Evidence for Selection Against Human Lung Cancers Bearing p53 Missense Mutations Which Occur within the HLA A*0201 Peptide Consensus Motif

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Abstract

Short peptide fragments of intracellular proteins that fit a defined sequence motif bind to the most common human major histocompatibility complex class I molecule, HLA A*0201, and mediate killing by cytotoxic T-cells [D. F. Hunt et al., Science (Washington DC), 255: 1261-1263, 1992; K. Falk et al., Nature (Lond.), 351: 290-296, 1991]. The existence of such a motif allows prediction of whether novel peptides derived from mutant oncoproteins might be presented on the surface of cancer cells bearing that HLA allele. Clinical cancer might develop only when these mutations occur outside a major histocompatibility complex binding motif or in those cells that acquire defects in antigen presentation. Here, we find that missense mutations of p53 from a variety of tumors fall within the HLA A*0201 motif less often than would be expected if the location of mutations and motifs were independent. When we analyzed the HLA subtype of lung cancer cell lines with known p53 missense mutations, we found that all of the mutant oncoproteins predicted to be presentable by HLA A*0201 came from tumors that either did not carry the A*0201 allele or had lost that allele in the process of tumorigenesis. Presentation of mutant oncoprotein peptides on class I major histocompatibility complex might thus represent a physiologically significant selection pressure in the development of human cancer.

Introduction

p53 is one of the most commonly mutated oncoproteins thus far discovered in human tumors (1). It is unique in that when mutant, it acts as a dominant oncogene and can cooperate with ras to transform primary cells yet when normal it will suppress tumor growth (2). In addition, there appears to be a selective advantage to the expression of mutant protein beyond simple loss of its tumor suppressor function, inasmuch as overexpression of p53 protein (which is highly correlated with the presence of missense mutations) is a strong adverse prognostic factor in breast and lung cancer (3, 4).

Can patients with cancer mount an immune response against mutant oncoprotein products such as p53? Some patients with p53 mutations in their tumors develop antibodies against p53 (5) but little is known about cellular immune responses. Recently we have found that cytotoxic CD8+ T-cells that specifically recognize and kill cells endogenously expressing mutant p53 can be generated in mice (6), indicating that a mutant nuclear oncoprotein such as p53 can be processed and presented by class I MHC. If mutant p53 proteins can be processed and presented on the cell surface, clones of cells with mutant oncoprotein epitopes that are negatively presented could escape immune surveillance and give rise to clinically evident tumors. This could be due to the inability to process and present the appropriate peptide due to the inheritance of particular polymorphic MHC alleles or the somatic loss of key elements in the processing or presentation pathway.

This hypothesis leads to a very specific prediction, that clinically evident tumors containing p53 mutations that fall in a known HLA consensus binding motif should lack that HLA haplotype. Conversely, tumors developing in a person with a given HLA haplotype should only exhibit p53 mutations not contained within the patient’s HLA consensus peptide binding motifs. To test these predictions we scanned the normal p53 protein sequence for the presence of nonamers (7, 8) matching the consensus motif of the most common HLA molecule, A*0201: X(ILM)XXXXXX(VLIA), where X represents any residue. Because 90% of p53 mutations occur in the 205-amino acid “conformational domain” in exons 5-8, this region is of particular interest and it was found to contain 6 HLA-A*0201 nonamer motifs (Fig. 1). Fifty amino acids fell within these motifs. Missense mutations that arise within these regions of p53 may be efficiently presented on HLA A*0201. We hypothesized that: (a) since HLA A*0201 is the most common allele [46% for the United States Caucasian population (9)], there should be a paucity of tumors containing p53 missense mutations falling in the HLA A*0201 nonamer motif; and (b) clinically evident tumors that have HLA A*0201 should not have p53 mutations which fit the HLA A*0201 motif.

Materials and Methods

Cell Lines and DNAs. Human lung cancer cell lines were established from patients seen at the Navy Medical Oncology Branch, National Cancer Institute, Bethesda, MD. Ninety % of these samples were from North American Caulasians. Appropriate informed consent was obtained in all cases.

