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Of mice and (wo)men: genotype–phenotype correlations in BRCA1

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To date, over 6300 mutations in *BRCA1*, involving 1100 distinct sites, have been described and reported in the BIC (breast cancer information core) database. Since the first *BRCA1* mutations in early-onset breast and ovarian cancer families were reported, several attempts to establish genotype–phenotype correlations for this gene have been reported. Moreover, *in vitro* data have suggested dominant-negative effects of putative mutant *BRCA1* proteins over wild-type proteins. Genotype–phenotype correlations are not only important for predicting the clinical course of the disease and to allow tailor-made surveillance of individuals at risk, but also have implications for the elucidation of the molecular genetic mechanisms underlying *BRCA1*-mediated tumorigenesis and the development of gene transfer-based therapies. Here, we discuss genotype–phenotype correlations at the *BRCA1* locus in mouse and man, and the functional aspects that may account for these observations.

BRCA1 MUTATIONS: MOLECULAR CONSEQUENCES AT THE mRNA AND PROTEIN LEVEL

The majority of mutations found in the human *BRCA1* gene predict the expression of a mutant protein, either due to truncating or missense mutations. Unfortunately, very little data is available on the *in vivo* stability of these alleged mutant proteins. Recently, it has been suggested that the majority of mutations leading to premature stop codons trigger nonsense-mediated decay (NMD) of the mutant mRNA (1). Again, although messenger abundance was clearly reduced (1.5–5-fold), the consequences of NMD on protein levels were not investigated.

To the best of our knowledge, the only known example of a *BRCA1* mutation leading to a decreased but detectable expression of the mutant protein is found in the tumor cell line HCC1937 (2,3). This mutation (exon 20; 5382insC) causes a frame-shift, resulting in a premature stop codon within exon 24 (the last exon of the gene). Perrin-Vidoz *et al.* (1) confirmed that this specific mutation does not trigger nonsense-mediated decay, most likely because the activating signal for NMD is an exon–exon junction downstream to the premature stop codon. Still, even if the majority of mutations were to affect mRNA stability, the establishment of genotype–phenotype correlations at the *BRCA1* locus implies that the residual expression of mutant proteins (truncated or missense) may have specific consequences for the clinical course of the disease.

BRCA1 GENOTYPE–PHENOTYPE CORRELATIONS IN MEN

Shortly after the identification of *BRCA1*, it became clear that mutations in sporadic breast cancer are extremely rare. However, although only a few percent of breast cancer cases are due to a germline mutation in *BRCA1*, a relatively large number of families are affected by germline mutations in this gene, and therefore represent a useful source for the establishment of genotype–phenotype correlations.

The first genotype–phenotype correlation described in *BRCA1* was the observation of a decreased ratio of ovarian to breast cancer among 33 families with germline mutations located 3′ to exon 12 (4) (Fig. 1). A subsequent study showed a similar correlation with mutations 3′ to the alleged granin consensus motif encompassed by exon 11 (5) (amino acids 1214–1223). In view of the close proximity of this sequence to the exon 12/13 boundary described by Gayther *et al.* (4), and of the fact that the granin motif is no longer believed to be functionally significant, the data described by Holt and

