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Short Communication

# Absence of rearrangements in the *BRCA2* gene in human cancers

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**Summary** Mutations of *BRCA2* in sporadic breast and ovarian carcinomas are exceedingly rare. This led to the suggestion that large genomic rearrangements could be involved. We performed Southern blots in genomic DNA from 130 primary breast cancers and 83 cancer cell lines (breast, ovarian, pancreatic and small cell lung carcinomas) and found no genomic rearrangements. These results suggest that a gene other than *BRCA2* is the target of the frequent 13q12.3 allelic deletions in human cancers. © 2001 Cancer Research Campaign http://www.bjcancer.com

**Keywords**: *BRCA2*; southern blot; human cancers; rearrangement; deletion

*BRCA2*, the second hereditary breast cancer gene, has been mapped to chromosome 13q12–13 (Wooster et al, 1994). *BRCA2* is a very large gene spanning more than 70 kb of genomic DNA encoding 3495 amino acids (Wooster et al, 1995; Tavtigian et al, 1996). To date more than 300 distinct germline mutations of *BRCA2* (http://www.nhgri.nih.gov/intramural_ research/lab_transfers/bic) have been identified that predispose carriers to breast cancer and to a lesser extent ovarian cancer (Rahman and Stratton, 1998). A small increase in risk for pancreatic and prostate cancer has also been reported in *BRCA2* pedigrees (Wooster et al, 1995; Lancaster et al, 1996; Phelan et al, 1996). Frequent loss of heterozygosity (LOH) at the *BRCA2* locus in a variety of sporadic cancers e.g. breast, ovarian (Lancaster et al, 1996), pancreatic, prostate (Cooney et al, 1996; Li et al, 1998), hepatocellular cancer (Kuroki et al, 1995), suggests this gene may behave as a tumour suppressor gene (Cleton-Jansen et al, 1995). However, no clear disease-causing somatic mutations have been described in *BRCA2* in sporadic breast cancers, and somatic mutations in ovarian cancers are very rare (Foster et al, 1996; Lancaster et al, 1996; Miki et al, 1996; Takahashi et al, 1996; Teng et al, 1996). The lack of somatic *BRCA2* mutations in sporadic breast and ovarian cancers could be due to the mutation detection assays used. Mutations may be missed if they are outside of the region of analysis and certain types of mutations (large deletions, insertions and duplications) may not be detected by PCR-based mutation detection assays. Southern blot analyses have identified 5 large Alu-mediated genomic deletions (Petrij-Bosch et al, 1997; Puget et al, 1997b; Swensen et al, 1997) and a 6 kb Alu-mediated duplication (Puget et al, 1997a) involving *BRCA1* in breast cancer families that would have been missed by conventional PCR-based mutation screening methods such as SSCP, PTT or direct sequencing using genomic DNA as template. A recent study found one case of sporadic breast cancer out of 81 studied with *BRCA1* genomic deletions (van der Looij et al, 2000). Similar large genomic deletions have also been described in other tumour suppressor genes e.g. *p53* (Masuda et al, 1987; Ruggeri et al, 1992), *hMLH1* (Nystrom-Lahti et al, 1995), *hMSH2* (Wijnen et al, 1998) and *Rb-1* (Ruggeri et al, 1992). Thus, we wanted to investigate whether similar genomic deletions occur in *BRCA2* which may have escaped detection using PCR-based techniques.

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## MATERIALS AND METHODS

We undertook Southern blot analysis of genomic DNA in a large series of 130 invasive breast tumours comprising ductal, lobular, mucinous, tubular, cribriform and squamous cell metaplastic carcinomas. These tumours were snap frozen at the point of collection. Genomic DNA from these primary tumours was digested with EcoRI. In addition, genomic DNA from established cell lines derived from breast (39), ovarian (29), pancreatic (7) and small cell lung (SCLC) tumours (8) was digested with 4 different restriction endonucleases (BamHI, EcoRI, HindIII and PstI). Digested DNA was size fractionated by electrophoresis and transferred onto nylon membranes. Filters were hybridized separately with two clones containing *BRCA2* cDNA. The first is a 5.8 kb fragment representing amino acids 1–1963 (*BRCA2*-front) and the second fragment is 4.6 kb from amino acids 1895–3495 (*BRCA2*-back).

