

Supplement :

Comparison of HPV-16 viral load between esophageal squamous cell carcinoma and cervical cancer

A total of 166 paraffin embedding esophageal squamous cell carcinoma (ESCC) specimens archived between March and May, 2004 and 90 paraffin embedding cervical squamous cell carcinoma specimens archived between 1995 and 2004 in Anyang Cancer Hospital were detected for HPV DNA by an SPF1/GP6+ mediated polymerase chain reaction (PCR) followed by sequencing. Twenty nine HPV-16 only infected samples from either ESCCs or cervical cancers were selected for viral load determination.

To estimate the amount of HPV-16 copies per human genome equivalent, a quantitative real-time PCR was performed using SYBR green master mix (Applied Biosystems). A 175 bp HPV16 E7 fragment was amplified using the following primer set, F: 5'-AATGACAGCTCAGAGGAGGA-3' and R: 5'-GTGTGCCCATTAACAGGTCT-3'. A 75 bp β -actin fragment was amplified using the following primer set, F : 5'-TCCACCTTCCAGCAGATGTG-3' and R : 5'-GCATTTGCGGTGGACGAT-3'. Standard curves were generated by plotting Ct values against known concentrations of input DNA; human blood DNA was used for the actin assay (500-0.05 ng dilutions) and HPV 16 plasmid DNA spiked with 100 ng human blood DNA for the HPV 16 assay (10^6 - 10^0 copy dilutions). The amount of actin DNA (ng) was calculated from the Ct values of sample DNA, using the standard

curve of human blood DNA. Similarly, the amount of HPV 16 DNA (copy) was calculated from the Ct values obtained for sample DNA, using the standard curve of HPV 16 plasmid.

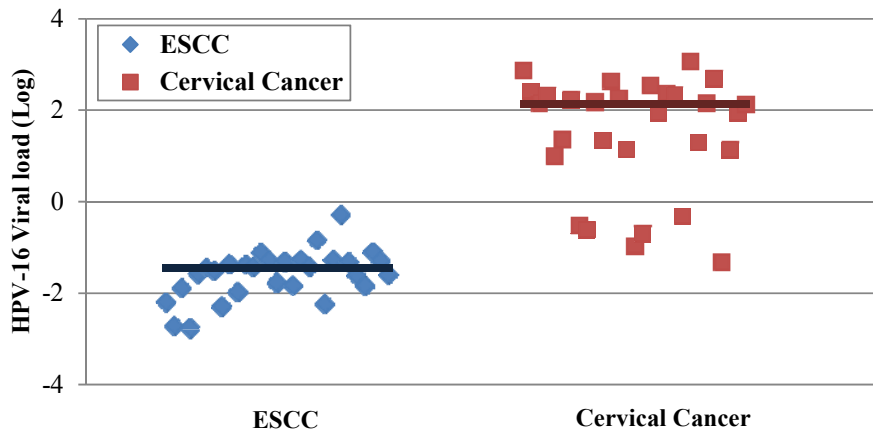


Figure 1. Comparison of HPV-16 viral load (log) between ESCC and cervical cancer specimens.

As shown in figure 1, the median copy number of HPV-16 in ESCC specimens was 0.04 copies/cell, which was nearly four orders of magnitude lower than that in cervical cancer (132.60 copies/cell) and the difference was statistically significant ($p < 0.001$).

Conclusion: Copy number of HPV-16 DNA in ESCC was significantly lower than that in cervical cancer and the discrepancy was at least two orders of magnitude by conservative estimates.