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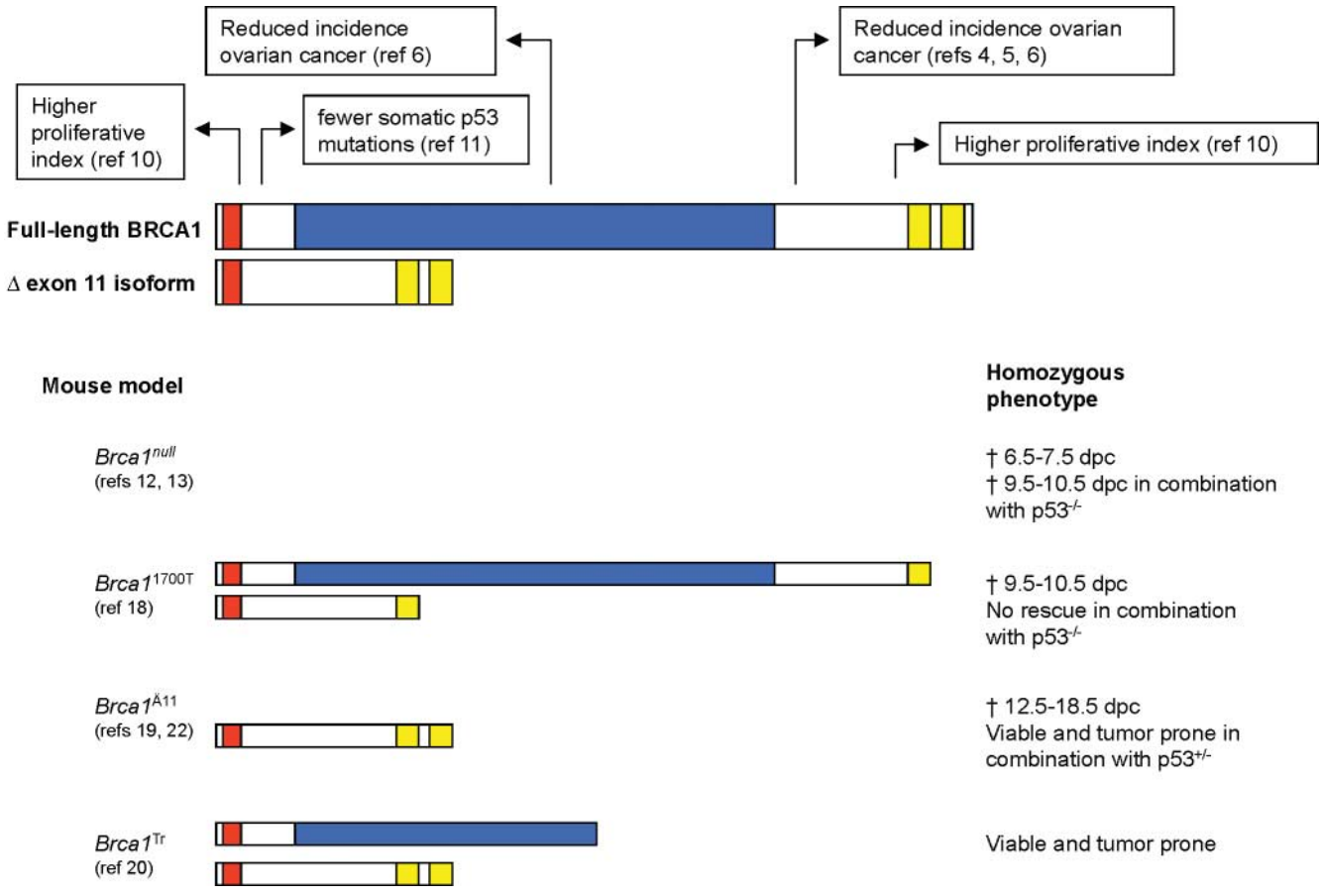

Figure 1. Genotype–phenotype correlations in mice and men. Wild-type human and murine BRCA1 is depicted in the middle, with the RING finger domain in red, the exon 11-encoded part of the protein in blue and the BRCT repeats in yellow. In the upper part the genotype–phenotype correlations described for human families are given. The lower part shows the four categories of targeted *Brca1* mouse models (see text) with their predicted resulting proteins and phenotypes.

co-workers (5) are likely to represent a refinement of the same genotype–phenotype correlations.

A more recent and comprehensive study of a cohort of 356 families characterized by protein-truncating mutations further confirmed this correlation (6). Here, families with mutations 3′ of nt 4191 (codon 1358), i.e. in between the boundaries described in the two above studies, showed a significantly lower incidence of ovarian cancer. This correlation is well within the 95% CI as determined in the study by Gayther *et al.* (4). Moreover, Thompson *et al.* (6) showed that families with a mutation in the 5′ region of *BRCA1* (upstream of nt 2401, codon 800) are also characterized by a decreased ovarian to breast cancer ratio.