## RESULTS

Initially, we detected aberrant-sized fragments in two primary tumours; tumour 386 with both probes and tumour 64 NT with *BRCA2*-front probe (Figure 1). There were 8 breast cancer cell lines that showed restriction fragment size fragment abnormalities with *BRCA2*-front and 4 with *BRCA2*-back. In ovarian cancer cell lines, abnormalities were only observed in 3 cell lines using *BRCA2*-front. No abnormalities were detected in any of the SCLC or pancreatic cell lines. A representative sample of these Southern blot experiments is presented in Figure 1.

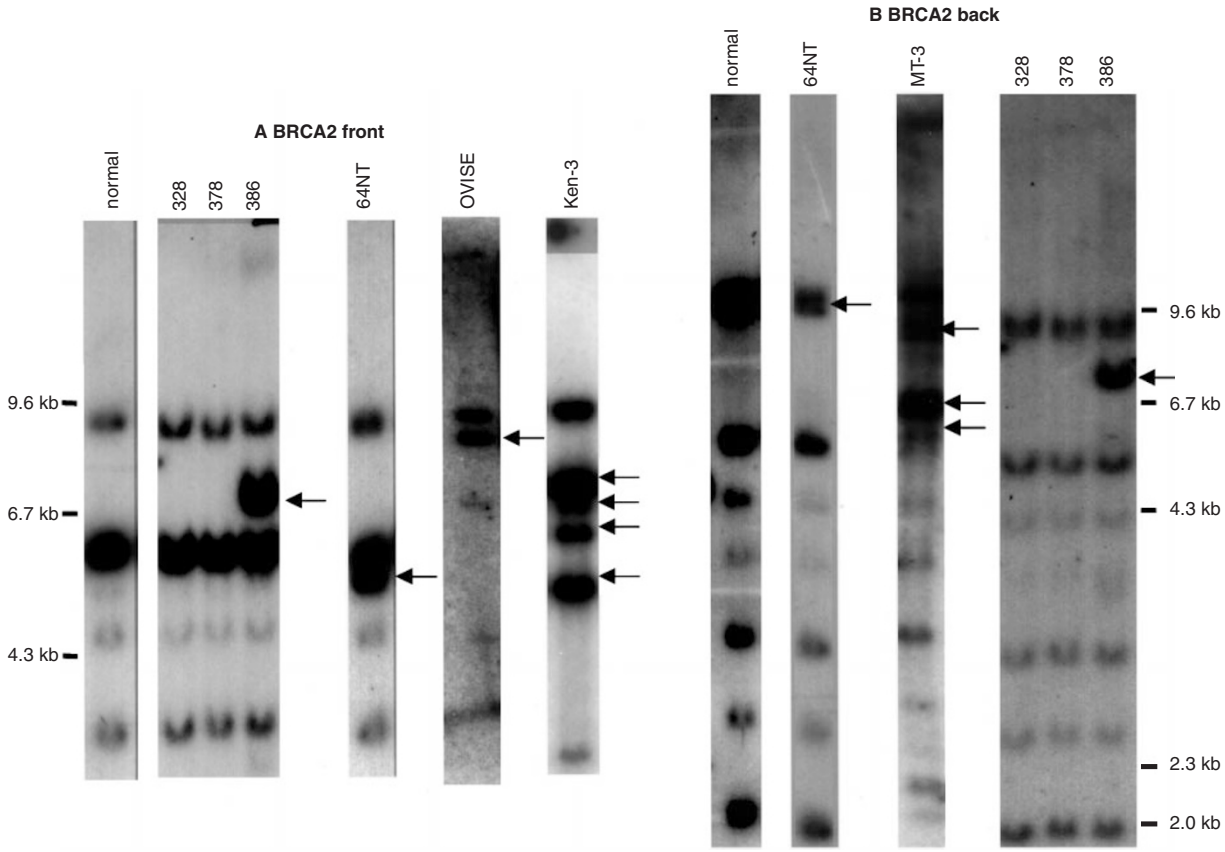


**Figure 1** Southern analysis of *BRCA2* gene in primary breast tumours (328, 378, 386, 64NT), breast cancer cell line (MT-3), and ovarian cancer cell lines (OVISE, KEN-3) digested with EcoRI. Hybridization with (**A**) *BRCA2*-front and (**B**) *BRCA2*-back shows aberrant restriction fragments

