

## REVIEW

## HPV infections and oesophageal cancer

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The first reports suggesting an involvement of human papillomavirus (HPV) in the development of both benign and malignant squamous cell tumours of the oesophagus date back to 1982. Since then, a substantial amount of literature has accumulated on this subject, summarised in this review. To date, 239 oesophageal squamous cell papillomas have been analysed in 29 separate studies using different HPV detection methods, with HPV being detected in 51 (21.3%) cases. Many more squamous cell carcinomas have been analysed: of the 1485 squamous cell carcinomas analysed by *in situ* hybridisation, 22.9% were positive for HPV DNA, as were 15.2% of the 2020 cases tested by the polymerase chain reaction. In addition, evidence derived from large scale serological studies, animal experiments, and *in vitro* studies is discussed in the light of the highly variable geographical incidence rates of oesophageal carcinoma worldwide. It may be that the (multifactorial) aetiology of oesophageal cancer differs greatly between those geographical areas with a low risk and those with a high risk for this disease. Oncogenic HPV types seem to play an important causal role, particularly in high risk areas.

Following these pioneering observations, HPV research has resulted in a rapidly expanding literature on both benign and malignant oesophageal lesions in different geographical regions.<sup>9–13</sup> The evidence accumulated during the past 20 years is strongly suggestive of a causal role for HPV in oesophageal carcinogenesis and will be reviewed here.<sup>10–17</sup> This discussion is strictly limited to reviewing the data on HPV only, and does not cover the other potential aetiological agents or the intriguing global epidemiology of this disease.<sup>9–10</sup> In addition, because HPV has only been implicated in the aetiology of squamous cell carcinoma (SCC) of the oesophagus, the discussion will be restricted to this malignancy only.

**SCC OF THE OESOPHAGUS**

One of the most intriguing features of oesophageal SCC is the wide variation in the disease incidence in different geographical regions of the world.<sup>18</sup> In most countries, the incidence rates are around 2.5 to 5.0/100 000 for men and 1.5 to 2.5 for women. However, in distinct areas the incidence rates are remarkably higher, varying up to 500 fold from one area to another.<sup>9–10 18 19</sup> Numerous epidemiological studies have identified the high risk countries for oesophageal cancer, namely: the People's Republic of China, Singapore, Iran, former USSR, Puerto Rico, Chile, Brazil, Switzerland, France, and South Africa. Among these high risk countries, the highest incidence rates have been reported in the northern parts of China, the Caspian littoral of Iran, and the Transkei area of South Africa, reaching up to 246/100 000.<sup>9–10 18 19</sup>

**RISK FACTORS**

The reasons for these major regional variations in the incidence of this disease are poorly understood.<sup>9–10 19 20</sup> Compelling epidemiological and experimental data suggest that some chemicals (such as nitrosamines, mycotoxins, cigarette smoke, excessive alcohol intake, opium abuse), nutritional deficiencies (for example, deficiencies of vitamins A, B, C, and trace elements), and physical factors (such as coarse and hot food) are associated with the development of this malignancy.<sup>9–10 19 20</sup> An aetiological role of certain microorganisms has been implicated (direct

The association of human papillomavirus (HPV) infections and squamous cell precancer lesions of the uterine cervix has been established since the late 1970s.<sup>1</sup> Oncogenic HPV types are regarded as the most important aetiological factor of cervical squamous cell carcinoma.<sup>2–4</sup> The early 1980s witnessed the rapid expansion of HPV research from the genital tract to cover the other squamous cell epithelia, thus widening the scope of HPV associated human tumours.<sup>1–2</sup> The squamous cell lining of the oral mucosa is in direct continuity with the oesophagus, and the first descriptions on HPV lesions in the oral mucosa<sup>5</sup> were slightly preceded by reports suggesting that this virus might be involved in the development of both benign<sup>6</sup> and malignant<sup>7</sup> squamous cell lesions of the oesophagus also. These early observations were based on the discovery of morphological similarities between HPV induced lesions in the genital tract (condyloma) and squamous cell tumours (papillomas and carcinomas) of the oesophageal mucosa.<sup>6–8</sup> These findings were soon substantiated by the demonstration of HPV structural proteins in these lesions using immunohistochemistry (IHC).<sup>8</sup>

