Are twenty human papillomavirus types causing cervical cancer?

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Conflict of interest

MA, MT and CD declare no conflicts of interest. JD declares having received research grants to his institution from Merck/SPMSD for studies on HPV vaccines. He declares no personal remuneration and no conflict of interest in the area of HPV screening

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Abstract

In 2012, the International Agency for Research on Cancer concluded that there was consistent and sufficient epidemiological, experimental and mechanistic evidence of carcinogenicity to humans for twelve HPV types (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58 and HPV59) for cervical cancer. Therefore these types were considered as 1A carcinogens. They all belong to the family of the alpha-papillomaviridae, in particular to the species α5 (HPV51), α-6 (HPV56), α-7 (HPV18, HPV39, HPV45, HPV59) and α–9 (HPV16, HPV31, HPV35, HPV52, HPV58). Less evidence is available for a thirteenth type (HPV68, α-7), which is classified as a 2A carcinogen (*probably* carcinogenic). Moreover, seven other phylogenetically-related types (HPV26, HPV53, HPV66, HPV67, HPV68, HPV70 and HPV73) were identified as single HPV infections in certain rare cases of cervical cancer and were considered *possibly* carcinogenic (2B carcinogens). Recently, Halec et al demonstrated that the molecular signature of HPV-induced carcinogenesis (presence of type-specific spliced E6*| mRNA, increased expression of p16; and decreased expression of cyclin D1, p53 and Rb) was similar in cervical cancers containing single infections with one of the eight aforementioned 2A or 2B carcinogens as in cancers with single infections with group I carcinogens. Ninety six percent of cervical cancers are attributable to one of the thirteen most common HPV types (group I and IIa). Including the additional seven HPV types (group IIb) added 2.6% to reach a total of 98.7% of all HPV-positive cervical cancers. From recently updated meta-analyses, it was shown that HPV68, HPV26, HPV66, HPV67, HPV73 and HPV82 were significantly more common in cancer cases than in women with normal cervical cytology, suggesting that for these 6 HPV types an upgrading of the carcinogen classification could be considered. However, there is no need to include them in HPV screening tests or vaccines given their rarity.

Introduction

The recognition that persistent high-risk human papillomavirus (hrHPV) infection is strongly linked to cervical cancer has opened new pathways of prevention. In 2012, the International Agency for Research on Cancer established that there was consistent and sufficient epidemiological, experimental and mechanistic evidence of carcinogenicity to humans of twelve HPV types (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58 and HPV59) for cervical cancer[1]. All these hrHPV types belong to the family of the alpha-papillomaviridae, in particular to the species α5 (HPV51), α-6 (HPV56), α-7 (HPV18, HPV39, HPV45, HPV59) and α–9 (HPV16, HPV31, HPV35, HPV52, HPV58), see Table 1. The epidemiological evidence evaluated was if the prevalence of the given HPV type is higher than that of HPV6 in cervical cancer and, in addition, whether the prevalence of the type was significantly higher in women with cervical cancer than among women with normal cervical cytology[1]. It was noted that other HPV types also belonging to the alpha-papillomaviridae were identified as single HPV infections in certain rare cases of cervical cancer (HPV26, HPV53, HPV66, HPV67, HPV68, HPV70 and HPV73).

Functional studies have shown that the oncogenicity of the hrHPV types is explained by their ability to promote cellular transformation and alter immune-related pathways. These hrHPV-induced events are mainly mediated by the products of two early genes, E6 and E7. Since

HPV16 and HPV18 are the most frequently detected types in cervical cancer worldwide, their E6 and E7 proteins have been extensively studied. These viral oncoproteins associate with a broad spectrum of cell cycle regulating and tumour suppressing proteins[2]. The best characterized HPV16 or HPV18 E6 and E7 activities are their ability to induce degradation of p53 and the retinoblastoma protein, respectively, via the ubiquitin pathway. A few studies have characterized the transforming properties of E6 and E7 from the other hrHPV types[3,4] although additional evidence is required to corroborate these in vitro studies.

Phylogenetic relatedness with established hrHPV types is an argument to suspect capacity for malignant transformation, but is insufficient to establish carcinogenicity. For this reason and because of their occurrence as single infections in rare cases of cervical cancers, the IARC working group considered the following types as *probably (class 2A carcinogen) (*HPV68 α-7) *or possibly (class 2B)* carcinogenic: HPV26 (α-6), HPV53 and HPV66 (α-6), HPV67 (α-9), HPV70 (α-7), HPV73 (α-11) and HPV82 (α-5).