Thus, in view of the above studies, the *BRCA1* gene can be subdivided in three fragments, mutations in the middle of which, delimited by nt 2401–4191, result in the highest ovarian to breast cancer ratio (Fig. 1). This may indicate that truncated proteins partly encompassing the functional domains located in the central part of a mutant BRCA1 exert more deleterious effects on ovarian then breast tissue homeostasis. Alternatively, mutations at the 5′ and 3′ end fragments of *BRCA1* may result in unstable polypeptides when compared with truncating mutations in the central fragment. A comparable situation has been described for the adenomatous polyposis coli (*APC*) tumor suppressor gene where mutations in the extreme 5′ end and in the 3′ half of the gene result in attenuated or atypical familial adenomatous polyposis (AFAP or AAPC), a FAP phenotypic variant characterized by a reduced polyp multiplicity and delayed age of onset. In this case, it was shown that AAPC mutations either result in nearly full-length proteins by internal initiation (5′ mutations) or in unstable mRNAs and/or truncated proteins (3′ mutations) (7–9). This emphasizes the need to study the effect of *BRCA1* mutations at the protein level in order to refine genotype–phenotype correlations.

Finally, BRCA1 studies with smaller cohorts showed that tumours from families with extreme 5′ or 3′ mutations have a higher proliferative index, whereas tumors from families with mutations 3′ to the RING finger domain are characterized by decreased incidence of somatic mutations in p53 (10,11) (see below and Fig. 1).

Brca1 GENOTYPE–PHENOTYPE CORRELATIONS IN MOUSE MODELS

Similar to the situation in man, genotype–phenotype correlations can be established in *Brca1* mouse models, notwithstanding the lack of clear-cut tumor susceptibility in the latter

animals. Homozygosity for all but one of the targeted *Brca1* mutations results in embryonic lethality at different developmental stages; this phenotypic variability is likely to be related to the specific *Brca1* genotypes. Several different mouse models have been generated, which can be grouped into four classes (Fig. 1). When trying to understand the effects of different mutations in *Brca1*-mutant mice, one should take into account that exon 11, the largest *Brca1* exon that encompasses 63% of the coding sequence and many of the known functional domains, is alternatively spliced in a broad spectrum of tissues.

The majority of targeted *null* alleles (here referred to as $Brca1^{null}$) will affect both exon 11-deficient and -proficient variants. This is the case of *Brca1* mouse models carrying deletions of exon 1–2 (12) and exon 5–6 (13). The antisense insertion of a neomycin resistance gene within exon 11 as reported by Liu *et al.* (14) is expected to result, analogous to the situation in *Apc* mouse models (15–17), in only residual amounts of the predicted mutant protein. However, this can only be confirmed by detailed biochemical analysis of mutant mouse cells. Likewise, it is at present not clear whether this targeted mutation also affects the *Brca1* mRNA species that do not encompass exon 11.

The second class of targeted alleles features a mutation 3′ of exon 11, thereby also affecting both exon 11-deficient and exon 11-proficient forms. This is represented by the $Brca1^{1700T}$ mouse model that we have generated by inserting the neomycin gene within exon 20 in the sense orientation (18).

Mice carrying targeted deletions of exon 11 form the third group ($Brca1^{\Delta 11}$). Xu *et al.* (19) generated such an allele by flanking exon 11 with LoxP sites and breeding the corresponding animals with a ubiquitous EIIA-Cre transgene.

Finally, a fourth category is represented by mice where a truncating mutation was targeted within exon 11, thereby affecting the exon 11-proficient isoform only and leaving the exon 11-deficienct form intact. The $Brca1^{Tr}$ mouse model has been obtained by inserting a loxP site within exon 11, leading to an in-frame stop codon at amino acid 924 (20).

Two additional models have been generated using constructs aimed at the deletion of the intron 10–exon 11 splice acceptor region. Possibly due to subtle differences in the targeting constructs, this approach has led in one case to a *null* (21), and to a Δ11 allele in the other (22).

