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LETTER TO THE EDITOR

Presence of MMTV-like *env* gene sequences in human breast cancer

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To the Editor,

Park et al. 2010 (No evidence of MMTV-like *env* sequences in specimens from the Australian Breast Cancer Family Study, Breast Cancer Research and Treatment online first) have reported lack of evidence for MMTV-like *env* gene sequences in breast cancer specimens from Australia.

We have recently addressed the question of differences in published results [1, 2] concerning MMTV-like *env* sequences in breast cancers. Besides obvious technical differences such as the ones considered here, there are major geographical variations in the incidence of breast cancer and in the frequency of viral sequences in the breast cancers. Ford et al. [3] reported that 40% of Australian women's breast cancers contained MMTV-like viral sequences but none could be detected in specimens from Vietnam. We have also found 30–40% positivity in breast cancer specimens from four countries in the Americas and three in Europe, but a sharply lower frequency in breast cancer specimens from Iran, Japan, and China where breast cancer is less common [unpublished]. This coordinate epidemiology of breast cancer and viral presence suggests a relationship that may turn out to be causal.

We have thus compared on the same American breast cancer specimens results obtained using the methodology of Park et al. with those analyzed under the experimental conditions we have previously described [1]. The results are shown in Fig. 1. The PCR product of MMTV-like *env* gene amplification following our conditions is shown (Fig. 1a). Although two bands in the gel are faint, three bands of molecular weight around 250 bp can be seen after hybridization with a labeled probe. The same three DNAs were used for amplification using the conditions of Park et al. In only one instance a 123 bp band in the gel and one hybridization band is seen (Fig. 1b). The amplified DNA was sequenced and shown to be 90% homologous to MMTV *env* gene.

Park et al. stated that their methodology can amplify five copies of a plasmid containing *env* gene sequences diluted in 10 ng of negative human DNA. This does not establish that the conditions reliably amplify viral sequences in human tumor DNA. Our technology can detect one copy of a plasmid containing 660 bp of MMTV-like *env* gene in 100 ng of genomic DNA. It also includes hybridization with a labeled oligonucleotide internal probe, which allows conclusive detection and identification of the specific band product. In addition, we usually sequenced the PCR products.

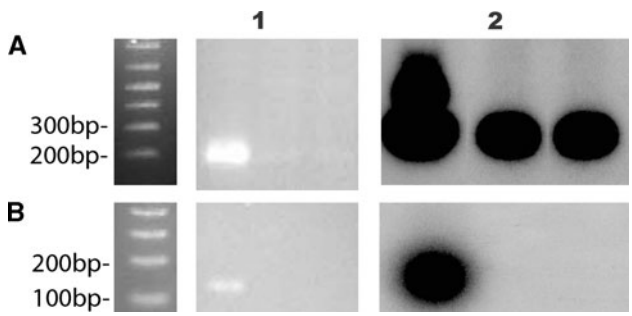

Fig. 1 **a** Nested PCR: (1) Amplification of 250 bp of MMTV-like *env* gene from breast cancer DNA using primers and conditions described in [1]. (2) Southern blot hybridization of the amplified PCR products using a $5'^{32}$P-labeled probe as described in [1]. **b** Nested PCR: (1) Amplification of 123 of MMTV-like *env* gene from breast cancer DNA using primers and conditions described by Park et al. (2) Southern blot hybridization of the amplified PCR products using a $5'^{32}$P-labeled probe (CAGGGGGAGTATAATTTCCAA) derived from Park et al. published sequences

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The results shown in Fig. 1 clearly indicate that the methodology of Park et al. is unable proficiently to amplify MMTV-like *env* gene sequences in breast cancer DNA. In only one of the three tumors were *env* sequences detected.

All our analyses are conducted in a laminar flow hood. The suggestion that positive results are due to contamination indicts many laboratories [3–10] whose efforts, like our own, are conducted with utmost care.

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REBUTTAL LETTER

Response to 'Presence of MMTV-like *env* gene sequences in human breast cancer'

Daniel J. Park · Melissa C. Southey · Graham G. Giles · John L. Hopper

To the Editor,

Pogo et al. in response to our recent publication [1], tested for MMTV *env* sequences in just three American breast cancer specimens using their previously published methods [2] and a modified version of the methods we described. They returned a signal from 3/3 cases using their previously published methods versus 1/3 using those published by us. However, Pogo et al. give no indication as to how these specimens were processed or selected or even whether controls were used, rendering impossible the meaningful interpretation of their data. Since our primer binding sites fall within the region amplified by their first round PCR but not their second round "nested" PCR, such results would be consistent with their template DNAs being contaminated with PCR amplicon. Pogo et al. state that the suggestion that positive results are due to contamination indicts many laboratories including theirs. While it is commendable that the authors conduct aspects of their analyses in a laminar flow hood, it is generally accepted that such a measure goes only some way towards the control of potential PCR product and non-PCR product-derived contamination. Potential sources of contamination, whether dust containing mouse tissue or PCR product, are present from the point of tissue collection onwards. Such contamination events do occur and should not be dismissed lightly.

We undertook this work because we were excited at the prospect of MMTV-like virus having a major aetiological role in human breast cancer in part due to the associated potential for vaccine intervention. We set out to verify previous reports of around 40% positivity in Australian breast cancer specimens [3] by testing similar Australian specimens but, in similar fashion to others before us [4], found null findings.

Convincing evidence for an association between MMTV-like virus and human breast cancer can only arise from carefully designed and rigorously conducted studies optimally involving multiple independent international groups and large numbers of independent breast cancer and control specimens. Peer-accepted alternative methods to PCR will be required in conjunction with PCR to build a convincing case.

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