Molecular signature of HPV-induced malignant transformation

The Catalan Institute of Oncology (ICO) recently collected more than ten thousand archived biopsies from women with histologically confirmed cervical cancer from all five continents [5]. Presence of HPV types was assessed with the SPF-10 PCR, which targets an amplicon of 65 basepairs in the L1 gene of a broad spectrum of HPV types. Amplified viral DNA was identified by DNA enzyme immunoassay and genotyped with the reverse hybridisation line probe ($LiPA_{25}$) assay[6]. DNA sequence analysis was done for HPV-positive samples which could not be genotyped with the $LiPA_{25}$ assay. Eighty five percent of samples were HPV-positive and 71% of them contained HPV16 or 18, whereas 95% contained DNA of high-risk HPV[5] (see Figure 1).

In the study described in the current issue of *J Pathol*, researchers of the German Cancer Research Center (Heidelberg) and ICO (Barcelona) assessed a set of biomarkers which are considered as a molecular signature of HPV-induced carcinogenesis[7]. The following five biomarkers, which demonstrate the expression and the effects of the oncogenes E6 or E7, were explored: a) presence of type-specific spliced E6*| mRNA[8]; b) increased expression of p16; and c-e) decreased expression of cyclin D1, p53 and Rb, using immunohistochemistry. Fifty five specimens contained a single infection with a class 2A/2B HPV type (HPV26, HPV53, HPV66, HPV67, HPV68, HPV70, HPV73 or HPV82) and 266 specimen were from cervical cancer patients containing a single HPV16 infection or a single infection with another class I HPV infection[7]. The biomarker patterns were very similar in cancer cases with a single class 2A/2B HPV as in those with a class I HPV infection. The authors concluded that their findings provide molecular evidence of carcinogenicity of eight more HPV types.

Clinical and public health significance of the additional carcinogenic HPV types compared to the twelve recognised carcinogenic HPV types?

Figure 1 shows the hrHPV types (A carcinogens, on top) followed by eight phrHPV types (2A and 2B carcinogens, at the bottom) ranked by increasing prevalence in cervical cancer and the incremental proportion of cervical cancers that can be attributed to these types as observed in the ICO study[5]. An estimated 530,000 cases of cervical cancer occurred in 2008[9], of which 320,822 (60.6%) can be attributed to HPV16 and 70.8% (10.2% more) to HPV16 or

HPV18. Ninety six percent of cervical cancers are attributable to one of thirteen HPV types (HPV16, 18, 33, 31, 45, 56, 35, 52, 56, 58, 59 and 68); incorporating seven more types, recognized by Halec *et al* as carcinogenic[7], adds another 2.6% to totalize 98.7% of all HPV-positive cervical cancers.

By comparison, an updated systematic review for 47 HPV types, comprising 423 studies with 372,000 women, found evidence that all 13 HPV types classified as class I or 2A carcinogens were more common among patients with cervical cancer than among women with normal cytology[10]. Six additional class 2A/2B HPV types were also more common in cervical cancer patients than women with normal cytology (HPV26, 67, 68, 69, 73 and 82)[10]. Together with the new mechanistic data[7], the evidence now suggests that at least for these 6 HPV types an upgrading of the carcinogen classification could be considered. It should be noted that the upgraded IARC systematic review did not find evidence of cervical cancer-association of HPV53 and HPV70, however[10].

In

Figure 2, the positivity rate is assessed in a screening population using an HPV assay targeting a cumulative series of types starting with one type (HPV16) and ending with all twenty types defined in Figure 1. Data are derived from a large population of women attending cervical cancer screening in Belgium all genotyped for twelve hrHPV types as well as for four phrHPV types (HPV53, HPV66, HPV67 and HPV68)[11]. The prevalence of HPV26, HPV70, HPV73 and HPV82 were estimated from the VALGENT study where the prevalence of 51 HPV types in a representative sample of the same screening population was assessed with the BSGP5+6+-PCR/MPG PCR[12]. HPV16 was present in 2.9%, HPV16/18 in 3.9%, and hrHPV infection in 10.9%. Adding seven types increased the test positivity rate to 12.1%.

