Growth retardation and tumour inhibition by *BRCA1*

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Inherited mutations in *BRCA1* predispose to breast and ovarian cancer, but the role of BRCA1 in sporadic breast and ovarian cancer has previously been elusive. Here, we show that retroviral transfer of the wild-type *BRCA1* gene inhibits growth *in vitro* of all breast and ovarian cancer cell lines tested, but not colon or lung cancer cells or fibroblasts. Mutant *BRCA1* has no effect on growth of breast cancer cells; ovarian cancer cell growth is not affected by *BRCA1* mutations in the 5' portion of the gene, but is inhibited by 3' *BRCA1* mutations. Development of MCF-7 tumours in nude mice is inhibited when MCF-7 cells are transfected with wild-type, but not mutant, *BRCA1*. Most importantly, among mice with established MCF-7 tumours, peritoneal treatment with a retroviral vector expressing wild-type BRCA1 significantly inhibits tumour growth and increased survival.

The breast and ovarian cancer susceptibility gene *BRCA1* is mutated in the germline and the normal allele is lost in tumour tissue from hereditary breast and ovarian cancer[1–4]. Somatic point mutations in *BRCA1* in sporadic tumours are very rare[5–7], but complete somatic deletion of one allele of *BRCA1* occurs in approximately 50% of sporadic breast cancers and 70% of sporadic ovarian cancers[8]. Expression of *BRCA1* mRNA is decreased in sporadic breast cancer, suggesting that *BRCA1* is altered at the genomic level in hereditary breast and ovarian cancer, but at either the genomic or transcriptional levels in sporadic cancer[9].

Most inherited *BRCA1* mutations produce truncated proteins that vary in length from 5% to 99% of full-length protein, although missense mutations are found which alter cysteines in the RING finger. The C-terminal end of the BRCA1 protein must be essential to normal BRCA1 function in breast epithelial cells, because patients inheriting 1853Stop develop very early onset breast cancer[10]. However, mutations in the 3' portion of *BRCA1* are less likely to lead to ovarian cancer than are mutations in the 5' portion of the gene[11].

BRCA1 expression increases in pregnancy, then declines after parturition[12,13]. Inhibition of *BRCA1* expression leads to growth acceleration of both normal and malignant breast epithelial cells[9]. Together these results suggest that BRCA1 may be a hormone-induced growth regulator. However, there has previously been no direct evidence that BRCA1 inhibits growth of breast or ovarian cancer at the cellular level, or that this effect is general and not limited to cases with inherited disease.

We carried out retroviral gene transfer of wild-type and mutant *BRCA1* to analyse the function of *BRCA1* in sporadic breast and ovarian cancer. Our results indicate that (i) wild-type *BRCA1* inhibits growth of breast and ovarian cancer cells *in vitro*; (ii) the C-terminal truncation mutant 1835Stop inhibits ovarian cancer cell growth, but not breast cancer cell growth, while other *BRCA1* mutants fail to inhibit either breast or ovarian cancer cell growth; (iii) MCF-7 breast cancer cells transfected with wild-type *BRCA1* are severely inhibited in their capacity to form tumours in nude mice; (iv) intraperitoneal injection of *BRCA1* as a retroviral construct into nude mice with established MCF-7 tumours increases expression of BRCA1 in these tumours; and (v) mice that have MCF-7 tumours when injected once with wild-type *BRCA1* retrovirus have significantly longer (3-fold increased) survival compared to mice injected with mutant *BRCA1* retrovirus.

