Mutations in the Arginine-rich Protein Gene, in Lung, Breast, and Prostate Cancers, and in Squamous Cell Carcinoma of the Head and Neck

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Abstract

Arginine-rich protein (ARP) is a highly conserved gene that maps to human chromosomal band 3p21.1. This gene contains an imperfect trinucleotide repeat which encodes a string of arginines. We previously detected a specific mutation (ATG50 —@ AGG) within this region of the gene in 10 of 21 sporadic renal cell carcinomas. Here, we report the detection of the same mutation in 5 of 21 squamous cell carcinomas of the head and neck, 1 of 2 small cell lung cancer cell lines, 6 of 18 non-small cell lung carcinomas, 9 of 22 breast tumors, and 5 of 13 prostate tumors. This mutation was seen in several early stage tumors and may thus be an early event in tumorigenesis. We also detected a mutation at codon 53 of this gene in both primary and metastatic tumors from one patient. Other nucleotide changes were observed in a few PCR subclones, but their frequency was the same in both tumor and control samples, suggesting that many of these changes were PCR or subcloning artifacts rather than mutations in the tumor cells themselves.

Introduction

A progressive accumulation of genetic changes in oncogenes and tumor suppressor genes leads to carcinogenesis (1–5). One of the major goals in the study of cancer is to identify early genetic changes that lead to tumorigenesis. Although both alleles of a tumor suppressor gene must be lost or inactivated to lead to carcinogenesis, activation or gain of function in only one copy of an oncogene is required (6–10). We identified a gene, from chromosomal band 3p21.1, which showed a specific mutation (ATG —@ AGG) or deletion of codon 50 in 10 of 21 sporadic RCC samples (11). In these tumors, we also observed the wild-type allele in 44 of 79 (56%) of the subclones from the PCR-amplified tumor DNA samples, consistent with the notion that most of the tumor cells were heterozygous for these mutations. These codon 50 mutations thus appeared to act as gain of function mutation, analogous to activation of an oncogene.

In this report, we describe our search for ARP mutations in primary tumors from SCCHN samples, SCLC cell lines, breast tumors, prostate cancers, and primary NSCLC tumors. We also analyzed several corresponding tumor cell lines, free of nontumor cells, as a check on the continued presence of a wild-type ARP sequence in tumor cells. We detected the same specific mutation, ATG50 —@ AGG, in all four tumor types. Mutations at codon 50 were seen in approximately one-third of all tumors tested, including several early stage tumors. This mutation may be an early event in the development of different solid tumors. In addition, we detected a mutation at codon 53 in a primary SCCHN sample and in a metastatic tumor sample derived from the same patient.

Materials and Methods

DNA from 18 NSCLC samples and matched normal tissue was obtained from the University of Pittsburgh School of Medicine (Pittsburgh, PA). DNA from the 22 breast samples was obtained from the Karmanos Cancer Institute, Detroit, MI. DNA from the 13 prostate tumors, 8 SCCHN cell lines, and 13 primary SCCHN tumors were obtained from Harper Hospital, Detroit Medical Center, Detroit, MI. Two lung cancer cell lines, HR2 and H128, were obtained from the American Type Culture Collection. DNA was isolated from frozen tissues and blood and subjected to PCR amplification, subcloning, and sequencing of a 125-bp region centered on the imperfect trinucleotide repeat of the ARP gene as described previously (11). This region encodes 50 of the 234 amino acids of the predicted ARP gene product. PCR amplification of this region was performed using primers G-42 and I-43 (11), to which synthetic tails were added at the 5' ends. A hot start PCR was performed using TaqStart Antibody from Clontech according to the manufacturer's protocol. PCR reactions contained 10 mm Tris-HCl (pH 8.3), 50 mm KCl, 1.5 mm MgCl2, 200 mm deoxynucleotide triphosphate, 0.2 mm each primer, 100 ng of genomic DNA, and 0.1 unit of Taq DNA polymerase (Promega) in a final volume of 25 μl. The PCR consisted of 30 cycles at 94°C for 1 min, 65°C for 2 min, followed by 7 min at 65°C. The PCR products were resolved on a 2% agarose gel and gene cleaned (BIO 101) according to the manufacturer's instructions. The gene-cleaned products were directionally cloned into the linearized pDIRECT plasmid vector using the ligation-independent cloning method according to the manufacturer's instructions (Clontech).