It was recently shown that the development of cervical (pre-)cancer (cervical intraepithelial neoplasia of grade 3 or more) is preceded by a steady increase in the viral load of a given

HPV type (transforming process)[13]. For 138 evaluated cases, 98.6% could be attributed to one of the thirteen HPV types and less than 1.4 % to phrHPV types. Both carcinogenic (12 types) and phrHPV types (HPV53, HPV66, HPV67 and HPV68) showed linear increases before detection of CIN3+[13]. Adding progressively more HPV types in an assay potentially increases the clinical sensitivity for cervical precancer and cancer but simultaneously decreases specificity[14]. The VALGENT study assessed the sensitivity for cervical intra-epithelial neoplasia of grade 2 or worse (CIN2+) as a function of the specificity of an HPV test targeting progressively more genotypes (up to the 20 types considered in Figure 1)[15]. A monotype HPV16 test showed a sensitivity and specificity of 50% and 94.6%, respectively. 100% sensitivity (with a corresponding specificity of 81.9%) was reached when the assay was restricted to eleven genotypes. Addition of nine more types resulted in the loss of specificity of 4.8% without any further gain in sensitivity.

Discussion

The careful molecular characterisation of cancer cases with a single infection with HPV26, HPV53, HPV66, HPV67, HPV68, HPV70, HPV73 or HPV82 and the demonstration that their profile is not different from that in cancer cases associated with established carcinogenic types provides mechanistic evidence of carcinogenicity[7], which fits with updated epidemiological evidence of cervical cancer association for 6 of these HPV types. However, the public health impact of these findings should not be exaggerated. Because of their scarcity in cancer (most are more rare then HPV6 in cervical cancer) and given the substantial loss in specificity, there are no reasons to include them in future screening tests or to propose 20-valent HPV vaccines. The observation that the eight most prevalent HPV types in cervical cancer is geographically stable precludes the need for local adaptations or inclusion of phrHPV types[16]. Also among the class I HPV types, there are strong differences in carcinogenicity and contribution to the disease burden (Figure I). Similarly, prospective studies found that only 7 HPV types contribute significantly to CIN grade 2 or 3 (HPV 16/18/31/33/45/52/58), all contributing 5% or more to the CIN2+ burden. Non-significant contributions to CIN2+ of about 2% were seen for HPV39/51/56[17]. Thus, it appears that (if anything) the composition of HPV screening tests could rather be reduced than expanded as this may improve specificity without significant decline in sensitivity. The most obvious example is HPV66, a common HPV type that is still included in several HPV tests in spite of repeated assessments of non-carcinogenicity [5,12,13,17].

An important concern is the occurrence of hrHPV-negative cancers which in fact are HPV-driven but a given HPV test on a cervical sample preceding the diagnosis yields a negative result. Reasons for this may be the loss of the L1 gene, latent infection, low HPV DNA copy number per cancer cell (analytical sensitivity under the detection limit), inadequate sample preparation or processing, or other laboratory error. A recent report from a woman who died from cervical cancer revealed repeated negative results using a L1-DNA test which were all positive for HPV16 DNA using real-time PCR targeting E6 or E7 genes[18]. Walboomers originally identified 92.1% hrHPV DNA (using the GP5+/6+ PCR) in an international series of cervical cancers[19]. Reanalysis of HPV-negative cases revealed that 38% were due to inadequate specimen, 58% were HPV-positive using alternative assays and only 4% still were HPV negative. Clinically validated HPV tests can currently be recommended in clinical practice in screening, triage and follow-up after treatment[20]. The choice of the assay should be based on based on an optimal balance of sensitivity and specificity of progressive neoplastic lesions. Their use should be submitted to strict quality control. Regular participation in internationally standardised proficiency panels has been shown to increase

laboratory performance[21]. Currently widening the spectrum beyond the set of high-risk does not seem rational from the public health point of view.

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Author contributions
MA: conception and writing of the manuscript
MT, CD, JD: critical review and contribution to writing of the manuscript

Figure 1. Cumulative proportion of cervical cancers in the World that are attributed to a ranked combination of 20 HPV types and the estimated number of cervical cancers in 2008 expected to be caused by these types. Adapted from de Sanjosé et al (2010)[5] and Arbyn et al. (2011)[9].