As mentioned above, the majority of the *Brca1* mouse models are homozygous lethal. However, the exact causes and developmental stage of their embryonic lethality appear to differ among the different targeted alleles. $Brca1^{null}$ models die around 6.5–7.5 d.p.c. because of a block in the cell cycle. $Brca1^{1700T/1700T}$ embryos die at 9.5–10.5 d.p.c. due to widespread apoptosis, although growth delay can be observed from as early as 7.5 d.p.c. The exon 11-deficient mice die late in gestation (12.5–18.5 d.p.c.) owing to apoptosis and neuroepithelial abnormalities (exencephaly). Finally, the exon 11 truncating mutation in the $Brca1^{Tr}$ model is compatible with adult life. Interestingly, 85% of the homozygous $Brca1^{Tr}$ mice develop tumors in different tissues.

It should be emphasized that, since antibodies recognizing murine Brca1 became available only recently (23), it is not yet clear whether the mutant proteins predicted to result from these targeted *Brca1* mutations are stably expressed. However, as is the case with the human genotype–phenotype correlations, the different developmental defects characteristic of each targeted mutation strongly suggest that at least some of these alleles are hypomorphic and affect Brca1 function in different fashions.

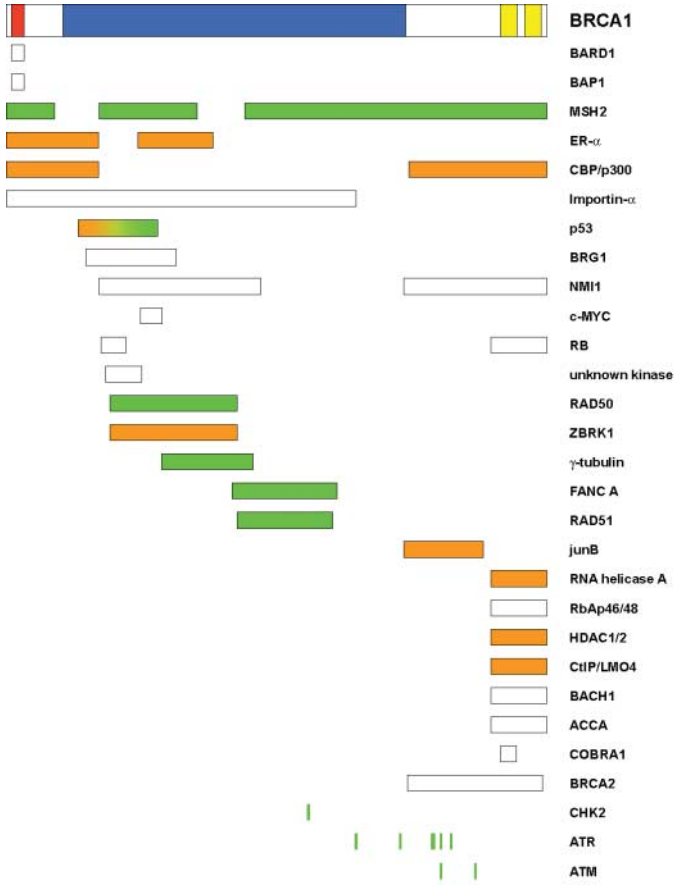

Figure 2. Overview of BRCA1-binding proteins. The RING finger is indicated by the red bar, whereas the blue and yellow bars represent the part of the protein encoded by exon 11 and the BRCT repeats, respectively. The filled boxes below the schematic representation of the full-length protein indicate the regions in BRCA1 where specific proteins bind. In general, proteins with a clearly defined function in DNA repair are depicted in green, while orange indicates a function in transcriptional regulation. The mixed color of p53 indicates close involvement in both processes. For CHK2, ATR and ATM, green bars indicate the positions of residues phosphorylated by the respective kinase.

FUNCTIONAL DOMAINS IN BRCA1

To understand how the above phenotypic differences arise, the functional domains found along the BRCA1 protein must be taken into consideration. As previously reviewed (24–26), the main function of BRCA1 is likely to be the control of genomic integrity and/or the transcriptional response to DNA damage. BRCA1's putative role in chromosome remodeling (27) may also be related to both these processes. Notably, the functional domains suggestive for a role in DNA repair are clustered around exon 11, while motifs involved in transcriptional regulation appear to mainly cluster within the C-terminal BRCT repeats (Fig. 2).

