q22–23, and 20q with a consensus region on 20q13, each in 3 of 14 cases (21%); and 2q22–31, 3q with a consensus region on 3q21–24, 6p with a consensus region on 6p21–23, 6q with a consensus region on 6q16–22, 7p15–22, 7q35, 10p14–15, 13q with a consensus

region on 13q22–32, 16q23–24, 17q24–25 and 19q13, each in 2 of 14 cases (14%). Gains of 2p24–25, 3p24–26, 5q12–15, 5q33–35, 11q14–22, 11q24–25, 11q32, 13q33–34, 15q25–26, 19p12–13, 21q22 and 22q were each found in one of fourteen cases (7%) (Table 1).

Regions of recurrent genetic loss included whole chromosome 11 and 20q12–13, each in two of fourteen cases (14%). Losses at 6q12–27, 9p24–q33, 13, 15, 18q12–22 and 19p12–13 were each found in one of fourteen cases (7%) (Table 1).

Figure 1 Summary of genetic aberrations found by comparative genomic hybridization in sporadic parathyroid adenomas. Gains are represented by lines at the right of the chromosomes and losses by lines at the left. Line lengths correspond to gains or losses of genetic material. Numbers on top of the lines represent case identification.

Discussion

We have found chromosomal imbalances in all PAs studied by CGH, with multiple abnormalities in most cases. Genetic gains were more frequent than losses in our series. Significant recurrent findings were genetic gains on 9p22–24, 9q34 and Xq26 in 43% of cases each, and on 4q21–28 and 8p22–23 in 29% of cases each. Some of these findings have previously been reported in PT neoplasms studied by CGH analysis, suggesting putative regions for oncogenes implicated in PT tumorigenesis. Chromosomal gain on 9p has been reported by Agarwal *et al.* (22) in one of 10 sporadic PAs (10%). Farnebo *et al.* (24) observed gain of whole chromosomes X and 4, each in one of 26 sporadic PAs (4%). Palanisamy *et al.* (23) found gain of whole chromosome 8 in one of the 53 sporadic PAs (2%) and Hemmer *et al.* (25) reported high-level amplification on chromosome 8 at a region involving 8p22 in one of 16 PAs (6%). Genetic gain of 9q, on a region including 9q34, has been reported in 7 of 29 PT carcinomas (24%) (28), and gain of whole chromosome 4 in 2 of 10 PT carcinomas (20%) (22). We have specifically investigated the presence of potential correlations between CGH findings and different clinical parameters, macroscopical and histological features and immunohistochemical expression of several proliferation markers and cell cycle proteins (29, 30) in our series. We have not been able to demonstrate any correlation (results not shown).

Four previously published reports have studied sporadic PAs by CGH (22–25). They found chromosomal imbalances in 62 to 90% of cases. The high number of genetic alterations detected by this technique in PAs, a benign neoplasm, contrasts with the findings in other types of endocrine tumors where they are correlated to the grade of malignancy (31), suggesting that the oncogenetic mechanisms may not be identical in different endocrine organs. In PAs, genetic losses are predominant. There are two frequent recurrent aberrations common to the four reports: losses on chromosome 11 and on chromosome 15, suggesting the existence of putative tumor suppressor genes implicated in PT tumorigenesis on these chromosomes. Losses on chromosome 11 have been found in 31 to 40% of cases in these four series. We found loss of chromosome 11 in 14% of our cases. As a whole, losses involving chromosome 11q have been reported in 39 of 119 sporadic PAs (33%) studied by CGH in the five published series, including ours. This alteration could be related to MEN1 gene abnormalities found by LOH and somatic mutation in these tumors (6–9). However, in a recent report by Hemmer *et al.* (25) the common deleted region on 11q was 11q23, with the presence of the two MEN1 alleles at 11q13 confirmed by fluorescent *in situ* hybridization. Furthermore, CGH frequently shows monosomy or extensive losses on chromosome 11 that include 11p. Two sporadic PAs

with 11p loss that was not accompanied by 11q loss have been reported (22, 23). These findings suggest the existence of additional tumor suppressor genes implicated in PT tumorigenesis on regions of chromosome 11 other than 11q13. LOH for several genes located on chromosome 11p has been reported in primary hyperparathyroidism (19), lending further support to this suggestion. Some known tumor suppressor genes located on 11q23 appear to be implicated in different human neoplasms (32–34), but their potential role in PT tumorigenesis is presently unknown.

Losses on chromosome 15q have been found in 12 to 30% of cases in the four previously published series (22–25) and in 7% of our cases. As a whole, 22 of 119 sporadic PAs (18%) studied by CGH have shown this aberration. LOH on 15q in PAs has been reported previously (17). These findings could suggest the existence of a tumor suppressor gene implicated in PT tumorigenesis located on 15q.

Up to now only a handful of primary PT hyperplasias has been investigated by CGH. Based upon the reported results and in contrast with PAs, PT hyperplasias show scarce chromosomal gains or losses, hindering the establishment of a common profile. The only significant recurrent chromosomal aberration appears to be loss of 11q in less than 10% of cases (25).

Only two reports address chromosomal aberrations in PT carcinomas by CGH (22, 28). In spite of having only partially overlapping findings, these reports suggest that loss of 1p, present in 40% of cases, could be a significant feature. This aberration is a less frequent finding in PAs (13%).

Based upon available evidence, it is not currently possible to pinpoint CGH abnormalities that could: (i) discriminate between PT carcinomas and PAs and (ii) forecast malignant transformation in PAs, otherwise an extremely infrequent event. In addition, as stated above, we have not been able to establish any significant CGH aberration in our series identifying clinical or pathological aggressive PAs.

In conclusion, our findings show chromosomal imbalances in all sporadic PAs studied by CGH, suggesting potential candidate locations for genes implicated in PT tumorigenesis on 9p22–24, 9q34, Xq26, 4q21–28, 8p22–23, 11 and 15q.

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