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

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 (SMSa; 20 mg every 28 days, for at least 6 months: AcroSMSa); 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 (Acro). Diagnosis was performed by analysis of variance (ANOVA).

Pancolonscopy

Patients were selected among Acro patients based on the presence of colonic polyps; all gave their informed consent. Colonoscopic examination was performed using an Olympus CFQ14SL apparatus by the same operator (A.C.). Polyps and extrapolyps mucosa samples were recovered during colonoscopy and placed in liquid nitrogen until further examination.

RNA and DNA extraction

Total RNA and DNA was prepared using an RNA-DNA mini-kit (Qiagen Corporation, Milan, Italy) according to the manufacturer’s instructions. Total RNA or DNA was resuspended in DEPC-treated water, and quantitated by spectrophotometry.

RT-PCR and sequencing analysis

Primers for RT-PCR, exons amplification and sequencing analysis are summarized in Table 2. cDNA 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.

RT-PCR and sequencing analysis

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

<table>
<thead>
<tr>
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<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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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.

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

Data are expressed as mean±SD; significant differences are indicated by asterisks: *p<0.002; **p<0.03.