HLA-A Allele Subtyping. Subtyping was performed by a combination of locus-specific PCR followed by allele-specific Southern hybridization as previously described with minor modifications (9). Briefly, PCR was carried out on a Perkin-Elmer 9600 for 32 cycles of incubation at 94°C for 20 s, 10 s at various annealing temperatures depending on the primer combination used, and 72°C for 20 s. The annealing temperature was 56°C for primer combinations AP31-AP2 and AP11-AP14, 60°C for combinations AP3-AP4 and AP13-AP14, and 66°C for combination AP11-AP12. Eight µl of each PCR product were loaded onto an agarose gel (0.8%) and run for 30 min at 120 V. The DNA was denatured by soaking the gel in 0.4 N NaOH for 45 min and double blotted onto Hybond N+ nylon transfer membranes (Amersham). After blotting, membranes were rinsed in 3x SSC and baked at 80°C for 2 h. Membranes were prehybridized at 42°C in 15 ml of a solution containing 0.75 µM NaCl, 50 µm sodium phosphate buffer (pH 7.7), 6 µm EDTA, 1% sodium dodecyl sulfate, 5% dextran sulfate, 15% deionized formamide, and 0.2 µg/ml of boiled salmon sperm DNA for at least 2 h. The prehybridization solution is then thrown away and the membranes are separated according to which probe they will be labeled with. Then, 7 pmol of 32P-labeled probe in 20 ml of prehybridization solution are added to each of the membranes and hybridization is continued for 45 min more at 42°C. The labeled membranes were then rinsed 3 times with 3x SSC at room temperature and washed with 3 ml tetramethyl-ammonium chloride, 50 µm Trit-HCl (pH 8.0), 2 µm EDTA, and 0.1% sodium dodecyl sulfate for 30 min. The washing temperature was 59.5°C. Membranes were then quickly rinsed in 3x SSC and dried in the oven at 80°C for 10 min.

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Results and Discussion

We analyzed the locations of 299 missense mutations in p53 occurring in all types of tumors of unknown HLA type (1) (Fig. 1). In order to determine whether there is negative in vivo selection against mutations that occur within a peptide binding motif, it is first necessary to estimate the fraction of mutations that would be contained within a nonamer motif if mutations were independent of motifs. Fifty of 205 codons in exons 5–8 of the normal p53 sequence are within a motif. Five of these 50 codons represent critical anchor residues, where mutations would be likely to destroy binding, reducing the target size to 45 amino acids. It is also possible that mutations would actually create new HLA-A*0201 nonamer motifs. If 299 mutations were distributed randomly over the 205-codon region, one would expect 66 of the 299 mutations to fall in 1 of the 6 motifs (45/205 \times 299 = 66), plus any additional mutations that create new motifs. Of the 299 missense mutations from the literature (1), there were only 30 that fell within these 45 codons. In addition, 6 mutations outside the 6 nonamer motifs created new HLA-A*0201 nonamer motifs, not present in the normal sequence giving a total of 36 of 299 missense mutations that could be presented by HLA-A*0201. This is significantly fewer than if mutations and motifs were independent (36 observed compared with 66 predicted, \( P = 0.004 \) by the two-tailed Fisher exact test). The inclusion of motifs created by random substitutions would increase the expected number of matches to greater than 66 and improve the significance of this correlation. Interestingly, codon 12 of K-ras, the most common site of ras mutation in lung cancer (10), also falls outside of A*0201 peptide binding motifs in the K-ras protein.

Because mutations in p53 occur in a nonrandom fashion for reasons unrelated to antigen presentation, the real test of our hypothesis lies in the prediction that individual tumors with p53 missense mutations matching the A*0201 motif would not carry that HLA allele. Thus, we examined 50 lung cancer tumor cell lines (11, 12) that we previously had shown to contain p53 missense mutations (13, 14), and 5 (in lung cancers NCI-H738, –H1373, –H1437, –H1450, and –H1672) had mutations occurring within an A*0201 motif. In addition, one new HLA-A*0201 motif (NLRPILTII) was created (in lung cancer NCI-H322, 248 R to L) by the mutation giving a total of six mutations that should be presentable by the HLA-A*0201 molecule. DNA HLA-A subtypes were determined on these 6 lung cancers, as well as 28 other lung cancer cell lines with p53 mutations falling outside of the motif. This was accomplished by a combination of locus- and allele-specific PCR with allele-specific oligonucleotide hybridization using DNAs of known HLA subtype as controls. An example of this analysis is shown in Fig. 2 and the results are summarized in Table 1. None of the 6 cell lung cancer lines that had p53 mutations in a A*0201 peptide binding motif contained A*0201 genes (or closely related alleles), significantly fewer than would be expected by chance (\( P = 0.02 \), binomial test), suggesting that these tumors contained HLA alleles incapable of presenting their mutant oncopeptide. In contrast, 10 of the 28 lung cancers with p53 mutations outside the A*0201 motif were A*0201, a frequency not significantly different from that expected in the general population.

