The Role of TP53 in Cervical Carcinogenesis

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For the p53 Special Issue

Functional loss of the tumor suppressor p53 by alterations in its TP53 gene is a frequent event in cancers of different anatomical regions. Cervical cancer is strongly linked to infection by high-risk human papillomavirus (HPV) types. The viral oncoprotein E6 has the ability to associate with and neutralize the function of p53. E6 interacts with a 100-kDa cellular protein, termed E6 associated protein (E6AP; also called ubiquitin-protein ligase E3A or UBE3A), which functions as an ubiquitin protein ligase. The dimeric complex then binds p53 and E6AP catalyzes multi-ubiquitination and degradation of p53. The ability to promote p53 degradation is an exclusive property of E6 from the high-risk HPV types. Indeed, the low-risk E6 proteins lack this activity, although they can bind p53. Consistent with the E6 function of the high-risk HPV types, the majority of cervical cancer cells have a wild-type p53 gene, but the protein levels are strongly decreased. Several independent studies have shown that in a small percentage of cervical tumors the p53 gene is mutated. However, this event appears to be unrelated to the presence or absence of HPV infection and the nature of the tumor. Hum Mutat 21:307–312, 2003.

KEY WORDS: cervical cancer; cancer; tumor; p53; TP53; human papilloma virus; HPV; E6-mediated p53 inactivation; E6AP; UBE3A

DATABASES:
TP53 – OMIM: 191170; GenBank: NM_000546 (mRNA)
http://p53.curie.fr/ (p53 Web Site at Institut Curie)
www.iarc.fr/p53 (IARC p53 Mutation Database)

CERVICAL CANCER DEVELOPMENT IS INTIMATELY LINKED TO HPV INFECTION

Carcinoma of the uterine cervix is one of the most common neoplasias among women worldwide [Pisani et al., 2002]. Clinical, epidemiological, and molecular data have clearly demonstrated that certain human papilloma viruses (HPVs) types, so-called high risk, are the etiological agents of cervical cancer [zur Hausen, 2002]. Indeed, they have been associated with more than 90% of cervical cancers [Walboomers et al., 1999]. A small percentage of tumors appear to be negative for the presence of HPV DNA, but the possibility that these invasive lesions contain an as yet unidentified HPV type cannot be excluded. HPV16 and HPV18 are the high-risk types most frequently found in malignant cervical lesions, covering approximately 75% of cases worldwide [Bosch et al., 1995; Walboomers et al., 1999].

HPV-induced cervical cancer is a multistep process (Fig. 1) [Ostor, 1993]. HPV is frequently detected in women during their active sexual life, however in the majority of cases, the HPV infection does not lead to a clinical manifestation and is cleared by the host immune system in a relatively short time (6–12 months). A small percentage of infections induce the development of low- and/or high-grade cervical intraepithelial neoplasias (CIN), which can still regress or progress to an invasive cervical carcinoma after a long period of latency (Fig. 1). The HPV genome encodes proteins that are able to induce unscheduled proliferation and prevent apoptosis [Tommasino, 2001]. These HPV-induced events facilitate the accumulation of mutations in the host genome, which possibly lead to activation of cellular oncogenes or inactivation of tumor-suppressor genes. The co-operation between viral and cellular oncogene

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products dramatically increases the possibility of transformation of a pre-malignant lesion to an invasive cancer. Thus, persistency of HPV infection represents a high-risk factor for the development of the malignant lesion. Several epidemiological studies have identified additional risk factors that play a role in progression of HPV-induced disease, most likely influencing the immune surveillance or acting as additional carcinogens. These include sexual habits, cigarette smoking, oral contraceptives, parity, and host genetic predisposition [Jones et al., 1990; Magnusson et al., 1999; Moreno et al., 2002; Moreno et al., 1995; Munoz et al., 2002; Schiffman et al., 1987].

