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INVITED COMMENTARY

Are 20 human papillomavirus types causing cervical cancer?†

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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 12 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 α-Papillomaviridae, in particular to the species α5 (HPV51), α6 (HPV56), α7 (HPV18, HPV39, HPV45, HPV59) and α9 (HPV16, HPV31, HPV33, HPV35, HPV52, HPV58). Less evidence is available for a thirteenth type (HPV68, α7), 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* [7] demonstrated that the molecular signature of HPV-induced carcinogenesis (presence of type-specific spliced E6*I 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 afore-mentioned 2A or 2B carcinogens to those in cancers with single infections with group 1 carcinogens. Ninety six percent of cervical cancers are attributable to one of the 13 most common HPV types (groups 1 and 2A). Including the additional seven HPV types (group 2B) 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 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 in cervical cancers.

Keywords: cervical cancer; human papillomavirus; carcinogenesis; biomarkers

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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 12 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 α-Papillomaviridae, in particular to the species α5 (HPV51), α6 (HPV56), α7 (HPV18, HPV39, HPV45, HPV59) and α9 (HPV16, HPV31, HPV33, HPV35, HPV52, HPV58) (see Table 1). The epidemiological evidence evaluated was if the prevalence of the given HPV type was 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 α-Papillomaviridae were identified as single HPV infections in certain rare cases of cervical cancer (HPV26, HPV53, HPV66, HPV67, HPV68, HPV70, HPV73 and HPV82).

Functional studies have shown that the oncogenicity of the hrHPV types is explained by their

Table 1. HPV types belonging to species and according to the level of evidence of carcinogenicity for cervical cancer

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						

Adapted from IARC [1].

Group 1 carcinogens.

Group 2A carcinogens.

Group 2B carcinogens.

Phylogenetic analogy with carcinogenic types.

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* (group 2A carcinogen) (HPV68 α7) or *possibly* (group 2B) carcinogenic: HPV26 (α6), HPV53 and HPV66 (α6), HPV67 (α9), HPV70 (α7), HPV73 (α11) and HPV82 (α5).

Molecular signature of HPV-induced malignant transformation

The Catalan Institute of Oncology (ICO) recently collected > 10 000 archived biopsies from women with histologically confirmed cervical cancer from all five continents [5]. Presence of HPV types was assessed by SPF-10 PCR, which targets an amplicon of 65 base pairs (bp) 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 hybridization 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; 85% 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 Centre (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 effects of the oncogenes *E6* or *E7*, were explored: (a) presence of type-specific spliced *E6**I mRNA [8]; (b) increased expression of p16; and (c–e) decreased expression of cyclin D1, p53 and Rb, using immunohistochemistry: 55 specimens contained a single infection with a group 2A/2B HPV type (HPV26, HPV53, HPV66, HPV67, HPV68, HPV70, HPV73 or HPV82) and 266 specimens were from cervical cancer patients containing a single HPV16 infection or a single infection with another group 1 HPV infection [7]. The biomarker patterns were very similar in cancer cases with a single group 2A/2B HPV to those with a group 1 HPV infection. The authors concluded that their findings provide molecular evidence of carcinogenicity for eight more HPV types.

Clinical and public health significance of the additional carcinogenic HPV types compared to the 12 recognized carcinogenic HPV types?

Figure 1 shows the hrHPV types (group 1 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%) could be attributed to HPV16 and 70.8% (10.2% more) to HPV16 or HPV18; 96% of cervical cancers were attributable to one of 13 HPV types (HPV16, 18, 33, 31, 45, 56, 35, 52, 56, 58, 59 and 68); incorporating seven more types, recognized by Halec *et al* [7] as carcinogenic, 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 group 1 or 2A carcinogens were more common among patients with cervical cancer than among women with normal cytology [10]. Six additional group 2A/2B HPV types were slightly more common in cervical cancer patients than in women with normal cytology (HPV26, 67, 68, 69, 73 and 82) [10]. It should be noted that Halec *et al* [7] did not assess HPV69. Together with the new mechanistic data [7], the evidence now suggests that, at least for five HPV types, an upgrading of the carcinogen classification could be considered. It should be recognized, however, that the upgraded IARC systematic review did not find evidence of cervical cancer association of HPV53, HPV66 and HPV70 [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