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**Abbreviations:** BPV 4, bovine papillomavirus 4; CI, confidence interval; DBH, dot blot hybridisation; HPV, human papillomavirus; IHC, immunohistochemistry; ISH, *in situ* hybridisation; OR, odds ratio; PCR, polymerase chain reaction; SBH, Southern blot hybridisation; SCC, squamous cell carcinoma; SCP, squamous cell papilloma; VLP, virus-like particle

carcinogens or promoters).<sup>9 10 21 22</sup> Fungal contamination of foodstuffs may produce nitrosamines and/or their precursors, in addition to mycotoxins, which are mutagenic and carcinogenic both *in vitro* and *in vivo*. Bacteria may be implicated by producing carcinogenic chemicals and increasing cell proliferation while stimulating the inflammatory process. In addition to HPV, herpes simplex virus, cytomegalovirus, and Epstein-Barr virus have been shown to infect the oesophageal epithelium, but so far no firm evidence for a role in oesophageal carcinogenesis has been provided.<sup>1 2 9 10 14-16 19 20 23</sup>

### EVIDENCE FOR HPV INVOLVEMENT

I was the first to suggest the association of HPV with both benign and malignant squamous cell lesions of the oesophagus,<sup>6 7</sup> opening up a new area of HPV research. The evidence for the involvement of HPV in oesophageal carcinogenesis has been provided by several distinct lines of research, namely: (1) the involvement of HPV in benign squamous cell tumours (papillomas); (2) evidence from animal studies (malignant transformation of oesophageal papillomavirus lesions in cattle); (3) the detection of HPV in oesophageal cancer and its precursor lesions by morphological IHC and DNA methods; (4) seroepidemiological evidence (HPV antibodies in patients with cancer); and (5) *in vitro* studies (transformation of oesophageal epithelial cells by oncogenic HPV types). This evidence has been discussed in a recent textbook,<sup>2</sup> and this review will provide an update to this discussion.

#### Evidence for HPV involvement in benign oesophageal papillomas

One line of evidence for the causal role of a suspected aetiological agent in any malignancy is the involvement of the same agent in benign lesions: squamous cell papilloma (SCP) in this case. Following my first report,<sup>6</sup> a large series of studies have reported the presence of HPV DNA in benign SCPs of the oesophagus.<sup>6 24-31</sup>

To date, 29 studies have been published, with a total of 239 cases having been analysed by different HPV detection methods, including morphology, IHC, dot blot hybridisation (DBH), *in situ* hybridisation (ISH), Southern blot hybridisation (SBH), and the polymerase chain reaction (PCR). In total, HPV has been detected in 51 cases (21.3%). The role of HPV in benign oesophageal SCPs still remains contradictory. There is a tendency towards higher detection rates when using PCR, although even then a wide variation (from 0% to 100%) in the figures is evident. However, this is not unusual when looking at the published data on HPV detection rates at other mucosal sites.<sup>2</sup> Because of the rarity of oesophageal SCPs, the number of published cases is still too small to draw definite conclusions on the role of HPV in the aetiology of oesophageal papillomas. As with some other mucosal sites, it may be that benign lesions with different aetiologies and pathogenesis exist in the oesophagus.<sup>2 14 15 23</sup>

#### Evidence from animal studies

Substantial evidence for the involvement of papillomavirus in oesophageal carcinogenesis has been obtained from studies on cattle, particularly in the Scottish Highlands, which is a high incidence area for upper alimentary tract papillomas and carcinomas.<sup>52-56</sup> Persistent and widespread papillomatosis and carcinomas can be experimentally reproduced with bovine papillomavirus 4 (BPV 4) infection in these animals.<sup>55 56</sup> Field studies in this region have revealed that up to 96% of the cancer bearing animals have concomitant papillomas, and 40% showed more than 15 papillomas in the alimentary tract. In many instances, the progression from benign papillomas to carcinomas could be clearly identified.<sup>52 56</sup>

The ingestion of bracken fern is the crucial factor in the malignant conversion of these papillomas. Bracken fern

contains carcinogenic agents (radiomimetics) and immunosuppressants (such as azathioprine).<sup>55</sup> High copy numbers of BPV 4 DNA sequences are regularly detected in both naturally occurring or experimentally induced papillomas. However, no viral DNA or viral antigens are present in naturally occurring or experimental cancers, indicating that the viral genomes are not required for the maintenance of the malignant state.<sup>52-59</sup>

Thus, these animal experiments suggest that: (1) BPV 4 may execute one of the early events in cell transformation, and its genetic information may not be required for malignant progression; (2) immunosuppression caused by the ingestion of bracken fern allows the spread and the persistence of BPV induced papillomas; and (3) the bracken fern supplies cocarcinogens and carcinogens, leading to cell transformation and progression to malignancy.