THE E6 PROTEIN OF THE HIGH-RISK HPV TYPES INDUCES DEGRADATION OF TP53

Numerous biochemical studies have elucidated the mechanism of action of the high-risk HPV types in cervical carcinogenesis [Tommasino, 2001]. They have unequivocally proved that the products of two early genes, E6 and E7, play a key role in the induction of pre-malignant and malignant cervical lesions [zur Hausen, 2002]. Both viral proteins have the ability to associate and subsequently inactivate several cellular proteins, including products of tumor-suppressor genes. As mentioned before, these HPV-mediated events lead to loss of control of fundamental cellular pathways, such as cell cycle and apoptosis [Tommasino, 2001]. The best characterized E6 activity of the high-risk HPV types (e.g., types 16 and 18) is its ability to induce degradation of the tumor suppressor protein p53 (TP53; MIM# 191170) via the ubiquitin pathway [Thomas et al., 1999b]. HPV16 E6 binds to a 100-kDa cellular protein, termed E6-associated protein (E6AP or UBE3A; MIM# 601623), which functions as a ubiquitin protein ligase. The E6/E6AP complex then binds the central region (also termed the core domain) of p53, which becomes rapidly ubiquitinated and is targeted to proteasomes [Huibregtse et al., 1991; Scheffner et al., 1993; Scheffner et al., 1990] (Fig. 2). Consequently, p53 levels are extremely low in cervical tumor cells. Since p53 plays a crucial role in...
safeguarding the integrity of the genome, cells expressing HPV16 E6 show chromosomal instability, which greatly increases the probability that HPV-infected cells will evolve toward malignancy.

The induction of p53 destabilization is an exclusive feature of E6 proteins from the high-risk HPV types. Indeed, E6 of the low-risk HPVs, which are seldom associated with malignant lesions, are not able to efficiently target the cellular protein [Lechner and Laimins, 1994]. Thus, this E6 in vitro property correlates with the potential oncogenicity of the corresponding HPV type.

In conclusion, inactivation of p53 represents a key step in cervical carcinogenesis, similarly to other human cancers, in which the p53 gene is frequently mutated. A recent study has further corroborated this conclusion providing direct evidence for the active role of E6-mediated p53 degradation in the survival of HPV-positive neoplastic cells [Butz et al., 2000]. Expression of E6-binding peptide aptamers in HPV16-positive cells resulted in abrogation of p53 degradation and induction of apoptosis [Butz et al., 2000]. Thus, therapeutic approaches aiming to inhibit the biological functions of the E6 protein may represent a successful strategy to induce regression of a HPV-positive lesion.

Recently, new members of the p53 family have been identified, e.g., p63 and p73 [Moll et al., 2001]. It is not clear yet whether E6 impacts on these p53-related proteins. Park et al. [2001] have reported that p73 can be functionally inactivated by E6 protein of low- and high-risk HPV types via direct binding, but without inducing its degradation. In contrast, another investigation found no interaction between E6 and p73 [Marin et al., 1998].

**TP53 POLYMORPHISM PRO72ARG REPRESENTS A POSSIBLE RISK FOR CERVICAL CANCER DEVELOPMENT**

A common p53 polymorphism at amino acid 72 has been characterized (Pro72Arg), resulting in either a proline residue, Pro72 (p53pro) or an arginine residue, Arg72 (p53arg). Both forms have wild-type biological activity [Thomas et al., 1999a]. However, it was recently proposed that the two p53 variants at codon 72 might contribute differently to the development of invasive cervical cancer. Storey et al. [1998] showed that the p53arg variant is more efficiently inactivated by the viral oncoprotein E6 of the high risk HPV types than the p53pro variant. In addition, they analyzed cervical specimens for the distribution of the two p53 variants in healthy women and women with cancer [Storey et al., 1998]. Their findings suggest that women with the arg/arg allelotype are at higher risk of HPV-associated cervical cancer than pro/pro or heterozygotes. However, this clinical issue remains a subject of debate. Several studies were unable to confirm the epidemiological data reported by Storey et al. [1998], while other investigations found an enrichment of the Arg72 allele in cervical cancers in comparison to the healthy controls [for instance see references Agorastos et al., 2000; Helland et al., 1998; Hildesheim et al., 1998; Josefsson et al., 1998; Lanham et al., 1998; Makni et al., 2000; Minaguchi et al., 1998; Zehbe et al., 1999, 2001].

