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Letter to the Editor

Is HPV-18 present in human breast cancer cell lines?

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Sir,

Although several studies have suggested an association between breast cancer and human papillomavirus (HPV) infection (de Villiers *et al*, 2005; Cazzaniga *et al*, 2009), two recent papers published in the *British Journal of Cancer* (Heng *et al*, 2009; Lawson *et al*, 2009) were particularly provocative. The authors claimed that both primary human breast cancers and two well-characterised breast cancer cell lines (MDA-MB-175VII and SK-Br-3) contained HPV-18, a type of HPV found with increased frequency in adenocarcinomas (Iwasawa *et al*, 1996; Burk *et al*, 2003).

To further characterise the MDA-MB-175VII and SK-Br-3 cell lines for HPV-18 gene content and expression, we performed PCR and RT – PCR to detect viral DNA and mRNA. We also included two cell lines as controls. The HeLa cervical cancer cell line contains HPV-18 and was used as a positive control and the C33 cervical cancer cell line contains no detectable HPV genomes. All cell lines were grown in 10% FBS DMEM media to approximately 85% confluency and then harvested for DNA and RNA isolation, after which we performed standard PCR and RT – PCR reactions using the indicated primer sets for the early, late, and non-coding regions of HPV-18 (see Figure 1A). For reference, we also used the exact L1

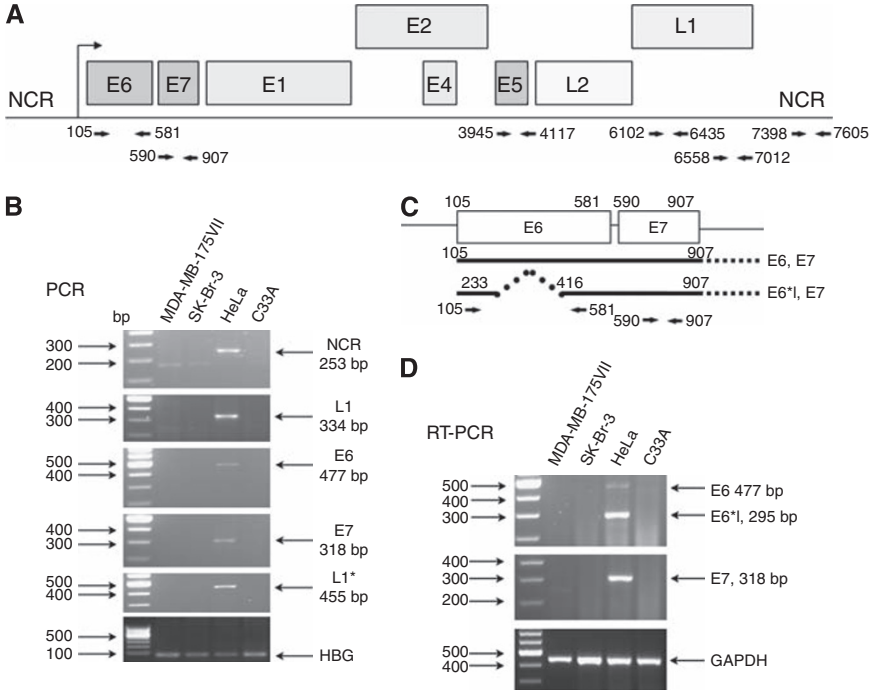

Figure 1 Absence of HPV-18 in the MDA-MB-175VII and SK-Br-3 breast cancer cell lines. (**A**) HPV-18 genome structure and PCR primer sets used in this study. (**B**) PCR for HPV-18 DNA in cell lines. The indicated primer sets were used to amplify the NCR, L1, E6 and E7 regions of HPV18 DNA. L1* indicates the primer set used in the article by Heng *et al*. (**C**) Early transcripts of HPV-18 and primer sets used for RT – PCR. (**D**) RT – PCR for mRNAs of HPV-18 early genes (*E6* and *E7*).

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primer sequence set that was used by Heng *et al* for detection of HPV-18 in MDA-MB-175VII and SK-Br-3 by *in situ* PCR.

In order to detect HPV-18 DNA, cellular DNA was isolated using a Qiagen DNA isolation kit. After performing PCR reactions (95°C for 5 min, 35 cycles: 95°C for 30 s, 55°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 min) using different sets of primers, the PCR products were separated on a 2% agarose gel. Consistent with the studies of Schneider-Gadicke and Schwarz (1986) that mapped the genomic fragments of HPV-18 present in HeLa cells, our primer sets were able to amplify the L1, NCR, E6 and E7 regions of HPV-18 DNA in HeLa cells (panel B). C33A cells were uniformly negative (panel B). Surprisingly, the MDA-MB-175VII and SK-Br-3 breast cell lines were completely negative for HPV-18 DNA (panel B), indicating either the absence or very low abundance of HPV-18 DNA in these cell lines. As an internal control for verifying DNA quality isolated from the above cell lines, we performed PCR with primers specific for human β globin (HBG). All samples were uniformly positive for the presence of this gene (panel B, bottom).

To address the possibility that the copy number of HPV-18 genomes was extremely low in these cells and undetectable by PCR, we also performed more sensitive RT–PCR reactions to detect HPV-18 mRNA. Cellular RNA was isolated by the TRIzol method, followed by one-step RT–PCR (42°C for 60 min, 95°C for 2 min, 35 cycles: 95°C for 30 s, 55°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 min). PCR products were separated on a 2% agarose gel. In HeLa cells we detected transcription products for the two major transforming genes of HPV-18, *E6* and *E7* (panel D). As expected, we also detected the long and short size variants of E6 mRNA that are generated by RNA splicing. Corresponding to our PCR data that indicated a lack of HPV DNA in the breast cells, we also found no evidence for expression of HPV-18 mRNA (panel D). To validate our RNA purification, we performed RT–PCR for GAPDH mRNA, which demonstrated that the RNA samples were of sufficient quality to detect the expression of a single copy gene.

To conclude, HPV-18 DNA and mRNA are not detectable in the MDA-MB-175VII and SK-Br-3 breast cancer cell lines, contradicting the study of Heng *et al*. As the *E6* and *E7* genes of the high-risk HPVs are retained and expressed in all HPV-induced cervical cancers (Androphy *et al*, 1987; Banks *et al*, 1987; Hawley-Nelson *et al*, 1989; Munger and Howley, 2002) and their cooperative interaction is required for efficient cell immortalisation and maintenance of the tumourigenic phenotype (Androphy *et al*, 1987; Banks *et al*, 1987; Hawley-Nelson *et al*, 1989; Munger and Howley, 2002), our results strongly indicate that HPV is not an aetiologic factor in the generation of these breast tumour cell lines. Although there may be a subset of breast cancers that are induced by HPV, the MDA-MB-175VII and SK-Br-3 cell lines clearly cannot be used to support this hypothesis and they are not valid cell lines for studying HPV-mediated transformation of breast cells.

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