#### Morphological similarities to established HPV lesions

In 1982, I examined a series of 60 oesophageal SCCs, and found epithelial changes fulfilling the criteria of both exophytic, inverted, and flat HPV lesions in 24 of 60 patients.<sup>7</sup> For the first time, the possibility was raised that HPV could be the agent responsible for the development of oesophageal SCC. This preliminary report was soon confirmed by other studies (table 1).<sup>60-64</sup>

#### Expression of HPV structural proteins

In 1986 Hille *et al* were the first to use IHC for the demonstration of HPV antigens in oesophageal SCCs, and they found antigen expression in seven of their 70 patients.<sup>60</sup> IHC was soon replaced by different DNA hybridisation methods, and the number of purely IHC studies remained limited (table 1).<sup>29 60 65-67</sup> In total, 182 cases were analysed by IHC and HPV antigen expression was shown in 23 cases (12.6%).

#### DETECTION OF HPV DNA IN OESOPHAGEAL CARCINOMAS

Table 1 summarises the detection of HPV in oesophageal carcinomas using different hybridisation techniques and PCR.

#### Filter *in situ* hybridisation

During the late 1980s filter *in situ* hybridisation (then abbreviated as FISH) was widely used to detect HPV DNA in various mucosal samples, particularly those of the genital tract. This technique was shown to have a low sensitivity and poor specificity, making it obsolete in the early 1990s. In total, this technique was used to analyse 129 carcinomas, and showed HPV DNA in 67 cases (51.9%), which is considerably higher than that shown using any other detection technique (as a result of the high false positive rate).<sup>2 68-70</sup>

#### DBH and SBH

There are only two studies using the DBH technique.<sup>71 72</sup> Divergent results were reported; no HPV DNA was detected in the 37 cases analysed from Hong Kong,<sup>71</sup> whereas 42% of cases were positive in a series collected from France.<sup>72</sup> This last figure is consistent with the results from Chinese patients examined by means of SBH (table 1).<sup>73-76</sup>

#### *In situ* hybridisation

Until now, 13 studies have been published on the detection of HPV in oesophageal carcinomas using ISH.<sup>11 46 63 72 77-84</sup> The numbers of patients in these studies have been relatively small, except for the study reported by Chang *et al*, which comprised 363 cases,<sup>78</sup> and another by the same authors, which comprised a total of 700 patients from the high incidence area of China (table 1).<sup>11</sup> In this last study, 117 of the 700 SCCs (16.9%) were HPV DNA positive, with HPV types 6, 11, 16, 18, and 30 accounting for 39.8% of the positivity. The involvement of other (possibly novel?) HPV types in a considerable proportion of the remaining positive lesions has been