Exon 11 encompasses the interaction domains with RAD51 (28), the RAD50 complex (29), FANCA (30) and two out of

three domains responsible for the interaction with MSH2 (31). Moreover, several of the residues phosphorylated upon different types of DNA damage by Chk2 (32), ATR (33) and ATM (34) lie within exon 11.

The C-terminal region was identified *in vitro* as a transcriptional co-regulator (35,36), with some specificity for p53 (37,38) and STAT1 (39) co-activation. Also, the transcriptional co-activators p300 and CBP bind both the N- and C-terminal BRCA1 regions (40), while the transcriptional repressor CtIP binds the C-terminal BRCT repeats (41–43). The interaction with the RNA polymerase II holoenzyme complex (44) might occur via the BRCT repeats-binding RNA helicase A (45).

The functional demarcation between the repair and transcription-related motifs in BRCA1 may not be as clear-cut as suggested by the above studies. In fact, different domains in different part of the protein might have common, coordinated functional roles. For instance, while the C-terminus is a p53 co-activator, p53 itself was shown to bind to the first part of exon 11 (37,38). Also, the estrogen receptor, negatively regulated by BRCA1, binds to amino acids 1–300 and 428–683 (46–48). This estrogen receptor-inhibiting effect is mediated by p300 (49), which on its turn binds residues 1–303 and amino acids 1314–1863 in BRCA1 (40).

BRCA1 was also found to associate with the SWI/SNF chromosome-remodeling complex through its interaction with BGR1 (27). However, the SWI/SNF complex is also involved in p53 coactivation (50), a function also attributed to the BRCA1 C-terminus. Finally, the protein encoded by the Fanconi anemia gene FANCA, which binds BRCA1 amino acids 740–1083, also interacts with the SWI/SNF complex via BGR1 (51).

The N-terminal RING finger has been functionally identified as an E3 ubiquitin ligase and, although former proof has not been shown yet, the nuclear colocalization of BRCA1 and FANCD2 and the DNA damage-dependent ubiquitination of the latter suggests that this function is linked to DNA damage repair (52).

Together, the putative roles of BRCA1 in DNA repair and transcriptional regulation might explain the different phenotypes observed among different *Brca1* mouse models. Complete or partial loss of DNA repair function may account for the observed early embryonic lethality in the $Brca1^{null}$ models. The developmental defects observed in these embryos closely resemble those of $Rad51^{-/-}$ and $Rad50^{-/-}$ mutant mice (53,54). At this stage of embryonic development, the onset of gastrulation at 6.5 d.p.c., mouse embryos are extremely sensitive to dsDNA damage and respond to it by triggering p53-dependent apoptosis (55). Accordingly, the early phenotype of both $Brca1^{null/null}$ and $Rad51^{-/-}$ embryos is partially rescued until 9.5–10.5 d.p.c. in a $Tp53^{-/-}$ background (12,53,56), showing that their early lethality is p53-mediated.

Notably, the phenotype of $Brca1^{null/null}/Tp53^{-/-}$ embryos is remarkably similar in timing and gross morphology to that described for $Brca1^{1700T/1700T}$ embryos (18). Growth retardation was observed in this model as early as 7.5 d.p.c., although lethality did not occur until 10.5 d.p.c. This suggests that the primary defect in $Brca1^{null}$ and $Brca1^{1700T}$ may be the same, but that in the latter model the p53-dependent developmental block is somehow bypassed. Possibly, the truncated $Brca1^{1700T}$ protein exerts a dominant-negative effect on p53 function. Since the p53-binding domain is retained in $Brca1^{1700T}$, p53 may still bind to the mutant protein and, in the absence of the Brca1 C-terminal co-activation domain, become sequestered in an inactive complex.

