Colonic polyps of acromegalic patients are not associated with mutations of the peroxisome proliferator activated receptor γ gene

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ABSTRACT. Peroxisome proliferator activated receptor (PPAR)γ plays a pivotal role in regulating adipocyte differentiation and metabolism, but also has an antiproliferative effect in several tissues, including colonic mucosa, where it is highly expressed. Loss-of-function mutations have been reported in about 10% of sporadic primary colon cancer. Acromegalic patients have an increased prevalence of colonic neoplasms and lower PPARγ levels in the colonic mucosa. Thus, PPARγ may act as a tumor suppressor gene, and its reduced expression or loss-of-function mutations may contribute to tumorigenesis. In this study the expression and mutations of the PPARγ gene in the colonic polyps and mucosa outside polyps were investigated in 10 acromegalic and 17 non-acromegalic patients. PPARγ expression was evaluated by RT-PCR. PPARγ was expressed in each sample, but expression appeared to be lower in polyps than in mucosa outside polyps from either acromegalic or non-acromegalic patients. All exons of the PPARγ gene were directly sequenced after PCR amplification: no mutations were found either in acromegalic or in non-acromegalic patients. In conclusion, the results of this preliminary study suggest that the lower expression of PPARγ rather than somatic mutations of this gene is involved in colonic tumorigenesis.


INTRODUCTION

Peroxisome proliferator activated receptor (PPAR)γ, a member of the nuclear receptor superfamily, is highly expressed in adipose tissue, where it plays a key-role in the regulation of adipocyte differentiation and fat metabolism (1-3). A growing body of evidence indicates that PPARγ is a functional receptor for the thiazolidinedione class of antidiabetic drugs and may function as a tumor suppressor gene (4, 5): a fusion of PPARγ to PAX8 was reported in thyroid papillary carcinomas (6); PPARγ activation by troglitazone induces differentiation of liposarcoma (7), prostate cancer (8), or several transformed cells, whose growth is inhibited (9, 10).

PPARγ levels in the colonic mucosa are similar to those found in adipose tissue, and increased expression is found during differentiation of colonic epithelial cells (11, 12). Activation of PPARγ in cultured colon cancer cells induces growth inhibition and increases markers of cellular differentiation (9). Furthermore, PPARγ activation decreases pre-malignant intestinal lesions in rats treated with azoxymethane (13). In humans, somatic mutations in one allele of the PPARγ gene associated with a loss of function of the receptor were found in 4 out of 55 patients with primary colorectal cancer, being localized exclusively in exon 5 and exon 3, which encode for the ligand binding domain and DNA binding domain, respectively; c472delA results in deletion of the entire ligand binding domain; Q286P and K319X mutations retain a total or partial ligand binding domain but lose the ability to activate transcription; R288H mutation was associated with a normal response to synthetic ligands but with impaired transcription and binding when exposed to natural ligands (14). On the other hand, others failed to detect mutations of the PPARγ gene in a large series of tumor samples and cell lines (15).
The increase in serum IGF-I levels found in acromegaly (Acro) was associated with the increased prevalence of colonic polyps (16, 17), proliferation of colonic epithelial cells (18) and development of colonic adenomas (19). We have recently observed that patients with active Acro have reduced expression of PPARγ in the colonic mucosa, which is related to the increased serum IGF-I levels (20). This reduced PPARγ expression might have the same role of loss-of-function mutations, contributing to colonic tumor development. In fact, the loss of one allele of the PPARγ gene has been associated with an increased sensitivity to chemical carcinogenesis (21).

The aim of the present study was to search for PPARγ gene mutations in the colonic polyps of Acro patients.