**Table 1** Detection of human papillomavirus (HPV) in oesophageal squamous cell carcinomas

Detection method	Area or country	HPV types detected	HPV positive		Authors	Ref
			N	%		
HB	Finland	–	24/60	40	Syrjänen 1982	7
HB	S Africa	–	23/70	33	Hille <i>et al</i> 1986	60
HB	S Africa	–	13/20	65	Hale <i>et al</i> 1989	61
HB	Venezuela	–	2/2	100	Matos <i>et al</i> 1990	62
HB	China	–	25/51	49	Chang <i>et al</i> 1990	63
IHC	S Africa	Ag	7/70	10	Hille <i>et al</i> 1986	60
IHC	Japan	Ag	2/15	13	Mori <i>et al</i> 1989	65
IHC	China	Ag	7/31	23	Mori <i>et al</i> 1989	65
IHC	Japan	Ag	5/61	8	Nakamura <i>et al</i> 1995	66
IHC, EM	Belgium	Ag, virions	1/1	100	van Cutsem <i>et al</i> 1991	29
IHC	Japan	Ag	0/4	0	Kuwano <i>et al</i> 2001	67
FISH	Australia	11, 13, 16, 18	5/10	50	Kulski <i>et al</i> 1986	68
FISH	China	11, 16, 18	3/80	66	Chang <i>et al</i> 1990	69
HISTOFISH	Australia	6, 11, 16, 18	9/39	23	Kulski <i>et al</i> 1990	70
Slot blot	Hong Kong	6, 11, 16, 18	0/37	0	Loke <i>et al</i> 1990	71
Dot blot	France	6/11, 16/18	5/12	42	Benamouzig <i>et al</i> 1992	72
SB	China	16	12/24	50	Li <i>et al</i> 1991	73
SB	China	11, 16, 18, 30	8/20	40	Chang <i>et al</i> 1992	74
SB, PCR	China	16, 18	0/35	0	Lu <i>et al</i> 1995	75
SB, PCR	China	16, 18	37/103	36	He <i>et al</i> 1996	76
ISH	China	6, 11, 16, 18	22/51	43	Chang <i>et al</i> 1990	63
ISH	France	6, 11, 16, 18, 31, 33	1/12	8	Benamouzig <i>et al</i> 1992	72
ISH	UK	6, 11, 16, 18, 31, 33	0/4	0	Ashworth <i>et al</i> 1993	77
ISH	China	6, 11, 16, 18, 30...	85/363	23	Chang <i>et al</i> 1993	78
ISH	S Africa	6, 7, 16, 18, 30	3/10	30	van Rensburg <i>et al</i> 1993	79
ISH	Japan	6, 11, 16, 18, 31, 33	24/71	34	Furhata <i>et al</i> 1993	80
ISH	Japan	16, 18	13/42	31	Ono <i>et al</i> 1994	81
ISH	S Africa	6, 11, 18, 31, 33	25/48	52	Cooper 1995	82
ISH	Korea	Wide spectrum	11/25	44	Woo <i>et al</i> 1996	46
ISH	China	Wide spectrum	3/36	8	Chang <i>et al</i> 1997	83
ISH	Japan	6, 11, 16, 18	37/123	30	Takahashi <i>et al</i> 1998	84
ISH	China	6, 11, 16, 18, 30, 53	117/700	17	Chang <i>et al</i> 2000	11
PCR	USA	16/18	0/13	0	Kiyabu <i>et al</i> 1989	85
PCR	S Africa	Various	6/14	43	Williamson <i>et al</i> 1991	86
PCR	Korea	16, 18	16/24	67	Kim <i>et al</i> 1991	87
PCR	Japan	16, 18, CP	3/45	7	Toh <i>et al</i> 1992	88
PCR	China	6, 11, 16, 18	25/51	49	Chang <i>et al</i> 1992	74
PCR	Slovenia	CP	2/20	10	Poljak and Cerar 1993	89
PCR	China	GP	24/40	60	Chen <i>et al</i> 1994	90
PCR	Sweden	GP	0/10	0	Lewensohn-Fuchs <i>et al</i> 1994	91
PCR	Different	CP, 16, 18	10/72	14	Togawa <i>et al</i> 1994	92
PCR	S Africa	E6, GP	6/9	67	Cooper <i>et al</i> 1995	93
PCR	France	6, 11, 16, 18, 31, 33	0/75	0	Benamouzig <i>et al</i> 1995	94
PCR	Japan	CP	0/31	0	Akutsu <i>et al</i> 1995	95
PCR	Japan	CP	3/45	7	Sugimachi <i>et al</i> 1995	96
PCR	Japan	CP	15/72	21	Shibagaki <i>et al</i> 1995	97
PCR	Holland	CP	0/61	0	Smits <i>et al</i> 1995	98
PCR	Portugal	16, 18	9/16	56	Fidalgo <i>et al</i> 1995	99
PCR	China	6, 16, 18	3/70	4	Suzuk <i>et al</i> 1996	100
PCR	USA	6, 16, 18	1/23	4	Suzuk <i>et al</i> 1996	100
PCR	USA	73	1/1	100	West <i>et al</i> 1996	101
PCR	France	–	0/75	0	Benamouzig <i>et al</i> 1996	23
PCR	China	16, 18	32/152	21	He <i>et al</i> 1997	102
PCR	Holland	CP	0/63	0	Kok <i>et al</i> 1997	103
PCR	Hong Kong	CP, SSCP	6/75	9	Lam <i>et al</i> 1997	104
PCR	Alaska	CP	10/22	45	Miller <i>et al</i> 1997	105
PCR	Japan	18	3/41	7	Mizobuchi <i>et al</i> 1997	106
PCR	UK	–	0/22	0	Morgan <i>et al</i> 1997	107
PCR	USA	–	0/11	0	Paz <i>et al</i> 1997	108
PCR	Italy	–	0/18	0	Rugge <i>et al</i> 1997	109
PCR	USA	CP, RFLP, 16	1/51	2	Turner <i>et al</i> 1997	110
PCR	Japan	CP, 16, 18	0/103	0	Saegusa <i>et al</i> 1997	111
PCR	Slovenia	CP, 6, 16, 18	0/121	0	Poljak <i>et al</i> 1998	15
PCR	Japan	CP, 16, 18	17/27	63	Khurshid <i>et al</i> 1998	112
PCR	Japan	CP, 16, 18	3/24	12	Takahashi <i>et al</i> 1998	84
PCR	China, S Africa	CP	19/63	30	Lavergne <i>et al</i> 1999	49
PCR	China	CP	20/117	17	de Villiers <i>et al</i> 1999	113
PCR	Italy	CP, 16, 18	0/45	0	Talamini <i>et al</i> 2000	50
PCR	Japan	CP	12/75	16	Kawaguchi <i>et al</i> 2000	114
PCR	Belgium	CP	1/21	2	Lambot <i>et al</i> 2000	115
PCR	China	CP	2/32	6	Peixoto-Guimaraes 2001	116
PCR	China	CP	17/101	17	Chang <i>et al</i> 2000	12
PCR	India	CP	25/40	63	Sobti <i>et al</i> 2001	117
PCR	Italy	CP, RFLP	8/17	47	Astori <i>et al</i> 2001	118

