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

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We thank Garland and colleagues for their interest in our study (1) and for raising the important issue of the sensitivity of the procedures we used to detect human papillomavirus (HPV) in esophageal squamous cell carcinoma (ESCC). More precisely, they have expressed concerns that our ability to detect HPV in ESCC tumor samples may have been compromised by degradation of DNA in formalin-fixed paraffin embedded (FFPE) tissue samples; modified $MgCl_2$ concentration; use of electrophoresis and sequencing instead of HPV type–specific probes; or potentially suboptimal histologic assessment of tumor samples. We have addressed each of these points below.

1. *Degradation of DNA*. We agree that internal controls of equal or longer length than the HPV amplicon would have been ideal to ensure that our samples contained longer sequences that could be amplified. However, we believe this potential source of bias is unlikely to explain our overall low rate of HPV detection, as DNA is known to be very stable (even in FFPE tissue), and the samples analyzed in our study were all collected recently (between 4–8 years old; refs. 2, 3). Furthermore, the 2 primer pairs $GP5^+/6^+$ and PCO3/PCO4 were designed to be used in combination to screen FFPE tissues for the presence of HPV and have been used in previous published studies (4–7).
2. *Change in $MgCl_2$ concentration*. We used a lower $MgCl_2$ concentration (2.0 mmol/L) than other reports (3.5 mmol/L). We have tested the sensitivity of the $GP5^+/6^+$ primer pair at the lower concentration and can detect down to 10 copies for certain HPV types including HPV-16 and HPV-18 (unpublished data). Furthermore, other studies (8, 9) have also used 2.0 mmol/L in their $GP5^+/6^+$ PCR and Chao and colleagues (8) report a similar sensitivity to ours (100 HPV copies detected).
3. *Use of electrophoresis and sequencing instead of HPV type–specific probes*. Our detection threshold was 10 HPV copies per sample for some HPV types (HPV-16 and HPV-18). It is questionable whether higher HPV sensitivity could be achieved using probes, which would likely come at the cost of lower specificity. A particular advantage of the sequencing strategy that we employed is the avoidance of cross-reactivity problems that are common when using probes (10). Finally, it must be considered whether detection thresholds of less than 10 HPV copies per sample are clinically important (11).
4. *Histologic screening and sample suitability for PCR*. The tumor samples used in our study were all confirmed histologically as ESCC by an expert gastrointestinal pathologist (I.S.B.) using a section cut adjacent to the unstained sections used for DNA extraction. We did not confirm the existence of ESCC tumor in a second section on the other side of the unstained section, although this is a highly unlikely source of error given the large size of the tumors in this series. Also, the overall low prevalence of HPV we detected would have changed little had we chosen exclude the 22 ESCC samples on slides (4.0% compared with 3.6%).

Thus, while we agree that methodologic issues must be considered as possible explanations for the overall very low prevalence of HPV DNA in ESCC tumor samples, we do not believe that low sensitivity explains the findings. The varying HPV prevalences reported in published ESCC studies are most likely explained by variations in laboratory technique and differences in sampling strategies for patients and tissues. These scientific issues require resolution before any public health implications can be addressed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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