The fact that the development of cervical cancer is a multi-step process, in which several host genetic and environmental factors may be involved, could explain the discrepancy of the different studies. It is possible that in the presence of additional risk factors, the contribution of p53 polymorphism becomes irrelevant for cervical cancer development. For instance, it has been reported that intratype HPV natural variations may be involved in determination of the progression or regression of HPV-induced lesions [reviewed in Zehbe and Tommasino, 1999]. Thus a study, which takes into consideration the risk factors identified so far, could clarify the possible involvement of p53 polymorphism in cervical carcinogenesis. In addition, the functional data reported by Storey and colleagues [Storey et al., 1998] show that both p53 variants are targeted by E6 protein, although the p53arg is a better substrate for E6 than the p53pro. It is possible that the different degradation efficiencies of the p53 polymorphic forms marginally impact on cervical carcinogenesis and, therefore, only a weak association between the p53arg allele and cervical cancer could be detected.

**HPV E6 ABROGATES TP53 TRANSCRIPTIONAL ACTIVITY INDEPENDENTLY OF ITS DEGRADATION**

Several lines of evidence indicate that HPV E6 proteins have developed different mechanisms to alter the biological functions of p53 [reviewed in Mavrantani and Banks, 2001]. For instance, it has been shown that E6 from low- and high-risk HPV types have the ability to bind the C-terminal region of p53 [Li and Coffino, 1996]. This interaction occurs in the absence of E6AP and is not required for the E6-induced p53 degradation. The biological significance of E6 binding to the C terminus of p53 is not entirely clear yet. However, evidence indicates that this association may influence the transcriptional activity of p53 [Lechner and Laimins, 1994]. Indeed, it has been shown that E6 proteins from the low-risk HPV types are able to inhibit the transcriptional repression of p53 in vivo and abrogate the p53/DNA interaction [Lechner and Laimins, 1994]. Since these E6 proteins do not promote p53 degradation, but retain the ability to bind its C-terminal region, most likely the E6 activities described above are a consequence of this interaction.
In addition, two independent studies have recently reported that HPV16 E6 also associates with the transcriptional regulators CBP and p300, with resulting inhibition of p53-driven transcription [Patel et al., 1999; Zimmermann et al., 1999].

**TP53 IS SELDOM MUTATED IN CERVICAL CARCINOMAS**

Consistent with the E6 activities, analysis of a limited number of cell lines derived from HPV-positive cervical cancers showed that the p53 gene is wild-type [Crook et al., 1991; Iwasaka et al., 1993; Scheffner et al., 1991; Srivastava et al., 1992; Yaginuma and Westphal, 1991]. In the same studies HPV-negative cervical tumor cells were shown to have a mutated p53, suggesting that p53 can be functionally inactivated in cervical cancer cells either by association with E6 or mutations of the gene. These initial data on cervical cancer cell lines were confirmed by a study in which 28 primary cancers of the cervix were analyzed for the status of HPV infection and p53 mutations [Crook et al., 1992]. Scaling up of the analysis of primary cervical tumors clearly showed that p53 mutations are very rare in cervical cancers. Table 1 shows the most frequent mutations detected [Olivier et al., 2002]. However, when these mutations occur they can be found in both in HPV-positive or -negative cervical tumors, indicating that there is no correlation between HPV and p53 status [Busby-Earle et al., 1994; Denk et al., 2001; Fujita et al., 1992; Helland et al., 1993; Kim et al., 1997; Milde-Langosch et al., 1995; Park et al., 1994]. In addition, p53 mutations are also rarely detected in recurrent cervical cancers, excluding the possibility that alterations in the p53 gene may determine a more aggressive phenotype of the high-risk HPV-induced cancer [Denk et al., 2001].