Lethality of $Brca1^{\Delta11/\Delta11}$ embryos occur at 12.5–18.5 d.p.c. (19,22), thus showing that the $Brca1^{\Delta11}$ protein retains the functional domains that allow normal embryonic development beyond 6.5 d.p.c., the time at which lethality is observed in $Brca1^{null}$ embryos. Accordingly, it has been shown that the $Brca1^{\Delta11}$ protein can still localize to the nucleus and form the characteristic Brca1 nuclear speckles during S-phase and after DNA damage. Notably, formation of Rad51 speckles is disturbed in these *Brca1* mutant cells (23). Also, homology-directed DNA repair is disturbed in $Brca1^{\Delta11/\Delta11}$ ES cells, but non-homologous end joining is unaffected, or possibly even increased (57). This might suggest that the resulting accumulation of DNA damage in the $Brca1^{\Delta11/\Delta11}$ cells is less than in $Brca1^{null/null}$, and insufficient for the early (6.5 d.p.c.) activation of the p53-dependent checkpoint. Interestingly, *Tp53* haploinsufficiency ($Brca1^{\Delta11/\Delta11}/Tp53^{+/-}$) rescues the late $Brca1^{\Delta11/\Delta11}$ lethality to post-natal viability (19). This suggests an explanation for the viability of the $Brca1^{Tr/Tr}$ mice described by Ludwig *et al.* (20). As mentioned before, in this mutant the exon 11-deficient splice variant is unaffected, potentially circumventing the 6.5 d.p.c. embryonic lethality as in the case of $Brca1^{\Delta11/\Delta11}$. Moreover, the truncated exon 11-proficient isoform may partially inactivate p53 in a dominant-negative fashion as suggested for $Brca1^{1700T}$, and, as in $Brca1^{\Delta11/\Delta11}/Tp53^{+/-}$, rescue the embryos to post-natal viability.

DISCUSSION

The genotype–phenotype correlations observed at the human *BRCA1* gene may also be explained by similar models, as discussed above. Most likely, the primary outcome of loss of BRCA1 function in tumors is, both in mouse and man, the accumulation of DNA damage and the altered transcriptional response to it. However, whereas in *Brca1* mutant mice loss of this function leads to a p53-dependent cell cycle block, in the developing tumor loss of the p53 response to DNA damage will represent a selective growth advantage. Accordingly, it was shown that *BRCA1*-mutant tumors often carry somatic p53 mutations (58).

Genotype–phenotype correlation between specific *BRCA1* mutations and organ-specific tumours is less obvious. The $Brca1^{Tr}$ model is the only conventional mouse model that is cancer-prone, although the tumor spectrum found in these mice is much broader than it was observed in families with *BRCA1* mutations. Rather than trying to accommodate the tumor spectrum of this model with the differences in ovarian to breast cancer ratio among carriers of different *BRCA1* gene mutations, we believe that the putative dominant-negative effects of truncated BRCA1 proteins on p53 function, similar to that postulated for $Brca1^{1700T}$, may explain the genotype–phenotype correlation reported by Sobol *et al.* (11). All of the tumors (5/5) arising in patients with 5′ *BRCA1* germline mutations acquire somatic loss of p53, whereas only 21% (3/14) of the tumors with 3′ mutations have similar p53 loss ($P = 0.0048$, note that a clear demarcation for the two phenotypes could not be given

in the dataset used). Non-BRCA1-related tumors showed frequencies of somatic p53 losses similar to tumors with 3′ *BRCA1* mutations (in this study 17.5%). Although other explanations are possible, these observation suggest that BRCA1-related tumors select for functional loss of p53 rather than its activation by missense mutations, and that, in the case of 3′ *BRCA1* germline mutations, analogous to the *Brca1*^{1700T} mouse targeted mutation, the truncated BRCA1 protein inactivates p53 in a dominant-negative fashion, thereby compensating for the growth advantage provided by somatic inactivation of the p53 gene.