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BRCA1 inhibits breast and ovarian cancer

Transfection experiments were carried out to determine whether *BRCA1* could inhibit growth of breast and ovarian cancer cells that came from different patients. MCF-7 and MDA-MB-157 breast cancer cell lines have very low expression of *BRCA1* mRNA[9] and BRCA1 protein (ref. 14 and unpublished data), and only one allele at each of the markers *D17S1185*, *D17S1320*, *D17S1321*, *D17S855*, *D17S1322*, *D17S1323* and *D17S1327*, indicating genomic loss of one copy of the 2 Mb region containing *BRCA1*. Transfection into these cell lines tests the effects of overexpressing *BRCA1* in tumours originally derived from patients who very likely have sporadic breast cancer. An ideal control would be a tumour cell line from a patient with an inherited *BRCA1* mutation and somatic loss of the remaining allele; such a cell

Table 1 Effect of *BRCA1* expression vectors on growth

Vector	Fibroblast (WI38)	Breast Ca (MCF-7)	Breast Ca (MB-157)	Ovarian Ca (CaOv-4)	Ovarian Ca (ES-2)	Ovarian Ca (PA-1)	Lung Ca (FK111)	Colon Ca (OK3)
LXSN	85+2.5	85+3.7	42+1.2	72+2.3	34+1.3	92+1.2	98+1.7	433+9.4
BRCA1	87+2.2	0+0[a]	0+0	0+0[a]	0+0	0+0.3	101+4.2	480+16.3
1835Stop	85+1.2	88+3.3	39+1.5	3+1.7	2+1.5	7+1.2	102+5.8	473+20.5
340Stop	87+1.4	89+3.3	44+2.1	80+2.7	32+1.7	92+1.4	99+5.0	483+33.0
Δ343–1081	84+1.4	96+3.7	40+1.5	76+4.9	32+1.5	93+1.9	97+3.7	460+29.4
Δ515–1092	88+2.4	93+15.9	48+2.5	77+4.2	34+1.5	96+1.9	99+5.0	473+28.7

G418-resistant transfectants per 10^7 cells. Mean + standard error.
[a]10–20 small colonies were identified in each transfection but these never grew beyond 30 cells per clone.

line would permit the test of growth inhibition in breast cancer cells definitively lacking any normal *BRCA1* expression. However, no long-term cell lines have yet been established from these patients.

Transfection of wild-type LXSN-*BRCA1* into breast cancer cell lines, MCF-7 and MDA-MB-157, and ovarian cancer cell lines, Caov-4, ES-2 and PA-1, significantly inhibited growth (Table 1). No colonies of the *BRCA1*-transfected breast or ovarian cancer cell lines grew beyond 30 cells per clone (we were eventually able to expand some of the MCF-7 clones in an enriched growth media containing GMS-A (Gibco), 10% fetal calf serum and 5 ng/ml EGF, in order to use these clones for subsequent studies). In contrast to the growth inhibitory effect of wild-type *BRCA1* on breast and ovarian cells, fibroblasts and lung and colon cancer cells were not inhibited by LXSN-*BRCA1*. Expression vectors producing a 340-amino acid truncated BRCA1 protein or either of two internally deleted proteins did not inhibit growth of any cell line, indicating that both the disease-associated mutations and the internal deletions produced functionally inactive BRCA1. However, a truncated 1,835-amino acid BRCA1 protein significantly inhibited growth of ovarian cancer cells, but not breast cancer cells. This suggests that different mechanisms mediate growth inhibition of ovarian cancer cells and breast cancer cells and that this difference depends on the length of the truncated protein.

Successful transfer of the *BRCA1* gene into the cancer cells was shown by both RT-PCR analysis and immunoblotting (Fig. 1). Transfectants expressed wild-type *BRCA1* or mutant *BRCA1* mRNA of the expected sizes (Fig. 1*a*). The 190-kD BRCA1 protein was expressed in transfected MCF-7 cells (Fig. 1*b*, lanes 3–4), but not in untransfected MCF-7 cells (Fig 1*b*, lanes 1–2). The level of BRCA1 expression in transfected cell lines and in tumours is still quite low (<20%) relative to that in normal breast or ovarian cells, probably because normal expression of the growth-inhibitory BRCA1 protein is incompatible with growth of MCF-7 cells *in vitro* or *in vivo*. To confirm that the unaffected growth of lung and colon cancer cells resulted from failure of inhibition, rather than simply poor expression or unstable BRCA1 protein, we measured the level of BRCA1 protein in lung and colon cancer cells stably transfected with LXSN-*BRCA1*. Transfected lung and colon cancer cells overexpressed BRCA1 protein, but their growth was not inhibited (Fig. 1*c*).