Ag, HPV antigens; CP, consensus primers; EM, electron microscopy; FISH, filter in situ hybridisation; GP, general primers; HB, histological biopsy; IHC, immunohistochemistry; ISH, in situ hybridization; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SB, Southern blot hybridisation.

suggested. Indeed, several novel types have been demonstrated recently by means of PCR and sequencing.<sup>49 113</sup>

In total, ISH has been used to study 1485 oesophageal SCCs, and HPV DNA has been found in 22.9% (341 of 1485) of these patients. HPV-16 was the most frequent HPV type detected. With regard to geographical regions, the HPV detection rate is much higher in lesions from the high risk areas than in those from Europe. This supports the view that oesophageal carcinoma might have a different aetiology in the low and high risk areas, as discussed in recent reviews.<sup>9 10 14 15 19 23 105 106 109</sup>

### Polymerase chain reaction

By 1998, 28 PCR studies had been reported, comprising a total number of 1183 oesophageal carcinomas.<sup>23 74-76 85-107</sup> These studies are discussed in detail elsewhere.<sup>10</sup> HPV DNA was found in 15.6% of the cases (185 of 1183). Since then, several other studies have been published, as summarised in table 1. By March 2002, 837 additional cases had been analysed, 123 of which were HPV positive.<sup>12 15 49 51 84 108-112 114-118</sup>

The detection rates of HPV DNA in oesophageal cancer samples with PCR are subject to a wide variation, as has also been noted in studies with other lesions. The detection of HPV has varied from 0% to 60–70% (table 1). There seems to be a common denominator for the low and high detection rates; namely, the geographical distribution of the material. Accordingly, almost all of the studies where HPV DNA could not be amplified in the tumours were carried out in low risk countries in Europe or in the USA. In contrast, the detection rates of HPV DNA in oesophageal carcinomas derived from the high risk areas (such as China, South Africa, Japan, and Alaska) are significantly higher, and only two studies that included Japanese patients reported no evidence of HPV.<sup>95 111</sup>

Surprisingly, the overall detection rate of HPV DNA (15.2%; 308 of 2020) is less than that found with ISH (22.9%), and significantly less than that reported with SB (40–50%) and FISH (51.9%) (table 1). The reasons for these divergent results may be purely technical (as a result of errors in the interpretation of the data) and, perhaps most importantly, may reflect the diversity of these lesions in different geographical regions. However, PCR should be regarded as the most sensitive HPV detection method, and the higher detection rate with hybridisation methods might indicate a cross hybridisation of HPV probes with human DNA or DNA from other microorganisms.<sup>1-3</sup>

### HPV DNA IN OESOPHAGEAL PRECANCER LESIONS

There is little doubt in that oesophageal SCC develops through distinct precursor lesions known as dysplasia and carcinoma in situ.<sup>119 120</sup> In the high risk areas of China, mass screening programmes have been established to detect cancer precursors by using balloon cytology.<sup>19 74 121 122</sup> However, these precancer lesions have not been systematically studied for the involvement of HPV until recently.<sup>9-13 69 122-124</sup>