To confirm the presence of genomic rearrangements in the abnormal samples, the experiments were repeated with longer incubation of the DNA with the respective restriction endonucleases to ensure complete digestion. No abnormal restriction fragments were detected in any of the samples suggesting that the initial abnormal bands were due to incomplete digestion (data not shown). While no large genomic deletions were observed in the *BRCA2* gene, restriction fragment length polymorphisms were observed in both primary tumours and cell lines (Figure 2).

## DISCUSSION

In summary, *BRCA2* does not undergo large intragenic deletions in human tumours. Only 8 somatic *BRCA2* mutations, 3 in breast tumours (Lancaster et al, 1996; Miki et al, 1996; Weber et al, 1996), 4 in ovarian cancers (Foster et al, 1996; Takahashi et al, 1996) and one in a hepatocellular carcinoma (Katagiri et al, 1996), have been reported since the discovery of the gene using PCR-based mutation detection assays. Similar to *BRCA1*, the region containing *BRCA2* undergoes loss of heterozygosity in a fraction of breast and ovarian tumours (Cleton-Jansen et al, 1995). In fact in another study using these cell lines, we found that 24/36 breast (67%), 6/30 ovarian (20%), 4/7 pancreatic (57%) and 5/8 SCLC (63%) used in this study had homozygosity for all markers tested in the region encompassing *BRCA2* (data not shown) suggestive of allelic deletions. Partial mutational analyis of *BRCA2* mutations was undertaken for some of the cell lines with LOH and to date, only one breast cancer cell line with LOH, MT-3, was found to have a 1 bp deletion in exon 23 of *BRCA2* (KL Gorringe & C Caldas, unpublished data). Like *BRCA1*, the lack of *BRCA2* mutations in sporadic breast and ovarian cancers

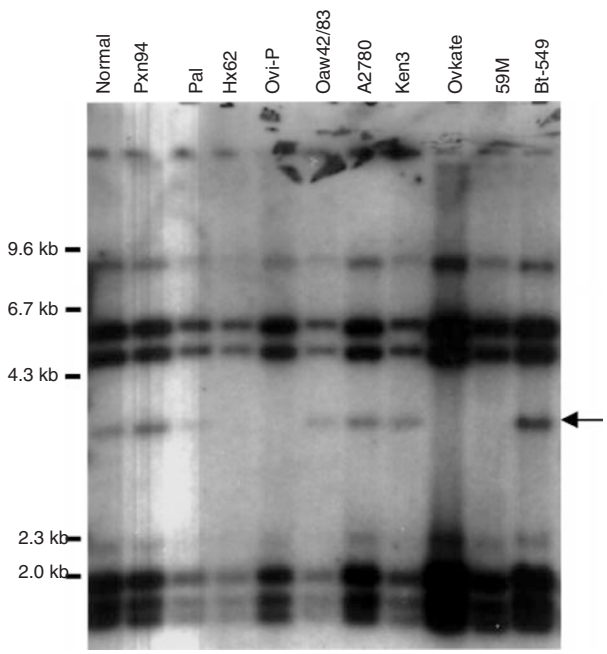


**Figure 2** Restriction fragment length polymorphism seen in the normal control, breast (BT-549) and ovarian cancer cell lines digested with Pst-1 and hybridized with *BRCA2*-front

suggest either distinct genetic pathways or different mechanisms for inactivating gene function compared to the familial forms (Rahman and Stratton, 1998). The high frequency of LOH on chromosome 13q could be explained by the close proximity of other tumour suppressor genes e.g. retinoblastoma (Lee et al, 1988), Brush-1 (Schott et al, 1994) or other putative tumour suppressor gene(s) that might be targeted instead of *BRCA2*.

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