Mutation site and ovarian cancer susceptibility

To determine whether the differential effects of 5' versus 3' *BRCA1* mutations on growth of ovarian cancer cell lines were paralleled in human patients, we calculated the relative frequency of ovarian versus breast cancer among 166 patients in our series inheriting *BRCA1* mutations (Table 2). Mutations inherited by 19 patients were nonsense alterations leading to transcript

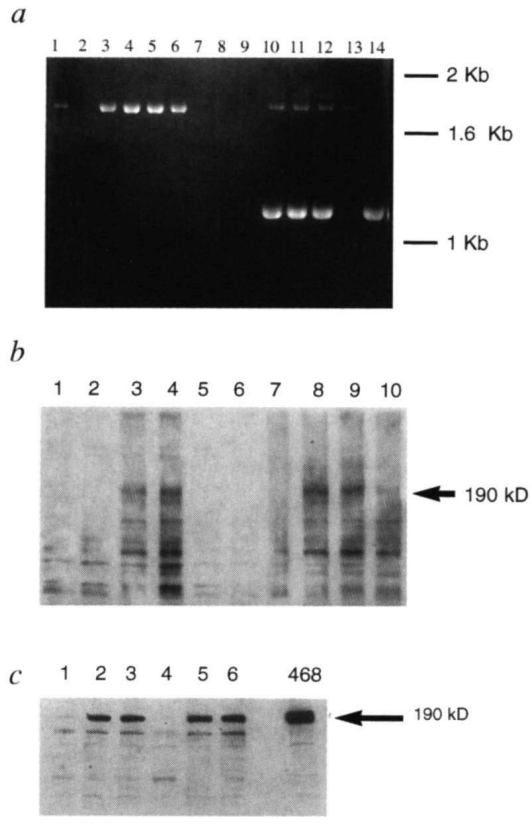

Fig. 1 Expression of transferred *BRCA1* genes *a*, Expression of *BRCA1* transcript in transfected MCF-7 cells. RT-PCR of RNA from MCF-7 breast cancer cells transfected with wild-type and mutant *BRCA1*. Primer pair 2a (see Methods) yields a 1.7-kb band in wild-type *BRCA1* transfected cells; primer pair 3 yields a 1.1-kb band in BRCA1 Δ343–1081 transfected cells. Lanes are as follows: untransfected MCF-7 cells tested with primer pair 2a (lane 1) or primer pair 3 (lane 2); MCF-7 cells transfected with wild-type *BRCA1* tested with primer pair 2a following reverse transcription (lanes 3–6) or without reverse transcription (lanes 7–9); MCF-7 cells transfected with mutant *BRCA1* tested with primer pairs 2a and 3 simultaneously (lanes 10–12); control amplification of wild-type LXSN-*BRCA1* plasmid DNA with primer pair 2a (lane 13); control amplification of LXSN-BRCA1 Δ343–1081 plasmid DNA with primer pair 3 (lane 14). *b*, Expression of BRCA1 protein in transfected MCF-7 cells and tumours. Whole cell lysates from MCF-7 tumours were probed with antibody (C-20, Santa Cruz Biotechnology) made to the C-terminal end of BRCA1 (ref. 14). Lanes are as follows: lysates from untransfected MCF-7 cells (lanes 1–2); lysates from MCF-7 clones transfected with wild-type *BRCA1* (lanes 3–4); lysates from uninfected MCF-7 tumours (lanes 5–7); lysates from MCF-7 tumours *ex vivo* transduced with wild-type LXSN-*BRCA1* (lanes 8–10). *c*, Immunoblot of lysates from FK111 lung cancer cells (Lanes 1–3) and OK3 colon cancer cells (Lanes 4–6) probed with C-20 antibody. Lanes 1 and 4 represent untransfected controls and lanes 2, 3, 5, and 6 represent separate sets of pooled stably transfected clones of *BRCA1*. 468 is a control lysate from MDA-MB-468 cells.