According to our experience, such dysplastic lesions frequently accompany invasive SCCs, and present with HPV suggestive cellular changes, such as koilocytes.<sup>10</sup> HPV DNA was recently detected in cytological brushings of the oesophagus in a substantial proportion (17%) of human immunodeficiency virus infected patients without clinically detectable lesions,<sup>125</sup> although it was not found in oesophageal acalasia lesions.<sup>126</sup> In our series of 700 SCCs from China, of which 117 (16.9%) were HPV DNA positive,<sup>11</sup> HPV signals were detected in the surrounding hyperplastic and dysplastic epithelia in 5.6% of patients, and in the resection margins of 0.2% cases, figures that agree with another recent report.<sup>118</sup> The presence of HPV in the adjacent normal and dysplastic epithelium fits in with the “condemned mucosa” concept, recently proposed to explain the pathogenesis of multifocal HPV induced

carcinogenesis in the genital tract and in the upper aerodigestive tract,<sup>127</sup> including oesophageal SCC.<sup>10 124</sup>

### SEROLOGICAL EVIDENCE

Virus-like particles (VLPs) have been used to detect HPV antibodies in the sera of patients with oesophageal SCC,<sup>128-131</sup> in both low risk and high risk areas for this disease. In the study from Finland, based on a serum bank of 39 268 samples, IgG antibodies against HPV-16 VLPs were measured.<sup>128</sup> Of the cases, eight of 39 were HPV-16 seropositive as compared with two of 78 matched controls ( $p < 0.001$ ; odds ratio (OR), 14.6; 95% confidence interval (CI), 1.8 to 117). For a low risk country for this disease, this high OR clearly suggests a significantly increased risk for this malignancy among HPV-16 seropositive subjects. Data are very similar from Norway, where an increased risk of oesophageal cancer among HPV-16 seropositive subjects was of the same order of magnitude (OR, 6.6; 95% CI, 1.1 to 71).<sup>129</sup> However, such an increased risk could not be confirmed in a study from Sweden, where the age and sex adjusted ORs for SCC were 1.0 (95% CI, 0.5 to 2.0) for HPV-16 seropositive patients and 0.5 (95% CI, 0.2 to 1.1) for HPV-18 seropositive patients.<sup>131</sup> The reasons for these discrepant results in these three low risk neighbouring countries remain unclear at the moment.

In Shaanxi Province, China, a high risk area for oesophageal cancer,<sup>130</sup> 90 patients with tumours and 121 cancer free matched control subjects were analysed for the presence of HPV-16 specific antibodies using VLP as the antigen. With the HPV-16 seropositivity cut off value similar to that used in cervical cancer studies, 24% of the patients and only 7% of the controls were seropositive (OR, 4.5; 95% CI, 1.8 to 11.9). OR increased with increasing HPV-16 seroreactivity. Thus, the serological results correlate with the HPV DNA detection data in the balloon cytology samples from the screening population,<sup>69 122</sup> suggesting that HPV-16 infection is an important risk factor for oesophageal carcinoma in this high risk area for the disease.<sup>9 10 19 130</sup>

### IN VITRO STUDIES

During the 1990s, the loss of function of the p53 tumour suppressor gene by mutation or interaction with the E6 protein of the oncogenic HPV types was established as a frequent event in oesophageal SCC.<sup>132 133</sup> The presence of p53 mutations in HPV positive SCCs suggests that HPV and p53 mutation are not mutually exclusive events.<sup>95 133</sup> The interactions between HPV-16 E7 and the retinoblastoma protein in clinical samples have not been studied systematically.<sup>2 10</sup> Similar results have been reported in HPV positive oesophageal cancer cell lines,<sup>134</sup> where different types of p53 mutations were a frequent occurrence. Interestingly, p53 seems to be mutated even in early precancer lesions, at least in the high risk area of China,<sup>135</sup> although p53 mutations are exceptional in benign SCPs.<sup>136</sup> The presence of frequent mutations of the p53 gene in both HPV positive and HPV negative carcinomas suggests an important role for environmental carcinogens in oesophageal carcinogenesis.<sup>20 95 104 132 133 135-140</sup>

