Preferential Retention of Codon 72 Arginine p53 in Squamous Cell Carcinomas of the Vulva Occurs in Cancers Positive and Negative for Human Papillomavirus

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Abstract

We have sought to determine the basis for preferential loss of the codon 72 arginine (72R) rather than the arginine (72R) allele in squamous cell carcinoma of the vulva with loss of heterozygosity (LOH) in p53. The proportion of cases containing human papillomavirus (HPV) 16 was not statistically different among individuals with either 72RR or 72RP in the germ line (P > 0.99), but p53 LOH was significantly more common in individuals heterozygous 72RP than in 72RR individuals (P = 0.04). LOH more commonly involved the 72P allele in both HPV-positive and HPV-negative cancers. Our results imply that preferential loss of the 72P allele in vulval squamous cell carcinoma occurs by HPV-dependent and -independent mechanisms.

Introduction

The frequent mutation of p53 in human cancer attests to its critical importance in carcinogenesis (1). p53 exists in two principal polymorphic forms that have either arginine (72R) or proline (72P) at codon 72 (2). This polymorphism is balanced, although the selective pressure maintaining this is not known (3). Representation of each allele within populations varies according to latitude, with a strong correlation between the R allele and increasing latitude (3). Functional differences between the two polymorphic forms of the wild-type protein have been described previously (4). Furthermore, the 72R polymorphic form of wild-type p53 is more sensitive to proteolysis mediated by the E6 protein encoded by oncogenic types of HPV than the wild-type 72P (5). Consistent with this observation, increased representation of individuals homozygous 72RR in the germ line was observed in patients with HPV-related SCC of the cervix (5). Other studies have, however, failed to observe such an effect (6, 7). Although the etiological association between HPV infection and cancer is most well established for cervical SCC, other cancers such as SCC of the vulva, head and neck, and esophagus may also be associated with HPV. We previously observed no significant differences in germ line representation of 72R and 72P between individuals with vulval or head and neck SCC, which suggests that germ-line possession of wild-type 72RR is unlikely to represent a significant risk factor for development of these cancers (8). However, analysis of vulval and head and neck SCC arising in individuals with germ-line 72RP revealed that p53 mutations preferentially target the 72R allele, whereas LOH in p53 more frequently targeted the 72P allele (8), p73, a gene with structural and functional homology to p53, was recently cloned (9), but mutations do not appear to be common genetic events in human cancer (10). However, methylation-dependent transcriptional silencing of p73 in some hematological malignancies suggests that loss of function in p73 may be an important event in oncogenesis, at least in some tissues (11). Some tumor-associated p53 mutants are able to associate with, and inhibit the function of, p73, raising the possibility that this interaction may be important in carcinogenesis in tissues that express p73 (12). Recently, we have shown that the ability of these mutants to form heterodimers with, and thereby inactivate, p73 is strongly influenced by the identity of the amino acid at codon 72 (8). Specific p53 mutations with R at codon 72 efficiently form complexes with p73 and inhibit its DNA binding and proapoptotic activity, but this property is absent or markedly reduced if the same mutants have P at position 72. Moreover, we detected such mutants in SCC of both vulva and head and neck (8). Taken together, these observations lead us to hypothesize that the preferential mutation of the R allele of p53 in vulval and head and neck SCC might reflect, at least in part, the selective interaction of mutant p53 72R but not p53 72P proteins with p73. Apparently nonrandom allele loss in 72RP germ-line heterozygotes has also been reported in esophageal SCC, in which the lost allele in six cancers with LOH was exclusively 72P (13). In this study, the authors suggested that loss of P reflected the preferential retention of the 72R form of wild-type p53, which has been shown to be more sensitive to HPV 6-dependent degradation (5).

In the present study, we have investigated whether the preferential loss of the 72P allele in SCC of the vulva (8) is associated with HPV status, as observed in esophageal cancer (13), or whether other selective pressure(s) might operate to favor loss of the 72P allele and/or mutation of the 72R allele.

Materials and Methods

Tumors. Tissues were obtained at surgical removal of cancers with matched normal tissue and snap-frozen in liquid N2 on harvesting prior to nucleic acid isolation. Diagnosis and presence of tumor within the resected tissue was confirmed by routine histopathological analysis.

Nucleic Acid Isolation and p53 and HPV Analysis. Genomic DNA was isolated from frozen tissues by proteinase K digestion and RNA using RNAzol B according to the manufacturer’s instructions (Biogenesis Ltd). p53 status of the vulval SCC has been described previously (8). HPV DNA sequences were sought using established PCR methodology (14). HPV typing was by sequencing of amplified products.