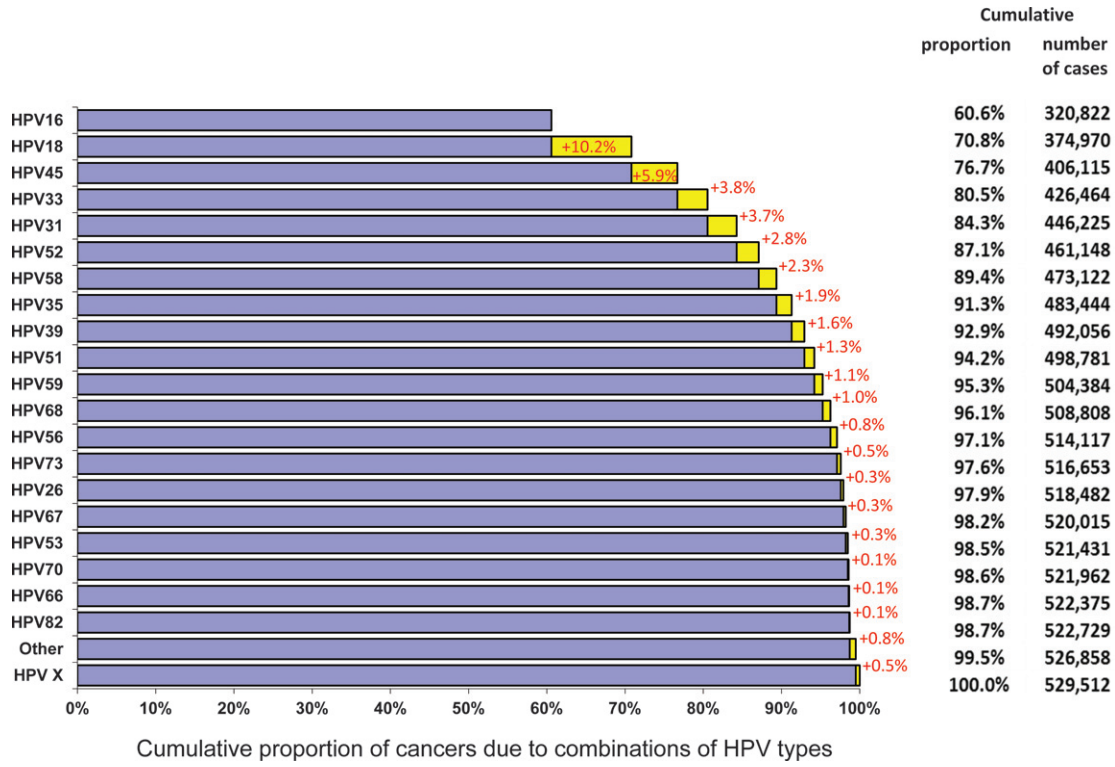

Figure 1. Cumulative proportion of cervical cancers in the world that are attributed to a ranked combination of 20 HPV types and the estimated number of cervical cancers in 2008 expected to be caused by these types. Adapted from de Sanjosé *et al* (2010) [5] and Arbyn *et al.* (2011) [9].

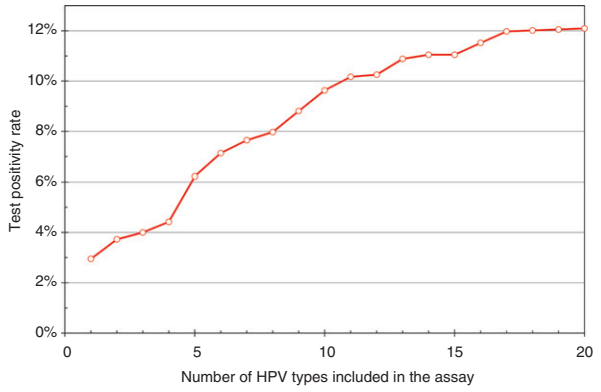

Figure 2. HPV test positivity rate using an assay including a cumulative series of types starting with one type (HPV16) and ending with 20 types, as defined in Figure 1. From Arbyn *et al* (2009) [11], extended up to the end of 2013. The prevalences of HPV26, HPV70, HPV73 and HPV82 were estimated from the VALGENT study [12].

(HPV16) and ending with all 20 types defined in Figure 1. Data are derived from a large population of women attending cervical cancer screening in Belgium, all genotyped for 12 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 intra-epithelial 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 13 HPV types and < 1.4 % to phrHPV types. Both group 1 (12 types) and group 2A/2B HPV 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 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 11 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 characterization 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 five 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 are geographically stable precludes the need for local adaptations or inclusion of phrHPV types [16]. Also among the group 1 HPV types, there are strong differences in carcinogenicity and contribution to the disease burden (Figure 1). Similarly, a prospective Swedish study found that only seven HPV types contribute significantly to CIN grades 2 or 3 (HPV 16/18/31/33/45/52/58), each 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 on 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 the *E6* or *E7* genes [18]. Walboomers *et al* [19] originally identified 92.1% hrHPV DNA (using the GP5$^+$/6$^+$ PCR) in an international series of cervical cancers. Re-analysis of HPV-negative cases revealed that 38% were due to inadequate specimens, 58% were HPV-positive using alternative assays and only 4% were still HPV-negative. Clinically validated HPV tests can currently be recommended in clinical practice in screening, triage and follow-up after treatment of cervical precancer [20]. The choice of the assay should be based on an optimal balance of sensitivity and specificity of progressive neoplastic lesions [20]. Their use should be submitted to strict quality control. Regular participation in internationally standardized proficiency panels has been shown to increase laboratory performance [21]. Currently, widening the spectrum beyond the high-risk set does not seem rational from the public health point of view.

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Author contributions

MA, conception and writing of the manuscript; and MT, CD and JD, critical review and contributions to writing the manuscript.

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