Recently, several HPV-16 and HPV-18 positive cells lines from oesophageal SCCs have been established,<sup>92</sup> and have been used to study HPV replication.<sup>141</sup> HPV-16 and HPV-18 genomes were independently transiently transfected into HCE-4 and HCE-7 cell lines with and without E1 and E2 genes under the control of heterologous promoters. These cell lines supported viral replication, and heterologous E1 and E2 were not required for HPV replication, suggesting that specific host nuclear factors in oesophageal squamous epithelial cells may support HPV replication.<sup>141</sup> Malignant transformation of human embryonic epithelial cells was induced in vitro by HPV-18 E6/E7 in synergy with TPA, and these cells were subsequently grown as an established SHEE (synergically infected human embryonic oesophageal cells) cell line.<sup>142</sup>

Three different passages of this cell line were used to study cytogenesis, telomere and telomerase, and the c-myc, ras, bcl-2, and p53 genes. The results revealed that changes of chromosomes, telomere length, telomerase activity, and the expression of certain genes occurred as a dynamic progressive process, suggesting that they are important events in the immortalisation of HPV infected oesophageal epithelial cells.<sup>140</sup> Immortal cell lines have been recently produced by the transduction of HPV-16 E6/E7 into primary culture of human oesophageal keratinocytes, using a recombinant adenovirus.<sup>143</sup> Oesophageal keratinocytes with an extended lifespan have upregulated telomerase activity and have acquired serum resistant growth. The high efficiency of E6/E7 induction by adenovirus vector also revealed the M1 and M2 stages of keratinocyte immortalisation. In vitro experiments will probably shed more light on the complex process of oesophageal carcinogenesis in the near future.

## CONCLUSIONS

The possible mechanisms of HPV associated carcinogenesis in the oesophagus have been discussed in more detail recently.<sup>2 9 10</sup> Despite the accumulating evidence on the presence of the HPV genome in cancer samples, and the malignant transformation of oesophageal epithelial cells by the oncogenic HPV types, several questions need to be answered before the causal role of HPV in oesophageal carcinogenesis can be as firmly established as in cervical cancer.<sup>2-4</sup> However, because of the limits of space only a brief account of some key issues is possible in this context.

One of the prerequisites for the development of cervical cancer seems to be persistent infection by the oncogenic HPV types.<sup>2-4</sup> Such persistent HPV infections probably occur in the oesophagus also, as has been suggested by the detection of HPV DNA in normal oesophageal epithelium and in cancer precursor lesions.<sup>11 118 124-127</sup> So far, however, no prospective follow up studies of such HPV positive lesions or normal oesophageal mucosa harbouring HPV are available, to establish whether such persistent HPV infections are associated with an increased risk of cancer.

There is no reason to doubt that oesophageal cancer develops secondary to multiple genetic events. Infections with microorganisms cause an acute and/or chronic inflammatory process, thereby increasing cell proliferation,<sup>144</sup> and produce carcinogens or promoters that act directly on oesophageal epithelial cells.<sup>19 144</sup> Of importance in this context is the chronic (non-reflux related) oesophagitis described as the most frequent finding in high risk populations for oesophageal SCC in Iran and China, and presumed to be a precursor lesion of this disease.<sup>9 10 19</sup> The extracts of several fungi isolated from foodstuffs in these high risk areas have been shown to have mutagenic and carcinogenic effects by both in vitro and in vivo studies.<sup>10 19</sup> In oesophageal carcinogenesis, some of the factors may be important in the initiation of the neoplastic process, whereas others may act in the promotion and progression of the lesions. In addition to chemical agents, nutritional deficiencies, and physical factors, the current data suggest that HPV may play an important role in the aetiology of oesophageal SCC.<sup>1-3 10 145 146</sup>

At this point, it is too early to speculate upon the detailed molecular mechanisms whereby HPV is involved in the development of malignant transformation in the oesophagus.<sup>141-143 147</sup> The experimental data accumulated so far suggest that similar mechanisms to those detected in cervical carcinogenesis are also involved in the oesophagus.<sup>2-4 9 10 14-17</sup> The analogy with BPV 4 induced carcinogenesis in cattle cannot be ruled out, with regard to synergistic actions between HPV and chemical carcinogens present in foodstuffs, particularly in high incidence areas of the disease,<sup>2 9 10</sup> although the papilloma-carcinoma sequence in the human oesophagus is not firmly documented. The occurrence of p53 mutations in