instability and no mutant protein. Mutations inherited by 13 patients were missense alterations in the RING finger leading to complete but aberrant protein. All other mutations were protein-truncating mutations at sites throughout the gene. Our hypothesis was that truncations on opposite sides of the granin motif at codons 1214–1223 (ref. 14) would be associated with different susceptibilities to ovarian cancer. The difference in ovarian and breast cancer distribution between the two groups was statistically significant; ovarian cancer formed a significantly lower proportion (2%) of the cancers in patients with mutant proteins that would include the granin motif (see accompanying paper on page 303 of this issue) compared to the proportion (25%) of cancers in patients with more severely truncated proteins ($\chi^2 = 11.12$, $P < 0.001$). This result is consistent with the previous observation that the site of *BRCA1* mutation is associated with relative susceptibility to ovarian versus breast cancer[11]. The granin consensus motif at codons 1214–1223 is well within the confidence limit for the estimated location of the optimal change point in that analysis[11].

Table 2 Inherited BRCA1 mutations and type of cancer

Termination codon of mutant protein	Cancer site Breast	Ovary
0[a]	16	3
36	2	
37	7	1
39	17	9
64	6	4
81	4	2
313	5	1
766	3	4
780	7	
901	14	4
915	4	3
1203	6	
1214–1223	Granin motif	
1265	5	
1364	12	1
1829	6	
1853	7	
1863[b]	13	
Totals:		
0–1223	91	31
Granin motif 1223–1863	43	1

[a]Nonsense mutations leading to loss of transcript.
[b]Complete protein: missense mutations in RING finger.

BRCA1 inhibits mouse breast tumorigenesis

Transfer of wild-type *BRCA1* into MCF-7 cells inhibited the capacity of MCF-7 cells to form tumours in nude mice. Supernatants containing 5×10^7 vector particles from LXSN control and LXSN-*BRCA1* PA317 producer clones were used to transduce 5×10^7 MCF-7 cells and OK3 colon cancer cells in culture. Transduced cells were injected into the flanks of six nude mice for each vector and each cell line (cells were not treated with G418 before injection, but analysis of Southern blots demonstrated that 70–80% of MCF-7 cells are transduced by this protocol[15]). Four weeks after injection, tumours developed in five of six mice injected with MCF-7 cells carrying LXSN control retrovirus, but no tumours developed in the six mice injected with MCF-7 cells carrying wild-type LXSN-*BRCA1* (Table 3). However, retroviral transduction by *BRCA1* had no effect on colon tumour formation (Table 3). Tumours ultimately developed in all LXSN control mice and 4/6 LXSN-*BRCA1* mice. However, the tumours in LXSN-*BRCA1* mice were significantly smaller (60 ± 24 mg) compared to tumours of the LXSN control mice (569 ± 60 mg) ($P = 0.0018$ (paired), 0.0007 (independent)).

Because these retroviral transduction studies did not result in 100% transduction rates and did not completely prevent tumour development, other mice were injected with stable MCF-7 transfectants expressing *BRCA1*. MCF-7 clones transfected with wild-type *BRCA1* required enriched media and grew far more slowly than MCF-7 clones transfected with mutant *BRCA1* (Fig. 2*a*). Growth inhibition of MCF-7 clones transfected with wild-type BRCA1 was cell-cycle dependent, reflecting either G_1/S inhibition or a prolonged G_0/G_1 phase. Three MCF-7 clones stably transfected with *BRCA1* Δ343–1081 and four MCF-7 clones stably transfected with wild-type *BRCA1* were each injected into five mice. None of the 20 mice injected with wild-type *BRCA1* transfectants developed tumours, whereas all 15 mice injected with mutant *BRCA1* transfectants developed tumours (Table 3), confirming that *BRCA1* inhibits tumorigenesis in nude mice.