Results

We analyzed a total of 76 tumors including 22 breast, 21 SCCHN (3/21 were cell lines), 18 NSCLC (1/18 was a cell line), 13 prostate samples, and 2 SCLC cell lines for mutations in the cryptic trinucleotide repeat of the ARP gene. One specific mutation, ATG50 —@ AGG, was seen in every type of tumor studied. However, it was present in only 92 of 174 (52%) of the tumor subclones sequenced from the 26 patients whose tumor samples had this mutation; the other 48% had the wild-type sequence. This could be the result of contaminating normal tissue in the tumor samples. However, it was also observed in one of the two long-term lung cancer cell lines, in which normal cells are not present, indicating the presence of a wild-type sequence in the tumor cells (11). Table 1 shows the frequency of the specific mutation, ATG50 —@ AGG, in the ARP gene in various cancers.

In one of the SCCHN cell lines, an ATG53 —@ ATA mutation was seen in both the primary tumor sample (S1) and its metastatic derivative from the lung (L1). The presence of this mutation in both primary and metastatic samples from the same patient indicates that it...
Fig. 1 shows the sequencing gel with the wild-type sequence, the observed in the SCCHN cell line, Si, and in the lung metastasis cell line, L1 (C).

been subsequently observed in several pancreatic adenocarcinomas.5

We had previously analyzed LOH with highly polymorphic mark-

ers in the NSCLC samples used in this study (12). The results indicated that, of the two 3p21.1 markers (D3S1289 and Not73) used, only D3S1289 showed any LOH, and in only four of 22 tumors used in this study (12). Of these four, only 101–877 had a mutation at codon 50. Only one of the 11 NSCLC tumors with a codon 50 mutation showed LOH of one 3p21.1 marker.

Discussion

We detected a possible gain of function mutation involving codon 50 of the ARP gene in 10 of 21 RCC samples (11). We have now detected the same mutation in 8 of 20 lung cancers, 5 of 21 SCCHN, 9 of 22 breast cancers, and 5 of 13 prostate cancers, i.e., in roughly one-third of all cancers tested. Our previous LOH results on the lung tumors (12) indicated that there was very little if any LOH of the 3p21.1 markers in these tumors.

Table 1 Frequency of a specific mutation, ATG → AGG, in codon 50 of the ARP gene in various cancers

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>Specific mutation (ATG50 → AGG)</th>
<th>No. of tumor samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Head and neck</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Lung-SCLC</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lung-NSCLC</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Prostate</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2 Mutations in subclones of PCR products of the ARP gene in breast, lung, prostate, and SCCHN tumors.