Two independent studies have shown that p53 mutations in HPV-positive cancers are preferentially associated with intermediate-risk virus infections [Kim et al., 2001; Nakagawa et al., 1999], suggesting that in the presence of these HPV types the p53 mutations represent a more important event for the development of an invasive cancer. This association can be explained by the fact that E6 proteins from intermediate-risk HPV types may have a reduced activity in targeting p53 in comparison to the high-risk HPV E6s [Lechner and Laimins, 1994]. Thus, inactivation of p53 by other means may facilitate the establishment of an invasive cervical lesion.

Most studies to date have analyzed only the central region of p53, which is the most frequently mutated domain in cancers of different anatomical regions and corresponds to the DNA-binding domain [Hollstein et al., 1991]. Therefore, the possibility that p53 is mutated in other regions in primary carcinomas of the uterine cervix cannot be completely excluded. However, a recent study, in which a larger region of p53 was analyzed by a yeast functional assay, argues against this hypothesis [Denk et al., 2001].

**CONCLUSIONS**

Different lines of evidence clearly demonstrate that inactivation of p53 represents a key step in carcinogenesis. In the development of cervical cancer high-risk HPV infection is the leading event and the viral protein E6 has developed different mechanisms to neutralize the biological functions of p53. The most efficient way is to mediate the degradation of this cellular protein via the ubiquitin pathway, which is an exclusive property of E6s from the high-risk HPV types. In addition, E6 is able to alter the transcriptional activity of p53 by distinct mechanisms, either by direct association with the C terminus of p53 or via binding to p300/CBP.

The fact that two p53 variants at codon 72, Pro72 (p53Pro) and Arg72 (p53Arg), are targeted by E6 with different efficiency suggests a possible involvement of p53 polymorphism in cervical carcinogenesis. However, the contradictory results reported by several studies on the distribution of the p53pro and p53arg variants in women with cervical cancer leaves this issue still unsolved. Despite the fact that several studies have shown that a small percentage of cervical tumors contains p53 mutations, they also provide evidence that this event is independent of the HPV status and does not impact on the nature of an invasive lesion.

In conclusion, apart from the cervical cancer cases containing intermediate-risk HPV types, which can be found associated with p53 mutations, inactivation of

<table>
<thead>
<tr>
<th>Codon number</th>
<th>Amino acid change</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td>175</td>
<td>Arg→His</td>
<td>4/94 (4.2)</td>
</tr>
<tr>
<td></td>
<td>Arg→Pro</td>
<td>1/94 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Arg→Cys</td>
<td>1/94 (1.1)</td>
</tr>
<tr>
<td>181</td>
<td>Arg→Cys</td>
<td>3/94 (3.2)</td>
</tr>
<tr>
<td></td>
<td>Arg→His</td>
<td>1/94 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Arg→Leu</td>
<td>1/94 (1.1)</td>
</tr>
<tr>
<td>213</td>
<td>Arg→Ter</td>
<td>2/94 (2.1)</td>
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<tr>
<td></td>
<td>Arg→His</td>
<td>1/94 (1.1)</td>
</tr>
<tr>
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<td>Gly→Val</td>
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<tr>
<td>248</td>
<td>Arg→Gln</td>
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<tr>
<td>273</td>
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<tr>
<td></td>
<td>Arg→Pro</td>
<td>1/94 (1.1)</td>
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The data were extracted from the IARC TP53 mutation database (R7 version, September 2002)[Olivier et al., 2002]. The database comprises 94 nucleotides substitutions, which lead to nonsense, missense or silent mutations. Only nonsense and missense mutations have been included in the table.
p53 in high-risk HPV-positive cancers appears to be exclusively mediated by the E6 protein.

REFERENCES