BRCA1 inhibits established tumours in mice

LXSN-*BRCA1* vectors were injected into the peritoneum of mice with established MCF-7 tumours to determine if wild-type *BRCA1* could be integrated into

Table 3 Inhibition of tumorigenesis in mice by *BRCA1*

	LXSN vector wild-type *BRCA1*	*BRCA1* del 343–1081	Control
Mice injected with MCF-7 cells transduced with BRCA1 *or control retrovirus*			
Number of mice with tumours 4 weeks after injection	0/6	5/6	6/6
Number of mice with tumours 8 weeks after injection	4/6	6/6	6/6
Weight of tumours at 8 weeks (mgms)	60 ± 24	569 ± 60	610 ± 82
Mice injected with transduced colon tumour cells			
Number of mice with tumours 4 weeks after injection	6/6	6/6	5/6
Weight of tumours (mgms)	1540 ± 128	1633 ± 110	1490 ± 92
Mice injected with MCF-7 cells stably transduced with retrovirus			
Number of mice with tumours 8 weeks after injection	0/20	15/15	ND
Mice with established MCF-7 tumours injected with BRCA1 *retroviral vectors*			
Days survival after injection (see also Fig. 3)	15, 18, 22, 26, 41	4, 8, 9, 11, 11	ND
Mean and standard error of survival (days)	24.4 ± 2.1	8.6 ± 1.3	

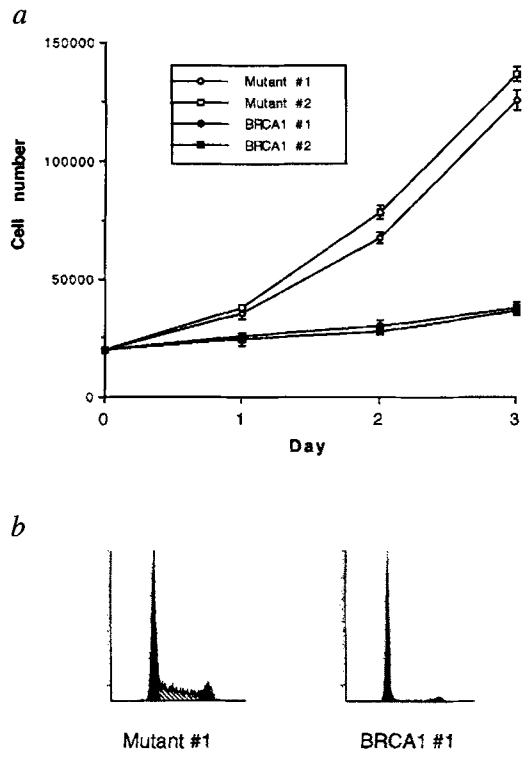

Fig. 2 Growth of MCF-7 breast cancer cells transfected with wild-type versus mutant *BRCA1*. *a*, Growth rates (mean ± standard deviation) of two MCF-7 clones stably transfected with wild-type *BRCA1* versus *BRCA1* Δ343–1081 in media containing 5 ng/ml EGF and 10% fetal calf serum. *b*, Proportion of MCF-7 cells in various stages of the cell cycle after stable transfection and expression of wild-type or mutant *BRCA1*. Wild-type *BRCA1* clone #1: G_0–G_1 89%, G_2–M 5%, S 6%; G_2/G_1 1.97; CV 2.88. Mutant clone #1: G_0–G_1 54%, G_2–M 9%, S 37%; G_2/G_1 1.96; CV 3.75.

tumour cells, inhibit tumour growth and improve survival. Five mice with established MCF-7 peritoneal tumours (multiple 3–5 mm palpable nodules) were injected with wild-type LXSN-*BRCA1*; five other mice with established MCF-7 tumours were injected with mutant LXSN-*BRCA1* Δ343–1081. Previous experience indicated that this experimental approach generally results in retroviral vector integration into 20 to 40% of tumour cells[15]. BRCA1 protein was expressed in MCF-7 tumours in mice treated with wild-type LXSN-*BRCA1* retroviral vector (Fig 1*b*, lanes 8–10). No BRCA1 protein expression was detectable in tumours of mice not treated with *BRCA1* (Fig. 1*b*, lanes 5–7).

