

High-Risk Human Papillomavirus in Esophageal Squamous Cell Carcinoma—Letter

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We read with interest the recent article excluding human papillomavirus (HPV) as a significant contributor in esophageal squamous cell cancers (ESCC), at least in Australia (1). This is in contradistinction to oropharyngeal cancers (OPC) where there is compelling epidemiologic and molecular evidence indicating HPV as the etiology of a subset of cases. Within Australia, there is a changing epidemiology of OPCs. In a recent study of 302 cases, the incidence of HPV-related OPCs increased from 19% in 1987 to 1990, 47% in 2001 to 2005, to 60% in 2005 to 2006 (2). Reports worldwide vary considerably in finding HPV in ESCC: with rates up to 81% (3). The clinical implications of excluding HPV as a major contributor to ESCC are important, particularly as HPV-related cancers have better prognosis and chemoradiation responses than their non-HPV counterparts.

We question whether the HPV rates have been fully determined in this ESCC series, due to potential methodologic problems. The authors used internal control primers which amplify a 110-bp region of the β -globin gene (1). This contrasted with the 150-bp HPV L1 amplicon generated with GP5⁺/6⁺ primers. It would be standard practice to use an internal control of longer or equal length to the HPV amplicon, to ensure DNA below 150 bp has not degraded (4). Also, there are several aspects of the described PCR method which could contribute to lower assay sensitivity. Firstly, the authors modified the MgCl₂ concentration to 2 mmol/L from the original assay

description of 3.5 mmol/L. This would increase PCR stringency, but reduce sensitivity. The assay sensitivity in detecting HPV in tissue should be stated.

Secondly, the amplicons were analyzed by gel electrophoresis, a less sensitive method than using HPV-specific probes. If original paraffin-embedded tissue blocks were small, faint amplicon bands could be missed: although would not explain so many negatives. (This would be further exacerbated if there were DNA degradation). In addition, if there were HPV integration there could be interruption of the site being amplified.

It is standard when looking for HPV DNA in tissue that the sample used for PCR also has samples collected either side, to be rechecked histologically for tumor. This ensures that the sample is adequate for HPV PCR (5). We note that 22 (10%) of the total samples analyzed were from slide sections: the remainder from tissue blocks. Amplification from slide sections is less sensitive than from tissue. This is of relevance given that none of the 22 slides examined were HPV positive.

Disclosure of Potential Conflicts of Interest

S. M. Garland, advisory board for cervical cancer vaccines, honoraria for speakers bureau, and through her institution received monies for research on cervical cancer vaccine phase 3 trials.

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References

1. Antonsson A, Nancarrow DJ, Brown IS, Green AC, Drew PA, Watson DI, et al. Study of high-risk human papillomavirus in esophageal squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 2010;19:2080–7.
2. Hong AM, Grulich AE, Jones D, Lee CS, Garland SM, Dobbins TA, et al. Squamous cell carcinoma of the oropharynx in Australian males induced by human papillomavirus vaccine targets. *Vaccine* 2010;28:3269–72.
3. Li T, Lu ZM, Chen KN, Guo M, Xing HP, Mei Q, et al. Human papillomavirus type 16 is an important infectious factor in the high incidence of esophageal cancer in Anyang area of China. *Carcinogenesis* 2001;22:929–34.
4. Greer CE, Wheeler CM, Manos MM. Sample preparation and PCR amplification from paraffin-embedded tissues. *PCR Methods Appl* 1994;3:S113–22.
5. Garland SM, Hernandez AM, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med* 2007;356:1928–43.