The presence of functionally linked domains along the BRCA1 protein may also result in additional dominant-negative effects, where the mutant protein affects the normal function of BRCA1 and/or other interacting proteins. If confirmed, the presence of dominant-negative BRCA1 truncated proteins in tumors would limit the possibilities of gene-transfer therapies based on the reintroduction of wild-type BRCA1 in BRCA1-mutant tumors (5,59,60). Accordingly, three recent publications showed dominant-negative effects of mutant BRCA1 constructs in human and mouse cells *in vitro* and *in vivo* by looking at specific cellular phenotypes. Fan and co-workers (61) used different truncated and full-length mutant cDNA constructs and found that several carboxyl-truncated constructs (encompassing the first 302, 771 or 1313 amino acids of human BRCA1 respectively) could inhibit the function of wild-type BRCA1 in many different functional assays. Sylvain *et al.* (62) showed that a 70 kDa truncated Brca1 protein (∼600 amino acids, thereby still encompassing the RING finger, binding domains for p53, BRG-1, c-myc, RB, part of the region involved in binding of Rad50 and one of the two NLSs) exerts a dominant-negative effect on chemoresistance and tumorigenicity in mouse ovarian carcinoma cells. Finally, Brown *et al.* (63) generated a transgenic mouse model expressing a 299 amino acid mutant Brca1 protein in mammary tissue and found a slight delay in mammary development during pregnancy. On the other hand, the absence of tumors in the *Brca1*^{1700T} model even after γ-irradiation, argues against dominant-negative effects sufficient for tumorigenesis, although environmental and genetic modifiers may account for differences in tumor incidence between mouse and man (18).

The above mechanisms, postulated to explain the observed genotype–phenotype correlations at the *BRCA1* locus in mouse and man, are admittedly speculative and necessitate additional experimental evidence. In particular, somatic mutation data from hereditary *BRCA1* breast cancers, relevant to the putative dominant-negative nature of mutant BRCA1 proteins are surprisingly scarce in the literature. A dominant-negative mode of action exerted by the germline first-hit at *BRCA1* may result in tumors without loss of the wild-type allele or with a specific subset of second-hit mutations. High percentages of LOH (up to 86%) have been reported in tumors from genetically predisposed families (64). However, much of this work was done before the cloning of *BRCA1*, and was based on linkage analysis. Since its identification, no systematic study of the status of the wild-type *BRCA1* allele in tumors has been reported. Similar studies of the adenomatous polyposis coli (*APC*) gene in colorectal polyps from FAP patients have shown a correlation between first- and second-hit *APC* mutations resulting from selective pressure on the residual activity of truncated proteins (65–67). Additionally, haploinsufficiency at tumor suppressor loci can have a bigger impact then previously thought (68). Similar mechanisms might be operational in *BRCA1*-related tumors as well. A full analysis of molecular aberrations on both *BRCA1* alleles in familial and non-familial cases is needed to fully understand the functional role of BRCA1 in breast cancer. Moreover, biochemical and functional analysis of mutant BRCA1 protein found in human and mouse tumors will allow the dissection of the pathways of DNA repair and response to DNA damage affected in human hereditary breast and ovarian cancer, essential for the development of tailor-made preventive and therapeutic approaches.

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NOTE ADDED IN PROOF

Recently, using a humanized mouse model, Yang *et al*. (69) showed that a missense mutation in the BRCA1 RING finger domain leads to expression of a short truncated protein as a result of disturbed splicing, functionally resulting in a null mutation. This finding again strongly emphasizes the need to study the effects of mutations in BRCA1 at the protein level.