All five mice treated with mutant *BRCA1* retrovirus died in less than two weeks (8.6 ± 1.3 days survival post injection); the five mice injected with wild-type *BRCA1* survived from 15–41 days (24.4 ± 2.1 days survival post injection) (Table 3; Fig. 3). This difference was not due to a systemic effect of retroviral infection because injection of mutant *BRCA1* retrovirus (Table 3) as well as two additional control retroviruses[15] did not inhibit tumour growth.

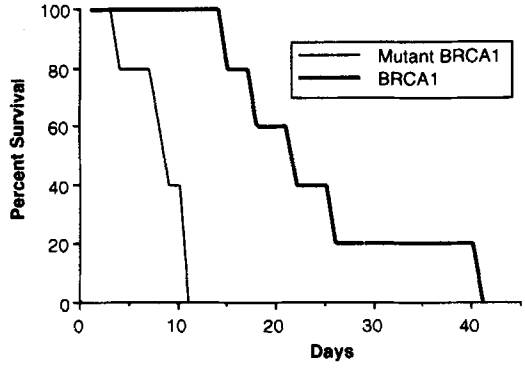

Fig. 3 Survival of mice with established intraperitoneal tumours treated once with retroviral construct containing wild-type *BRCA1* (upper curve) versus mutant *BRCA1* Δ343–1081 (lower curve).

Necropsies were carried out on all mice. The five mice treated with mutant *BRCA1* retrovirus all died of cancer, with tumours ranging in size from 650 to 840 mg at death. Of the five mice treated once with wild-type *BRCA1* retrovirus, three had detectable tumours at death, with sizes of 460 mg (15 days survival; cause of death probably the tumour), 320 mg (18 days survival; cause of death uncertain) and 290 mg (22 days survival; cause of death uncertain). The two mice surviving 26 and 41 days had no detectable tumour and died of pneumonia or unknown cause. Future experiments will include more than one peritoneal treatment with wild-type *BRCA1* retrovirus.

Discussion

Overexpression of *BRCA1* inhibits the growth of breast and ovarian cancer cells and suppresses tumorigenesis of MCF-7 breast cancer cells. Near full-length truncated BRCA1 proteins do not inhibit breast cancer cell growth, but do inhibit ovarian cancer cell growth, in parallel with the observation that human patients with near-full length truncated BRCA1 proteins may develop very early-onset breast cancer but rarely ovarian cancer. The mechanism of tumour suppression by *BRCA1* may differ for breast versus ovarian cancer, and this may have implications for diagnostic screening and therapy.

Disease-associated truncation mutations eliminate growth inhibition, providing support for the hypothesis that growth inhibition constitutes a tumour suppressive function of *BRCA1* and providing a functional assay for distinguishing mutations from polymorphisms. In-frame deletion mutants which delete a highly charged region containing potential glycosylation sites indicate that this region of the protein is required for growth inhibition. These data provide support for the hypothesis that glycosylation of BRCA1 contributes to its function as a granin[14]. This growth inhibition assay will allow further analysis of missense mutations and detection of potential structural domains.

BRCA1 growth inhibition is specific for certain cells and appears to be cell cycle dependent. This tissue specificity is unlike previously described tumour suppressors, notably p53 and Rb, which interact with general cell cycle mechanisms. We have recently shown that BRCA1 is present in secretory vesicles and is secreted[14] suggesting that BRCA1 may only inhibit growth of cells that either contain a regulated secretory

pathway or possess an appropriate receptor. Inhibition of tumour growth observed in mice was also specific to certain tissues, since breast tumours were inhibited by injection of wild-type LXSN-*BRCA1*, but colon tumours were not. The mechanism responsible for this tumour suppression was not merely prevention of tumour formation or early angiogenesis because injection of wild-type LXSN-BRCA1 retrovirus slowed the growth of established tumours.

