p53 codon 72 polymorphism and human papillomavirus associated skin cancer

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Abstract

Background/Aims—Non-melanoma skin cancers frequently harbour multiple human papillomavirus (HPV) types. A recent report suggests that a polymorphism of the p53 tumour suppressor gene that results in the substitution of a proline residue with an arginine residue at position 72 of the p53 protein might act as a risk factor in HPV associated malignancies. This study aimed to determine the following: (1) the relation between HPV infection and the development of cutaneous squamous cell carcinoma (SCC), and (2) whether there is a correlation between p53 codon 72 polymorphism and the development of SCC.

Methods—Blood samples were taken from 55 patients with skin cancer (both renal transplant recipients and immunocompetent patients with skin cancer) and 115 ethnically matched volunteers. A polymerase chain reaction based assay was used to determine p53 codon 72 genotypes. In addition, 49 benign and malignant lesions from 34 of the patients with skin cancer and 20 normal human skin samples from 20 of the control volunteers were examined for HPV.

Results—The proportions of p53 codon 72 genotypes found were 78% arginine homozygous, 2% proline homozygous, and 20% heterozygous among patients with skin cancer and 79% arginine homozygous, 3.5% proline homozygous, and 17.5% heterozygous among the control population. Statistical analysis showed no significant differences in the distribution of the two p53 isoforms between the patients with skin cancer and the control population. The predominant viral types detected in both the patients and the control group were EV associated HPVs, although the incidence was lower in normal skin samples than in malignant lesions or viral warts.

Conclusions—These results suggest that in a Celtic population there is no correlation between the presence of HPV, the p53 codon 72 arginine polymorphism, and the development of skin cancer.

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Keywords: p53 codon 72 polymorphism; human papillomavirus; skin cancer

Non-melanoma skin cancers (NMSCs) are the most frequent cancers in white populations. NMSCs often harbour multiple human papillomaviruses (HPVs), although a causal role for the virus in cutaneous tumorigenesis is yet to be confirmed. A recent report[1] suggests that a common polymorphism at codon 72 of the p53 tumour suppressor gene[2] might be a risk factor in the development of HPV associated cancers. The replacement of a proline residue with an arginine residue at position 72 of the p53 gene product marginally increased the susceptibility of the protein to degradation by the E6 oncoprotein of high risk HPV types 16 (HPV-16) and 18. The presence of an arginine residue was also found to increase greatly the susceptibility of p53 to degradation by the E6 protein of low risk HPV type 11.

Subsequent reports[3 4] demonstrated that individuals who were homozygous for the arginine variant of the protein were at no greater risk of developing cervical cancer. However, one report suggests that in Swedish and Italian women with HPV-16 positive cervical disease, the incidence of cancer was higher in those patients homozygous for the arginine isoform.[5]

Because cutaneous malignancies often harbour multiple low risk HPV types,[6–8] and the arginine isoform of p53 is susceptible to low risk HPV E6 mediated degradation, individuals with the arginine variant of p53 might have an increased risk of developing skin cancer.

Here, we examine the possible role of this polymorphism in the development of skin cancer. The distribution of p53 codon 72 alleles was determined in 55 patients with skin cancer (both renal transplant recipients and immunocompetent patients with skin cancer) and in 115 ethnically matched control volunteers.

The HPV status of 34 of the patients with skin cancer and 20 of the control volunteers was also examined to determine the prevailing viral types in each group.

