Assignment¹ of Breast Cancer Associated 1 (*BRCA1*) to tammar wallaby (*Macropus eugenii*) chromosome 2q3 by in situ hybridization

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¹ To our knowledge this is the first time this gene has been mapped in tammar wallaby.

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Rationale and significance

BRCA1, located at 17q21.31 in humans, is a significant gene in familial breast and ovarian cancer. *BRCA1* functions as an important regulator of pathways governing DNA repair, cell-cycle progression, ubiquitylation and transcriptional regulation in mammals. Orthologs are not found in the yeast, fly or worm genomes (Venkitaraman, 2002; Narod and Foulkes, 2004). Previously *SOX9*, located at 17q24.3 in humans, was mapped to the long arm of chromosome 2 in the tammar wallaby (Pask et al., 2002). Therefore mapping of the tammar wallaby *BRCA1* homologue to chromosome 2q3 has extended the region of synteny with human chromosome 17q by 29 megabases, identifying a large segment of a chromosome block (C14) that is conserved in marsupial evolution (Rens et al., 2003).

Materials and methods

A 1.4-kb tammar wallaby (MEU) probe spanning exon 11 of *BRCA1* was amplified from male MEU genomic DNA with the forward primer 5'-ATTGCCCAACACAGACAGAATG-3' and reverse primer 5'-GAAACAA-TGAGGTACTGTCTGTAG-3'. Cycling parameters included a denaturation step of 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 90 s. This probe was labeled with ³²P and used to screen a male MEU genomic BAC library (Arizona Genomics Institute). PCR and sequencing of the 1.4-kb product was performed on DNA extracted from positive clones. A single significant tblastx hit to human chromosome

17 (NC_000017.9 at base 38499118, E-value 2e-32) confirmed that the clone Me-KBa-354-P-19 contained the MEU *BRCA1* homologue. This clone was labeled with digoxigenin-11-dUTP by nick translation, pre-annealed with 1 µg tammar wallaby C₀t-1 DNA at 37°C for 30 min and hybridized overnight at 37°C to male MEU metaphase chromosomes that had been denatured for 75 s at 70°C in 70% deionised formamide:2× SSC. Hybridization signals were detected with Cy3 conjugated to anti-digoxigenin antibodies; chromosomes were counterstained with DAPI.

Probe name: Me-AGI-354-P-19

Probe type: genomic BAC clone

Vector: pCUGI-BAC1

Proof of authenticity: Partial sequencing

Fig. 1. Localisation of *BRCA1* to tammar wallaby chromosome 2q3. A male *Macropus eugenii* metaphase spread showing the BAC clone Me-KBa-354-P-19, which contains sequences homologous to human *BRCA1*, hybridized to the long arm of chromosome 2.

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Results

Mapping data:

Most precise location: 2q3

No. of cells examined: 30

Number of cells with specific signal: 1 (0), 2 (4), 3 (19), 4 (7)
chromatids per cell

References

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