Results

Loss of 72P Allele Occurs in HPV-Negative and -Positive SCC. We previously reported (8) that the germ-line frequencies of 72RR and 72RP were similar in individuals with SCC of the vulva and that,
in these cancers, LOH in p53 occurred more commonly in the P allele in individuals germ-line 72R/P. To ascertain whether the preferential loss of 72P was associated with HPV status, genomic DNA was analyzed for the presence of HPV DNA sequences. The characteristics of each cancer are shown in Table 1. HPV DNA was detected in 13 of 36 cancers (Table 2A), and sequencing revealed that this was HPV 16 in each case. The proportion of HPV-positive cancers was not significantly different in 72RR and 72RP germ-line individuals (5 of 14 versus 8 of 21; P > 0.99; Table 2B). The frequency of p53 LOH was similar in HPV-positive and HPV-negative vulval SCC (7 of 13 versus 5 of 14; P = 0.04) (Table 2A). In the R/P germ-line heterozygotes with LOH, the lost allele was 72P in 4 of 5 HPV-positive SCC and in 10 of 11 HPV-negative SCC (Table 2C).

p53 Status in Vulval Cancers. Because preferential loss of the 72P allele occurs in both HPV-positive and -negative cancers, we next considered the hypothesis that the apparently nonrandom loss of the 72P allele actually represents selection for mutation in R, with subsequent loss of the wild-type 72P allele. We, therefore, analyzed each cancer for p53 mutation. Missense mutations were identified in 17 of 23 HPV-negative and 4 of 13 HPV-positive cancers. These results are consistent with previous reports of p53 mutation occurring more commonly in HPV-negative vulval SCC (16), and with a high incidence of mutations in clinically aggressive and/or recurrent vulval SCC (17). HPV p53 mutations occurred significantly more commonly in the 72R allele rather than the 72P allele in individuals R/P in the germ line (8).

Table 1 Characteristics of vulval cancers

<table>
<thead>
<tr>
<th>Cancer</th>
<th>HPV</th>
<th>p53 germ line</th>
<th>p53</th>
<th>Mutant allele</th>
<th>LOH</th>
<th>Lost allele</th>
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</table>

* NA, not applicable; M, metastasis; WT, wild-type.

Table 2 p53 and HPV status of vulval cancers

<table>
<thead>
<tr>
<th>Arg/Arg (n = 14)</th>
<th>Arg/Pro (n = 21)</th>
<th>P</th>
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<tr>
<td>HPV-negative RR</td>
<td>3/9 (0.39)</td>
<td>1/3 (0.33)</td>
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<td>HPV-negative RP</td>
<td>11/13 (0.85)</td>
<td>1/11 (0.10)</td>
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<tr>
<td>HPV-positive RR</td>
<td>2/5 (0.50)</td>
<td>1/2 (0.50)</td>
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<tr>
<td>HPV-positive RP</td>
<td>5/8 (0.63)</td>
<td>1/5 (0.20)</td>
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</table>

Discussion

We and others have previously demonstrated that the 72P allele of p53 is preferentially deleted in squamous cell cancers arising in individuals with germ-line 72R/P heterozygosity (8, 13). In the present study, we have sought to clarify which selective pressure underlies the preferential loss of the 72P allele. Kawaguchi et al. (13) reported that allele loss exclusively involved the 72P allele in esophageal cancer and proposed that this was attributable to the relatively increased sensitivity of the 72R wild-type p53 protein to proteolysis mediated by HPV E6 (13). In the present study, we show that preferential loss of the 72P allele occurs in both HPV-positive and HPV-negative vulval SCC. There are several possible explanations as to why this is the case, and these are not mutually incompatible. Firstly, it is possible that cancers that lack detectable HPV DNA are initially HPV positive and that the HPV genome is lost during tumorigenesis. In this scenario, selection would favor the retention of the more easily degraded 72R p53, as proposed for esophageal cancer. Subsequent loss of the HPV genome would then require mutation of the p53 allele to functionally substitute for loss of HPV E6-dependent p53 inactivation, and this would inevitably occur in the remaining (72R) allele. Such a “hit and run” mechanism has been proposed previously in HPV-negative vulval SCC (17) and in cervical SCC in which p53 mutation was convincingly demonstrated after loss of HPV DNA (18). It is also consistent with the observation that p53 mutation in cervical and anal SCC occurs almost exclusively in HPV-negative cases (19, 20). A second plausible explanation is that the apparent nonrandom loss of the 72P allele actually represents selection for mutation in the 72R allele. Such a hypothesis is consistent with functionally significant interactions between some p53 mutants and the p53-related protein p73 (12) and the demonstration that, at least for some p53 proteins, this activity is significantly greater when R is the amino acid at codon 72 (8). On the basis of our previous work (8), mutant p53 proteins that occur in 72R rather than 72P would be predicted to have greater ability to be complexed with and to inactivate p73 α and β. Both isoforms of p73 are expressed in normal vulval epithelium and are often abundantly overexpressed in vulval cancers (data not shown). It is of interest that many of the p53 72R mutants that preferentially inactivate p73 are those with “gain of function” and that four of five metastatic vulval cancers from the present series expressed 72R p53 mutant proteins.

In conclusion, our data suggest the possibility that distinct selective pressures operate in HPV-negative and HPV-positive cancers. In
HPV-positive vulval cancers, loss of 72P may occur as a result of the increased sensitivity of the wild-type 72R p53 protein to degradation that is mediated by HPV E6, as suggested for esophageal carcinomas. In HPV-negative cancers, pressure may be for mutation in the 72R allele (rather than for selective loss of the 72P). The frequent presence of p53 mutations in metastatic vulval cancers reported in this and other studies is consistent with the hypothesis that gain of function, perhaps mediated via interaction with p53 family members, more readily occurs when mutations occur in the 72R allele.

References

Negative for Human Papillomavirus

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