The simplest explanation for these observations of both growth inhibition and tumour suppression is that BRCA1 inhibits tumour development by slowing or stopping growth of tumour cells *in vivo*. The combination of effectiveness and tissue specificity of growth inhibition by *BRCA1* retroviruses may enable BRCA1-mimetic agents or *BRCA1*-based gene therapy to be used to treat human breast and ovarian tumours.

Methods

Vector construction. The LXSN-*BRCA1* retroviral expression vector was constructed by cloning a *Sal*I-linkered *BRCA1* cDNA (nucleotides 83–5711) (ref. 2) into the *Xho*I site of the retroviral vector LXSN for transfection and retroviral transduction studies. Two nonsense mutations which produced predicted proteins of 1835 and 340 amino acids were constructed by restriction digestion of the parent vector LXSN-*BRCA1* with *Apa*I and *Bgl*II, respectively, followed by insertion of a termination codon linker and re-ligation. The two internal deletion mutants, which eliminated the region of *BRCA1* containing acidic residues and putative glycosylation sites, were constructed by restriction enzyme digestion and re-ligation because the selected restriction enzymes deleted fragments which conserved the reading frame. Δ343–1081 was constructed by digestion of LXSN-*BRCA1* with *Bsm*I followed by re-ligation, and Δ515–1092 was constructed by digestion of LXSN-*BRCA1* with *Bsu*36I followed by re-ligation. Constructs were confirmed by DNA sequencing.

Cell culture, gene transfer methods and nude mice studies. Cancer cell lines were obtained from American Type Culture Collection (ATCC), Rockville, MD. For the growth inhibition experiments, cells were plated in six well microtiter plates, transfected at 80% confluence by calcium phosphate coprecipitation for 4 h followed by glycerol shock, and then selected for 7–10 d with 500 μg/ml G418. All transfection studies were performed at least in triplicate.

The nude mice studies were performed with retroviral vector preparations obtained from cloned PA317 producer clones with a titer of 5–9 × 10^6 virions/ml. PA317 producer clones for the nude mice transduction studies were produced by transfecting Psi2 cells with LXSN-*BRCA1* or LXSN- Δ343–1081 *BRCA1* and then infecting PA317 cells with the resulting Psi-2 supernatant, as described[15]. For the *ex vivo* transduction studies, cultured MCF-7 breast cancer cells and OK3 colon cancer cells were transduced *in vitro* with LXSN-*BRCA1* or LXSN-Δ343–1081 *BRCA1* and then the transduced cells were injected subcutaneously into the left flank of 4 w old female nu/nu mice containing slow-release estrogen pellets[16]. Tumour size was determined weekly and animals were necropsied at 8 w after injection for determination of tumour weight and RT-PCR analysis for gene expression[9]. For evaluation of effects of *BRCA1* and mutant retroviral vectors on established tumours, 10^7 MCF-7 cells were injected into mice intraperitoneally and followed until palpable tumours were identified. At this point the mice were injected intraperitoneally with high titer retroviral vector stock (10^7 virions/ml) as described[15]. The possibility of viral spread of retroviral infection was eliminated by demonstrating that the retroviral vector preparation contained no replication-competent retrovirus by S+L- assays following amplification in *Mus dunni*[15].

Cell growth and gene expression. Cell growth studies were performed with early passage cells analysed at cell concentrations between 5 × 10^4 and 2 × 10^6 cells/ml. The DNA content studies were performed with stable MCF-7 transfectants grown in enriched growth media containing GMSA, 10% fetal calf serum and 5 ng/ml EGF. Following methanol fixation, the cells were incubated with propidium iodide and DNA content determined by flow cytometry. Cell lysates were prepared and immunoblotting performed as described[14,17]. RNA was isolated by our published methods[18] and RT-PCR performed with the indicated primers (Fig. 1*a*) by our published methods[9]. Primer pair 2a (forward primer: 5'–TGGCAAAGGCATCTCAGGAA–3'; reverse primer: 5'–GTGGGGGATCTGGGGTATCA–3') within exon 11 detects a 1.7-Kb fragment in wild-type *BRCA1*; primer pair 3 (forward primer: 5'–GAACATCATCAACCCAGTAAT–3'; reverse primer: 5'–CCCGTTCCTCTTTCTTCATCAT–3') detects a 1.1-kb fragment in mutant *BRCA1* del 343–1081.