Methods

PATIENTS AND VOLUNTEERS

Peripheral blood for the p53 codon 72 polymorphism analysis was taken from 55 patients with skin cancer attending the dermatology clinic at Beaumont Hospital and from 115 matched ethnic Irish control volunteers. Forty nine benign and malignant skin lesions comprising viral warts (19), squamous cell carcinomas (SCCs; 21), and normal skin (nine) from 34 of the 55 patients with skin cancer were examined to determine the HPV types present. Twenty normal skin samples from 20 of the control volunteers were also examined for HPV. We obtained ethics committee approval for our study.
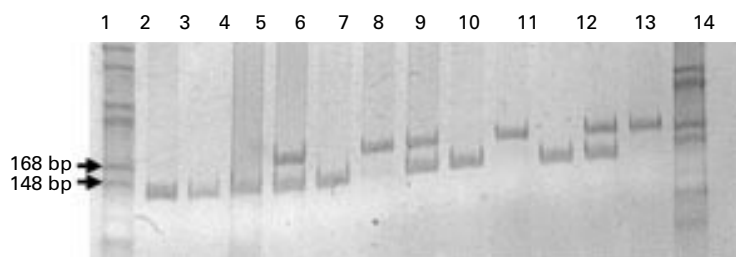
Figure 1   Sample 12% (wt/vol) acrylamide gel depicting the three p53 codon 72 genotypes detected in the patients with skin cancer and the control population. Lanes 1 and 14 contain molecular weight markers (HaeII digested pBluescript). Lanes 2, 3, 6, 9, and 11 show the single 144 bp band amplified from individuals who are arginine homozygotes for the codon 72 polymorphism. Lanes 7, 10, and 13 show the single 171 bp band amplified from the p53 gene of individuals who are proline homozygotes for the codon 72 polymorphism. Lanes 5, 8, and 12 show both the 144 bp arginine band and the 171 bp proline band amplified from the p53 gene of heterozygous individuals. The gel was stained with ethidium bromide and visualised under UV light.
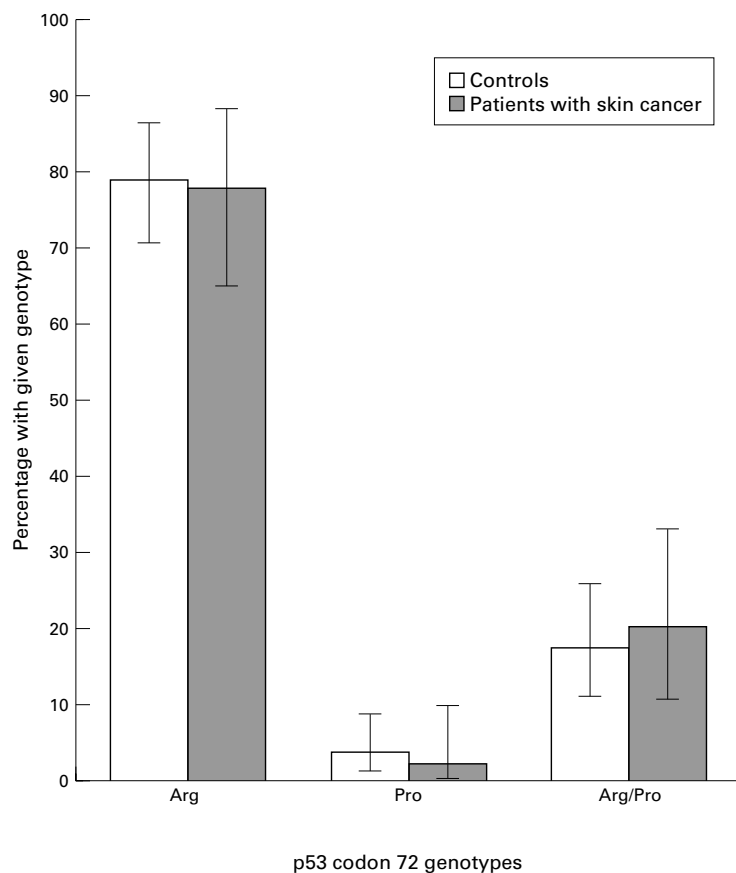

Figure 2   Bar chart depicting the distribution of p53 codon 72 genotypes in patients with skin cancer and in controls. Error bars represent exact binomial 95% confidence intervals. Arg, arginine homozygotes; Pro, proline homozygotes; Arg/Pro, heterozygotes.

PCR ANALYSIS OF p53 CODON 72 POLYMORPHISM

The amplification of p53 proline and arginine sequences was performed as previously described by Storey et al.[1] The products of each of the two polymerase chain reaction (PCR) amplifications were combined and analysed by 12% (wt/vol) polyacrylamide gel electrophoresis (sample gel shown in fig 1).

STATISTICAL ANALYSIS

Chi squared analysis was used to examine the differences in proportions of the p53 codon 72 genotypes between the patients with skin cancer and the control group. The odds ratio

(OR) for having the arginine variant and the development of skin cancer was also calculated. Exact binomial 95% confidence intervals (CI) were calculated for the proportions of the genotypes in each group.