<table>
<thead>
<tr>
<th>Tumor sample</th>
<th>Mutation frequency</th>
<th>Mutation type</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>249° B</td>
<td>4/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>486° B</td>
<td>4/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>3/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>ATG50 and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACG deleted</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARG deleted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>739° B</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>1047° B</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>114° B</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>1203° B</td>
<td>2/3</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>1254° B</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>Sim® B</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>Sau® B</td>
<td>3/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>361 B</td>
<td>1/5</td>
<td>ATG50 → TGG</td>
<td>ARG48 → TRP</td>
</tr>
<tr>
<td>444 B</td>
<td>1/3</td>
<td>ATG48 → AGT</td>
<td>ARG48 → TRP</td>
</tr>
<tr>
<td>402 B</td>
<td>2/3</td>
<td>1 ARG inserted</td>
<td>1 ARG inserted</td>
</tr>
<tr>
<td>1003 B</td>
<td>2/4</td>
<td>1 ARG inserted</td>
<td>1 ARG inserted</td>
</tr>
<tr>
<td>A1° H (CL)</td>
<td>4/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>W1° H (CL)</td>
<td>3/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>214° H</td>
<td>2/3</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>294° H</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>354° H</td>
<td>2/5</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>8862 H</td>
<td>2/4</td>
<td>2 ARG deleted</td>
<td>2 ARG deleted</td>
</tr>
<tr>
<td>9152 H</td>
<td>2/4</td>
<td>2 ARG deleted</td>
<td>2 ARG deleted</td>
</tr>
<tr>
<td>3-897° L</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>6567° L</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>6607° L</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>31-907° L</td>
<td>3/7</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>101-877° L</td>
<td>3/7</td>
<td>1 ARG deleted</td>
<td>1 ARG deleted</td>
</tr>
<tr>
<td>49-907 L</td>
<td>3/8</td>
<td>2 ARG's deleted</td>
<td>2 ARG's deleted</td>
</tr>
<tr>
<td>H2° SCLC (CL)</td>
<td>2/4</td>
<td>ATG50 → AGG</td>
<td>MET50 → ARG</td>
</tr>
<tr>
<td>955-234E10° P</td>
<td>2/4</td>
<td>ATG50 to AGG</td>
<td>MET50 to ARG</td>
</tr>
<tr>
<td>955-825D22° P</td>
<td>2/4</td>
<td>ATG50 to AGG</td>
<td>MET50 to ARG</td>
</tr>
<tr>
<td>955-864C13° P</td>
<td>2/4</td>
<td>ATG50 to AGG</td>
<td>MET50 to ARG</td>
</tr>
<tr>
<td>955-881C10° P</td>
<td>2/4</td>
<td>ATG50 to AGG</td>
<td>MET50 to ARG</td>
</tr>
<tr>
<td>595-212D14° P</td>
<td>3/4</td>
<td>ATG50 to AGG</td>
<td>MET50 to ARG</td>
</tr>
</tbody>
</table>


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Our analysis of PCR subclones from primary tumor tissue shows that tumors generating one or more subclones with mutations also generated multiple subclones with the wild-type sequence. This could be due to contaminating normal cells in each of the samples, although this seems unlikely in view of the high frequency of the wild-type sequence, roughly 50%, in the samples, and that an equally high frequency of the wild-type sequence was observed in two SCCNH cell lines and one SCLC cell line, H82, that showed the ATG50 → AGG mutation. We observed a similar frequency of clones with the wild-type sequence (44/79, or 56%) in 10 RCC samples (11). One explanation for these findings is that only a single copy of this gene is mutant in any cancer cell; another is that a mutation has occurred in a region of the gene not included in the 125-bp region that was analyzed. We favor the first explanation because of the extreme focus of mutations in codon 50 and also because mutational analysis of the remainder of the ARP gene did not reveal any changes in several RCC samples that had the codon 50 mutation (11).

This is reminiscent of the codon 12 and codon 61 mutations in the ras cellular oncogene seen in many cancers and responsible for its activation (13). It will be interesting to determine whether the specific mutation in the ARP gene confers a corresponding gain of function to the cells that harbor it.

The presence of the wild-type sequence in subclones from the tumor samples is compatible with the presumed role of ARP as an oncogene. Our initial attempts to introduce this specific mutation in the pcDNA3 vector into NIH3T3 cells have not resulted in a distinct phenotype. We are currently testing different vectors, driven by stronger promoters, to see whether the introduction of this specific mutation into cells with the wild-type ARP sequence has any phenotypic effects consistent with the hypothesis that ARP is an oncogene.

It is interesting that the ATG50 → AGG transversion abolishes a methionine residue and gives rise to an uninterrupted string of 10 AGG trinucleotides (codons 43–52) versus 7 seen in the wild-type sequence. The stretch of uninterrupted arginines in ARP would increase from 8 to 11, since codon 42 (CGG) also encodes an arginine. Deletion of the ATG50 codon, seen particularly in some RCCs, would also remove the methionine residue and increase the length of uninterrupted arginines residues to 10 (11). It is unclear whether the loss of the methionine or the lengthening of the stretch of arginines is more important. In either case, these observations provide a possible rationale for the apparent selection of this specific mutation in these tumors.

We detected the ATG50 → AGG mutation in several early stage tumors of the six primary lung tumors that had this specific mutation, five were stage I tumors (Table 2), suggesting that this mutation may be an early event in the development of some cancers. We are currently analyzing premalignant lung lesions and erythroplakias to determine whether this gene has mutated in these premalignant cell types.

Acknowledgments

We thank Hakim Shaid and Sarah Yatooma for expert technical assistance.

References

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