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1. Hall, J.M. *et al.* Linkage of early-onset breast cancer to chromosome 17q21. *Science* **250**, 1684–1689 (1990).
2. Miki, Y. *et al.* A strong candidate gene for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* **266**, 66–71 (1994).
3. Smith, S.A., Easton, D.F., Evans, D.G.R. & Ponder, B.A.J. Allele losses in the region 17q12–q21 in familial breast and ovarian cancer non-randomly involve the wild-type chromosome. *Nature Genet.* **2**, 128–131 (1992).
4. Cornelius, R.S. *et al.* High allele loss rates at 17q12–q21 in breast and ovarian tumors from BRCAI-linked families. The Breast Cancer Linkage Consortium. *Genes Chrom. Cancer* **13**, 203–210 (1995).
5. Futreal, P.A. *et al.* *BRCA1* mutations in primary breast and ovarian carcinomas. *Science* **266**, 120–122 (1994).
6. Merajver, S.D. *et al.* Somatic mutations in the *BRCA1* gene in sporadic ovarian tumours. *Nature Genet.* **9**, 439–443 (1995).
7. Hosking, L. *et al.* A somatic *BRCA1* mutation in an ovarian tumour. *Nature Genet.* **9**, 343–344 (1995).
8. Takahashi, H. *et al.* Mutation analysis of the *BRCA1* gene in ovarian cancers. *Cancer Res.* **55**, 2998–3002 (1995).
9. Thompson, M.E., Jensen, R.A., Obermiller, P.S., Page, D.L. & Holt, J.T. Decreased expression of *BRCA1* accelerates growth and is often present during sporadic breast cancer progression. *Nature Genet.* **9**, 444–450 (1995).
10. Friedman, L.S. *et al.* Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nature Genet.* **8**, 399–404 (1994).
11. Gayther, S.A. *et al.* Germline mutations of the *BRCA1* gene in breast/ovarian cancer families: evidence for a genotype/phenotype correlation. *Nature Genet.* **11**, 428–433 (1995).
12. Marquis, S.T. *et al.* The developmental pattern of *BRCA1* expression implies a role in differentiation of the breast and other tissues. *Nature Genet.* **11**, 17–26 (1995).
13. Lane, T.F. *et al.* Expression of *BRCA1* is associated with terminal differentiation of ectodermally and mesodermally derived tissues in mice. *Genes Dev.* **9**, 2712–2722 (1995).
14. Jensen, R.A. *et al.* BRCA1 is secreted and exhibits properties of a granin. *Nature Genet.* **12**, 303–308 (1996).
15. Arteaga, C.L. & Holt, J.T. Tissue-targeted c-fos retroviral vector inhibits established breast cancer xenografts in nude mice. *Cancer Res.* **56**, 1098–1103 (1996).
16. Soule, H.D. & McGrath, C.M. Estrogen responsive proliferation of clonal human breast carcinoma in athymic mice. *Cancer Lett.* **10**, 177–189 (1980).
17. Jensen, R.A., Beeler, J.F., Heidaran, M.A. & LaRochelle, W.J. Purification and characterization of baculovirus-expressed human α and β platelet-derived growth factor receptors. *Biochemistry* **31**, 10887–10892 (1992).
18. Jensen, R.A., Page, D.L., & Holt, J.T. Identification of genes expressed in premalignant breast disease by microscopy-directed cloning. *Proc. Natl. Acad. Sci. USA* **91**, 9257–9261 (1994).