HPV TYPING

DNA was extracted from viral warts, SCCs, and normal skin by conventional methods. Two PCR strategies were used for the detection of HPV: a degenerate PCR using MY09 and MY11 degenerate primers, as described previously by Manos et al,[9] and an EV specific nested PCR, using CP65–70-EV specific HPV primers, as described previously by Berkhout et al.[10] Using both the degenerate and EV specific primer sets for the detection of HPV has recently been shown to be much more effective than using either in isolation.[6] All PCR protocols contained positive, negative, and contamination controls, and were performed in a DNA Engine™ thermal cycler (MJ Research, Massachusetts, USA), initially using Taq polymerase (Promega, Madison, Wisconsin, USA), followed by reamplification with Pfu polymerase (Stratagene, Cambridge, UK). All Pfu generated PCR products were purified by TAE agarose (2% wt/vol) gel electrophoresis and the amplified DNA recovered with the Wizard™ PCR prep kit (Promega), according to the manufacturer's instructions. All purified fragments were then cloned into pCR Script AMP SK(+) (Stratagene) and cycle sequenced using CY5 labelled M13 universal primer and the Thermosequenase fluorescent labelled primer cycle sequencing kit (Amersham, Little Chalfont, Buckinghamshire, UK). Cycle sequenced products were then analysed on an ALF express genetic analyser (Pharmacia, Uppsalla, Sweden). All sequences generated were aligned with known HPV sequences in the Genome Sequence Database using the Basic Local Alignment Search Tool (BLAST).[11]

Results

ANALYSIS OF THE DISTRIBUTION OF p53 CODON 72 GENOTYPES AMONG PATIENTS AND CONTROLS

The proportions of p53 codon 72 genotypes found were 78% arginine homozygous, 2% proline homozygous, and 20% heterozygous among patients with skin cancer and 79% arginine homozygous, 3.5% proline homozygous, and 17.5% heterozygous among the control population (fig 2). Statistical analysis showed that there were no significant differences in the distribution of the various alleles between the control group and the skin cancer group (χ^2 = 0.49; degrees of freedom (df) = 2; p = 0.78). The OR for the association of the arginine variant with the development of skin cancer was calculated at 1.06 (95% CI, 0.49 to 2.3).

HPV TYPING

EV associated HPV types predominate in SCCs (both from renal transplant recipients and immunocompetent patients with skin cancer) and both EV associated HPV types and common cutaneous HPV types are detected in viral warts, again from both skin cancer groups

Table 1 Human papillomavirus (HPV) types detected in renal transplant recipients (RTRs) and skin lesions from immunocompetent patients (ICPs)

Patient	Immune status	Lesion	HPV typing using MY09/MY11 degenerate primers	HPV typing using CP65–70 EV specific primers
1	RTR	Wart	HPV-2a/HPV-57	HPV-23 rel
		NHS	-ve	-ve
		SCC	-ve	HPV-15
		SCC	-ve	HPV-36/RTRX1
		SCC	HPV-17/HPV-38-rel	HPV-36
		SCC	HPV-22	RTRX1/HPV-38 rel
2	RTR	Wart	HPV-57	HPV-38 rel
		Wart	HPV-10	HPVx14b
		NHS	-ve	-ve
3	RTR	Wart	HPV-10	RTRX5/RTRX6
4	RTR	Wart	HPV-17	HPV-23-rel
		NHS	-ve	-ve
5	RTR	Wart	HPV-10	HPV-2a/HPV-23-rel/RTRX10
6	RTR	Wart	ND	HPV-23 rel
		Wart	ND	RTRX10
7	RTR	Wart	-ve	RTRTX10 rel
8	RTR	Wart	HPV-57	Unknown HPV type
9	RTR	Wart	-ve	HPV-23 rel
10	RTR	SCC	-ve	HPV-38 rel
11	RTR	SCC	-ve	HPV-38 rel
12	RTR	SCC	-ve	-ve
13	RTR	SCC	-ve	HPVx20
14	RTR	SCC	-ve	HPV-15
15	RTR	NHS	-ve	-ve
16	RTR	NHS	-ve	RTRX9/HPV-37/ HPV-15 rel
17	ICP	Wart	-ve	HPV-2a/HPV-10 rel
18	ICP	Wart	-ve	HPV-20
		Wart	-ve	HPV-5-rel/HPV-15 rel
19	ICP	Wart	-ve	HPV-23 rel
		Wart	-ve	HPV-23 rel
		Wart	-ve	HPV-20/HPV-38 rel
20	ICP	Wart	-ve	HPVx2b
21	ICP	Wart	-ve	HPV-23 rel
22	ICP	SCC	-ve	HPV-20
23	ICP	NHS	-ve	HPV-24/HPV-17
24	ICP	SCC	-ve	-ve
		NHS	-ve	-ve
25	ICP	SCC	-ve	HPV-20
26	ICP	SCC	-ve	HPV-21
27	ICP	SCC	-ve	-ve
		NHS	-ve	HPV-7
28	ICP	SCC	-ve	HPVx2b
		NHS	-ve	-ve
29	ICP	SCC	-ve	Unknown HPV type
30	ICP	SCC	-ve	RTRX1
31	ICP	SCC	-ve	Unknown HPV type
32	ICP	SCC	-ve	HPV-23 rel
33	ICP	SCC	-ve	HPV-23 rel
34	ICP	SCC	-ve	Unknown HPV type

NHS, normal human skin; SCC, squamous cell carcinoma; ND, not done.

