p53 FUNCTIONAL STATUS IN HPV-POSITIVE HUMAN PRIMARY CERVICAL CARCINOMA

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Experimental evidence suggests that loss of p53 function is a critical event in cervical carcinogenesis. The mechanisms of p53 inactivation might include acquired gene mutations or interaction with the protein products of the integrated genome of high risk human papillomaviruses (HPV) (1). The high risk HPV types implied in cervical carcinogenesis encode the oncoproteins E6 and E7 which, at least "in vitro", bind to p53 and RB gene products respectively. It has been proposed that formation of p53/E6 and pRB/E7 complexes abrogates the negative cell cycle regulation of p53 and pRB (2,3), thus contributing to carcinogenesis and/or progression of cervical carcinoma. Furthermore, an inverse correlation between oncogenic HPV infection and TP53 mutations has been found in cervical cancer cell lines (4). However, studies conducted in primary cervical tumours produced conflicting results, engendering new controversy about the role of HPV and p53 in cervical carcinogenesis (5).

The role of p53 as a growth suppressor seems to be related to its function as a sequence-specific transcription factor that can induce expression of target genes, such as the recently identified WAF1/CIP1. WAF1/CIP1 gene encodes a 21 kD protein ($p21^{WAF1/CIP1}$) which is a potent inhibitor of G1 cyclin-dependent kinases (CDKs) whose activity propels cells into S phase (6). Therefore, $p21^{WAF1/CIP1}$ appears to act as the key effector of p53 in cell cycle control. Thus $p21^{WAF1/CIP1}$ expression can be considered to be an index of the presence of functional p53 protein. To further test the hypothesis that inactivation of p53 by interaction with HPV E6 oncoprotein plays a critical role in cervical carcinogenesis, we have analysed TP53 structure and expression in a series of 60 primary cervical carcinomas and correlated the findings with HPV status. As an index of p53 function, we have further measured $p21^{WAF1/CIP1}$ expression in the same samples.

Samples were screened for point mutations in the "hotspot" regions of the TP53 gene by PCR-SSCP analysis, and expression of p53 and $p21^{WAF1/CIP1}$ was detected by immunohistochemical analysis. Specific HPV genotypes were detected by PCR amplification.

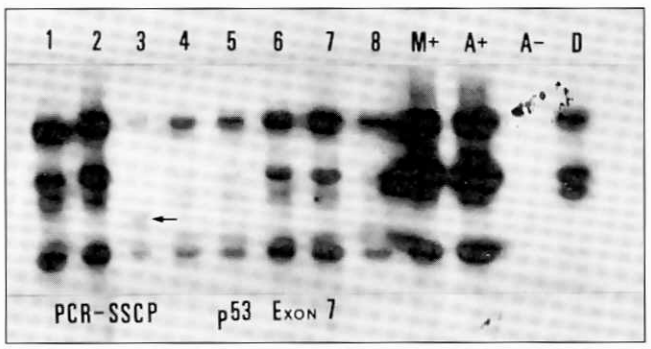

Figure 1. PCR-SSCP analysis of the TP53 gene.

A point mutation in exon 7 in sample 3 is revealed by a mobility shift of the PCR amplified fragment (arrow) in the SSCP electrophoresis.

We have found evidence supporting that p53 retains its function as a transcriptional activator in HPV associated primary cervical tumours. Presence of high risk HPV sequences is not functionally equivalent to the loss of p53 function through somatic mutations of the TP53 gene. Altogether, our data suggest that loss of wild-type p53 function is not a critical event in development of cervical carcinoma.

References

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