## Take home messages

- Oesophageal squamous cell carcinoma has a highly divergent geographical distribution, with up to 500 fold variations between low and high risk areas
- Epidemiological and experimental data suggest that some chemicals, nutritional deficiencies, physical factors, and infectious agents are associated with the development of this malignancy
- Evidence for the involvement of human papillomavirus (HPV) in oesophageal carcinogenesis has been provided by several distinct lines of research: (1) HPV involvement in benign squamous cell tumours (papillomas); (2) evidence from animal studies (malignant transformation of oesophageal papillomavirus lesions in cattle); (3) the detection of HPV in oesophageal cancer and its precursor lesions by morphological immunohistochemistry and DNA methods; (4) seroepidemiological evidence (anti-HPV antibodies in patients with cancer); and (5) in vitro studies (transformation of oesophageal epithelial cells by oncogenic HPV types)
- HPV detection rates in oesophageal carcinomas are highly variable in different geographical areas of the world, being significantly higher in high risk areas than in low risk regions. Of the several thousands of carcinomas analysed, HPV detection rates are 23% using in situ hybridisation and 15% by the polymerase chain reaction
- The experimental data accumulated so far suggest that similar mechanisms to those that occur in cervical carcinogenesis are also involved in the oesophagus—both the E6 and E7 oncogenes interfere with cell cycle regulation—and analysis of the expression of these viral oncogenes should be the major focus of HPV research in oesophageal carcinogenesis
- It may be that the aetiology of oesophageal cancer differs greatly between those geographical areas with a low risk and those with a high risk for this disease. This would be the most feasible explanation for the highly divergent detection rates of HPV in oesophageal carcinomas from these different geographical areas

oesophageal carcinomas is a frequent phenomenon, both in HPV negative and HPV positive lesions,<sup>83 132 133 147</sup> but much less attention has been paid to the interactions of HPV E7 and the retinoblastoma protein so far. In the cervix, it is clear that both E6 and E7 crucially interfere with cell cycle regulation, and the role of E7 might be even more important.<sup>2-4</sup> Analysis of the expression of these viral oncogenes should be the major focus of HPV research in oesophageal carcinogenesis.

It may be that the aetiology of oesophageal cancer varies greatly between those geographical areas with a low risk and those with a high risk for this disease.<sup>1 2 10</sup> This would be the most feasible explanation for the highly divergent detection rates of HPV in oesophageal carcinomas from these different areas, as discussed earlier. This divergent prevalence of HPV in oesophageal carcinomas in different geographical areas also makes it extremely difficult to speculate on the proportion of oesophageal carcinomas that are associated with HPV. An approximate estimate could be obtained by using the average HPV prevalence rate (15.2%) reported by the studies using PCR (table 1) and the current number of incident cases, 412 000<sup>18</sup>: approximately 62 000 cases annually. This figure is certainly biased by the unbalanced global distribution of the disease (341 000 of the cases being detected in the developing countries) and the highly divergent HPV prevalence rates in the different global regions, making any such estimation merely speculative.

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## ECHO

### Tandem assays may help to diagnose rheumatoid arthritis



Please visit the Journal of Clinical Pathology website ([www.jclinpath.com](http://www.jclinpath.com)) for link to this full article.

Combining two assays for rheumatoid factor (RF) may improve the diagnosis of rheumatoid arthritis (RA), according to the first study to measure performance of commercial test kits for IgM or IgA RF.

Three IgM kits and two IgA kits with superior sensitivity, specificity, and agreement in an initial screening failed to outperform each other when tested further.

By combining one each of the three IgM tests with the best IgA test in tandem, diagnostic performance improved for test results that agreed but was not useful for differing results, which occurred at a level of 15–27%. Each combination satisfied only one criterion: overall balance, confirmation of RA, or minimising discrepant results.

Twelve assays were evaluated initially for their sensitivity, specificity, and percentage likelihood agreement in measuring RF in 62 patients with RA and 91 healthy controls. The conditions were challenging: many patients had a negative latex test result and many controls had a positive result. The latex test was compared with a global ELISA (for IgA, IgM, IgG RF) and six IgM and four IgA assays. Two IgM, and two IgA assays were devised by the researchers. The best performers were tested further in 146 patients with a more typical RF profile and 75 controls. Three were IgM tests (two commercial), two were IgA tests (one commercial), and one was a global ELISA.

Numerous assays, based on different primary antibodies, are available for testing for RF, but published results reporting 30–90% of RA patients positive for RF illustrate their inaccuracy.

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## HPV infections and oesophageal cancer

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