Normal DNA corresponding to 3 of the tumor samples with mutations that fell within the consensus motif was available (H1450, H1672, and H1437). DNA HLA analysis showed that H1450 was positive for A*0201 in the normal DNA but negative in the tumor, indicating loss of the A*0201 allele in the process of tumorigenesis.

Fig. 1. Histogram depicting 299 missense mutations in exons 5 through 8 of the p53 open reading frame (1) related to the 6 HLA A*0201 consensus peptide binding motifs. Horizontal bars, the location of the consensus peptide binding motifs. The nonamer motifs extend from codons 129 to 137, 187 to 195, 193 to 201, 256 to 264, 264 to 272, and 322 to 330.

Fig. 2. Example of PCR/oligonucleotide hybridization subtyping of tumor cell line DNAs. HLA-A allele subtyping was accomplished using a panel of locus- and allele-specific PCR primers and oligonucleotide probes as described previously (9). Four primer pairs and 15 probes were used to fully type each of the DNA samples and only a single PCR product and probe combination is shown here. The ethidium bromide stained gel of the PCR products from a variety of tumor and normal DNAs using primer pair AP3 and AP4 is shown above an autoradiogram of the Southern blot of this gel which was probed with 32P-labeled oligonucleotide AP2. All of the positive signals detected with this combination were proved to probe combination is shown here. The ethidium bromide stained gel of the PCR products from a variety of tumor and normal DNAs using primer pair AP3 and AP4 is shown above an autoradiogram of the Southern blot of this gel which was probed with 32P-labeled oligonucleotide AP2. All of the positive signals detected with this combination were proved to
Such loss is consistent with immune selection by HLA-A*0201-restricted cytotoxic T-lymphocytes.

Three of the tumor DNAs (H1450, H650, and H2106) showed no PCR product of the predicted size when amplified with primer pair AP3-AP4 and no hybridization with any of the typing probes used. These three DNAs all yielded a product of the expected size when amplified with a pan HLA-A class I exon 2 primer pair, demonstrating PCR product of the predicted size when amplified with primer pair AP3-AP4 and no hybridization with any of the typing probes used.

Table 1 Comparison of the frequency of HLA A*0201 alleles in tumors bearing missense p53 mutations that either lie within or outside the consensus peptide motif [X(ILM)XXXXXX(VLIA)]

<table>
<thead>
<tr>
<th>Fraction with A*0201 allele</th>
<th>p*</th>
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<tr>
<td>Mutation in motif</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Mutation not in motif</td>
<td>10/28 (36)</td>
</tr>
<tr>
<td>General population</td>
<td>46</td>
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*a Calculated using the binomial test. NS, not significant.

b Percentage.

Fig. 3. Model of oncogene peptide presentation leading to tumor rejection, and class I MHC allele loss allowing tumor formation. Three hypothetical situations are represented.

In A, a mutant oncopeptide is effectively presented on a class I MHC molecule of a tumor and eliminated by cytotoxic T-lymphocytes. B and C, mechanisms for tumor escape, B by for which we have no probe. These three DNAs all yielded a product of the expected size when amplified with a pan HLA-A class I exon 2 primer pair, demonstrating PCR product of the predicted size when amplified with primer pair AP3-AP4 and no hybridization with any of the typing probes used.

This analysis is consistent with a model that human mutant oncogene products might undergo selection for those that cannot be presented on the MHC class I molecules expressed by the tumor in vivo (Fig. 3). Tumor escape by specific loss of the class I allele presenting a tumor antigen has been seen in an animal model tumor (15), and these data support the hypothesis that the same phenomenon occurs in the development of human lung cancers. Expression of mutant oncogene peptides on class I MHC could therefore represent a physiologically significant selection pressure in the development of human cancer.

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References

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