(table 1). Normal skin from the patients with skin cancer and the control volunteers was also found to harbour EV associated HPV types; however, the frequency of detection was much lower in the control volunteers than in patients with SCCs or viral warts (table 2). Table 3 summarises our findings.

Table 2 Human papillomavirus (HPV) types detected in the general population

Volunteer	HPV typing using MY09/MY11 degenerate PCR primers	HPV typing using CP65–70 "EV specific HPV primers"
1	-ve	-ve
2	-ve	-ve
3	-ve	-ve
4	-ve	-ve
5	-ve	-ve
6	-ve	-ve
7	-ve	-ve
8	-ve	HPV-38 rel/unknown HPV
9	-ve	HPV-23
10	-ve	-ve
11	-ve	HPV
12	-ve	-ve
13	-ve	-ve
14	-ve	-ve
15	-ve	-ve
16	-ve	-ve
17	-ve	-ve
18	-ve	-ve
19	-ve	-ve
20	-ve	-ve

Discussion

The p53 codon 72 polymorphism has been implicated as a risk factor in several human cancers.[12–14] individuals who are proline homozygotes have been shown to have a greater risk of developing lung cancer.[15 16] A recent study[1] suggests that the arginine isoform of p53 facilitates degradation by the E6 protein of low risk HPV-11. Because cutaneous carcinomas frequently harbour multiple low risk HPV types and not high risk HPV-16/18 it would seem that the presence of an arginine instead of a proline could be an important risk factor in the development of these tumours. All of the patients with skin cancer analysed in the study by Storey *et al* were either arginine homozygotes (75%) or heterozygotes (25%),[1] implying that the arginine isoform of p53 confers a susceptibility to tumorigenesis, although only a small number of patients were examined. A second study conducted on 29 immunocompetent patients who had developed skin cancer also suggests that possession of the arginine isoform of p53 is associated with an increased risk of developing HPV associated skin cancer.[17]

Table 3   Human papillomavirus (HPV) types detected in patients with skin cancer and in the control population

Disease status	HPV negative	HPV positive	HPV types detected
Controls (n = 20)	17/20	3/20	EV associated HPV types
Normal tissue from cases (n = 9)	7/9	2/9	EV associated HPV types
Warts (n = 19)	0/19	19/19	EV associated HPV types, common cutaneous HPV types
SCCs (n = 21)	3/21	18/21	EV associated HPV types

SCC, squamous cell carcinoma.

Our results do not confirm this hypothesis. We have shown that, at least in a Celtic population, there is no apparent link between the presence of HPV, the p53 codon 72 genotype, and the development of cutaneous carcinomas in both renal transplant recipients and in immunocompetent patients with skin cancer. This conclusion is supported by a recent study that also found no apparent link between a susceptibility to skin cancer and the arginine variant of p53 after renal transplantation.[18] It is possible, however, that in populations less prone to skin cancer, where the arginine isoform of p53 is less prevalent,[19] that it could still confer increased susceptibility to malignancy because both our study and that of Marshall and colleagues[18] were conducted on northern European populations. However, it has been shown that in some populations where the proline isoform predominates, individuals homozygous for the arginine isoform are at no greater risk of developing cervical carcinoma.[20–22] Whether or not this remains the case for cutaneous malignancies is yet to be established.

The HPV types demonstrated in the patients with skin cancer were also found in the control group, albeit at a much lower frequency. However, EV associated HPV types have been detected in the hair follicles of 46% of healthy volunteers,[23] so that a reservoir of EV HPV might exist in a latent or subclinical state in the population as a whole to a greater degree than that found in our study. Recently, intratype variation in the E6 gene of HPV-16 has been suggested as a possible additional factor in determining the susceptibility to cervical cancer associated with the p53 arginine isoform.[24] HPV types with a G instead of a T at position 350 of the E6 gene were found to be significantly over-represented in arginine homozygous women with cervical cancer.

We conclude that in a Celtic population there is no correlation between the presence of HPV and the development of skin cancer and that p53 codon 72 polymorphism is not associated with cutaneous malignancies.

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