MATERIALS AND METHODS

Patients

The study included the following groups of subjects: a) 10 Acro patients [5 men and 5 women; mean age (±SD) 49±11 yr; 4 with GH-secreting microadenoma, 6 with macroadenoma], referred to our Institution in the years 2001-2002: 4 patients had active, untreated Acro (AcroUntr); 2 patients had active Acro under somatostatin analogs therapy (SSMs; 20 mg every 28 days, for at least 6 months: AcroSSMs); 4 patients were in remission after pituitary adenomectomy (AcroRem); b) 17 non-Acro patients investigated because of colonic polyps (10 men and 7 women; mean age 53±7 yr: controls). Three patients of the latter group had positive family history for colorectal neoplasia, whereas no Acro patient had colonic cancer nor a positive family history for colonic neoplasia. Diagnosis of Acro was based on clinical and laboratory features, including an increase in serum IGF-I levels and the lack of suppression of serum GH levels below 2 μg/l after a 75-g oral glucose tolerance test (Table 1).

Pancolonscopy

Patients were selected among Acro patients based on the presence of colonic polyps; all gave their informed consent. Colonscopic examination was performed using an Olympus CFQ14SL apparatus by the same operator (A.C.). Polyps and colonic examination was performed using an Olympus

Table 1 - Clinical and biochemical features of the study groups.

<table>
<thead>
<tr>
<th></th>
<th>Acromegaly</th>
<th>Controls</th>
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<tbody>
<tr>
<td>No. (M/F)</td>
<td>10 (5/5)</td>
<td>17 (10/7)</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>49±11</td>
<td>53±7</td>
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<tr>
<td>Serum IGF-I (μg/l)*</td>
<td>531±277</td>
<td>203±67</td>
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<tr>
<td>Serum GH (μg/l)**</td>
<td>12.5±13.7</td>
<td>0.8±0.7</td>
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<tr>
<td>Estimated duration of acromegaly (months)</td>
<td>69±57</td>
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</tbody>
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Acromegaly: 4 patients had active untreated acromegaly, 2 had active disease under somatostatin analog therapy and 4 patients had acromegaly in remission after transphenoidal adenomectomy. Extrapolys mucosa samples were recovered during colonoscopy and placed in liquid nitrogen until further examination.

RT-PCR and sequencing analysis

Primer sequences and RT-PCR conditions are summarized in Table 2. DNA was amplified through 34 cycles of: 94 C, 1 min; 55 C, 1 min; 72 C, 2 min. The quality of cDNA and PCR products was evaluated on a 1% agarose gel. All exons of the PPARγ gene were amplified. The PCR products were purified on 1% Nusieve gel with Wizard PCR purification system (Promega, Madison, MA, USA). Both strands were sequenced directly after PCR amplification, using FS Ampli Taq DNA polymerase and fluoresceinate nucleotides. An ABI Prism 310 (Applied Biosystem, CA, USA) apparatus was employed. Sequence analyses were performed using Sequencing Analysis 3.0 software.

Assays

Serum GH and IGF-I (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) were determined by commercial kits. Normal values in our laboratory are as follows: GH, 0-5 μg/l; IGF-I, 182-780 μg/l, 16-24 yr; 90-492 μg/l, 25-50 yr; 71-290 μg/l, >50 yr. Serum GH assay had a sensitivity of 0.15 μg/l and serum IGF-I assay of 0.3 μg/l; inter- and intra-assay coefficients of variation were 2.9-4.5 and 2.4-4.0% for GH, 7.6-15.5 and 10.1-15.7% for IGF-I, respectively.

Statistics

Results of serum GH and IGF-I measurements were expressed as mean±SD. Comparison of parameters among the study groups was performed by analysis of variance (ANOVA).

RESULTS

As illustrated in Table 1, Acro patients had significantly higher serum GH and IGF-I levels than controls. Histological examination, performed in 8 polyps, revealed a tubular adenoma in 7 and a hyperplastic pattern in 1 Acro patient; 2 very small polyps were not used for histological examination. Non-Acro patients had tubular adenoma (no.=16) or hyperplastic polyps (no.=1). All patients were analyzed for somatic mutations in the PPARγ gene by examining either genomic DNA or cDNA of the colonic polyps. All colonic samples (polyps and mucosa outside polyps) of Acro and non-Acro patients expressed PPARγ as assessed by RT-PCR (Table 3). However, as assessed by RT-PCR, PPARγ expression appeared to be lower in the polyps than in mucosa outside polyps of either Acro or non-Acro patients. When all PPARγ gene exons were evaluated, no somatic mutations were detected neither in the genomic DNA nor in