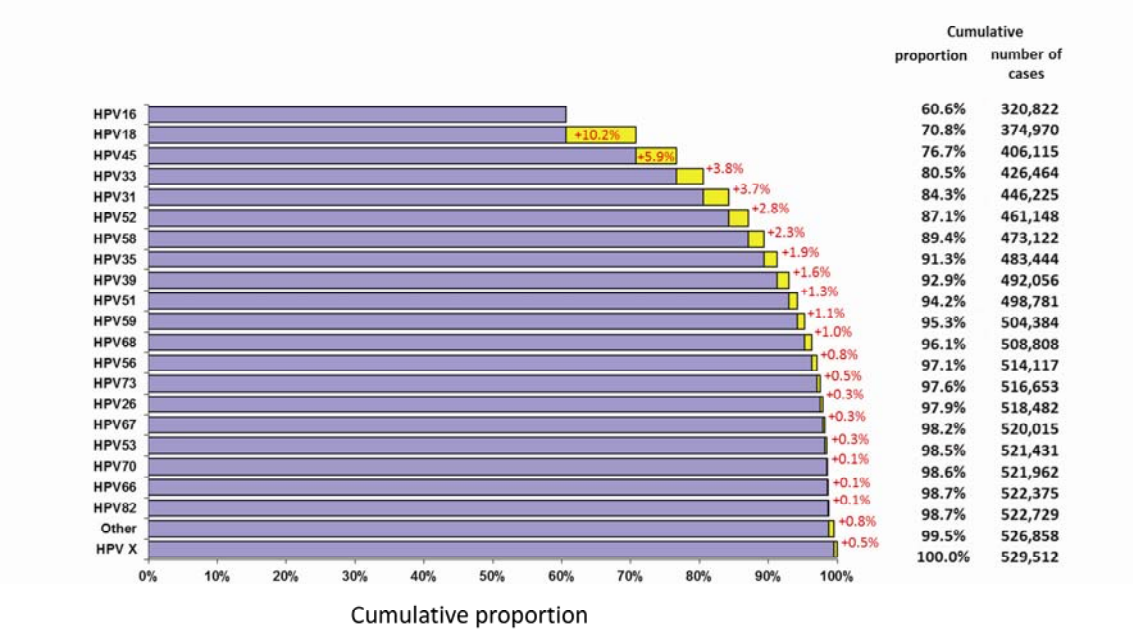

Figure 2. HPV test positivity rate using an assay including a cumulative series of types starting with one type (HPV16) and ending with twenty types as defined in Figure 1. (Source: Arbyn et al, 2009[11], extended up to end 2013. The prevalence of HPV26, HPV70, HPV73 and HPV82 were estimated from the VALGENT study[12]) .

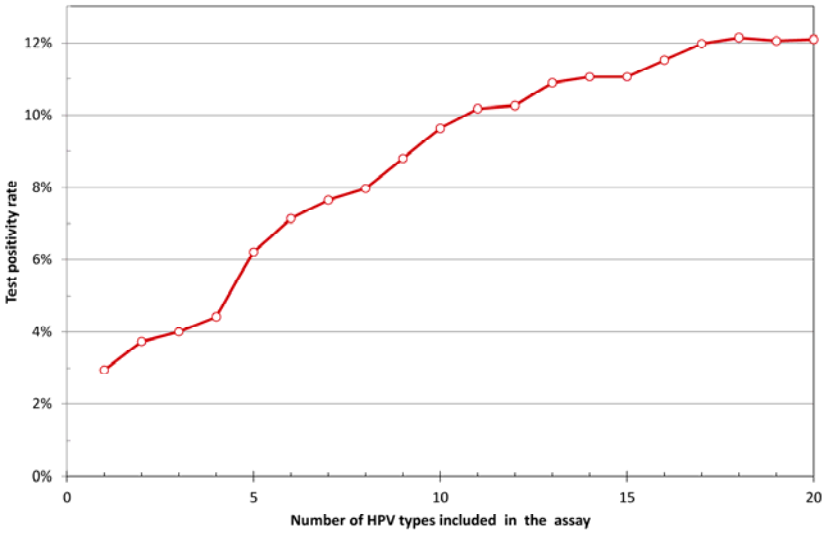

Table 1. HPV types belonging to species α5, α6, α7, α9, and α11 according to the level of evidence of carcinogenicity for cervical cancer (adapted from IARC Monograph, vol 100B[1]).

Species	Types							
α5	26	51	69	82				
α6	30	53	56	66				
α7	18	39	45	59	68	70	85	97
α9	16	31	33	35	52	58	67	
α11	34	73						

Group 1 carcinogens, Group 2A carcinogens, Group 2B carcinogens, Phylogenetic analogy with carcinogenetic type

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