REFERENCES

1. Perrin-Vidoz, L., Sinilnikova, O.M., Stoppa-Lyonnet, D., Lenoir, G.M. and Mazoyer, S. (2002) The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. *Hum. Mol. Genet.*, **11**, 2805–2814.
2. Tomlinson, I.P.M., Novelli, M.R. and Bodmer, W.F. (1996) The mutation rate and cancer. *Proc. Natl Acad. Sci. USA*, **93**, 14800–14803.
3. Scully, R., Ganesan, S., Vlasakova, K., Chen, J., Socolovsky, M. and Livingston, D.M. (1999) Genetic analysis of BRCA1 function in a defined tumor cell line. *Mol. Cell*, **4**, 1093–1099.
4. Gayther, S.A., Warren, W., Mazoyer, S., Russell, P.A., Harrington, P.A., Chiano, M., Seal, S., Hamoudi, R., Van Rensburg, E.J., Dunning, A.M. *et al.* (1995) Germline mutations of the *BRCA* 1 gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. *Nat. Genet.*, **11**, 428–433.
5. Holt, J.T., Thompson, M.E., Szabo, C., Robinson-Benion, C., Arteaga, C.L., King, M.C. and Jensen, R.A. (1996) Growth retardation and tumour inhibition by *BRCA1*. *Nat. Genet.*, **12**, 298–302.
6. Thompson, D. and Easton, D. (2002) Variation in BRCA1 cancer risks by mutation position. *Cancer Epidemiol. Biomark. Prev.*, **11**, 329–336.
7. Spirio, L., Olschwang, S., Groden, J., Robertson, M., Samowitz, W., Joslyn, G., Gelbert, L., Thliveris, A., Carlson, M., Otterud, B. *et al.* (1993) Alleles of the APC gene: an attenuated form of familial polyposis. *Cell*, **75**, 951–957.
8. van der Luijt, R.B., Meera Khan, P., Vasen, H.F., Breukel, C., Tops, C.M., Scott, R.J. and Fodde, R. (1996) Germline mutations in the 3′ part of APC exon 15 do not result in truncated proteins and are associated with attenuated adenomatous polyposis coli. *Hum. Genet.*, **98**, 727–734.
9. Heppner Goss, K., Trzepacz, C., Tuohy, T.M. and Groden, J. (2002) Attenuated APC alleles produce functional protein from internal translation initiation. *Proc. Natl Acad. Sci. USA*, **99**, 8161–8166.

10. Sobol, A., Stoppalyonnet, D., Bressacdepaillerets, B., Peyrat, J.P., Kerangueven, F., Janin, N., Noguchi, T., Eisinger, F., Guinebretiere, J.M., Jacquemier, J. *et al.* (1996) Truncation at conserved terminal regions of BRCA1 protein is associated with highly proliferating hereditary breast cancers. *Cancer Res.*, **56**, 3216–3219.
11. Sobol, H., Stoppalyonnet, D., Bressacdepaillerets, B., Peyrat, J.P., Guinebretiere, J.M., Jacquemier, J., Eisinger, F. and Birnbaum, D. (1997) BRCA1 p53 relationship in hereditary breast cancer. *Int. J. Oncol.*, **10**, 349–353.
12. Ludwig, T., Chapman, D.L., Papaioannou, V.E. and Efstratiadis, A. (1997) Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev.*, **11**, 1226–1241.
13. Hakem, R., de la Pompa, J.L., Sirard, C., Mo, R., Woo, M., Hakem, A., Wakeham, A., Potter, J., Reitmair, A., Billia, F. *et al.* (1996) The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell*, **85**, 1009–1023.
14. Liu, C.Y., Fleskennikitin, A., Li, S., Zeng, Y.Y. and Lee, W.H. (1996) Inactivation of the mouse *Brca1* gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev.*, **10**, 1835–1843.
15. Fodde, R., Edelmann, W., Yang, K., van Leeuwen, C., Carlson, C., Renault, B., Breukel, C., Alt, E., Lipkin, M., Khan, P.M. *et al.* (1994) A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. *Proc. Natl Acad. Sci. USA*, **91**, 8969–8973.
16. Smits, R., Kielman, M.F., Breukel, C., Zurcher, C., Neufeld, K., Jagmohan-Changur, S., Hofland, N., van Dijk, J., White, R., Edelmann, W. *et al.* (1999) Apc1638T: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorigenesis and development. *Genes Dev.*, **13**, 1309–1321.
17. Kielman, M.F., Rindapaa, M., Gaspar, C., Van Poppel, N., Breukel, C., Van Leeuwen, S., Taketo, M.M., Roberts, S., Smits, R. and Fodde, R. (2002) Apc modulates embryonic stem-cell differentiation by controlling the dosage of beta-catenin signaling. *Nat. Genet.*, **11**, 11.
18. Hohenstein, P., Kielman, M.F., Breukel, C., Bennett, L.M., Wiseman, R., Krimpenfort, P., Cornelisse, C., van Ommen, G.J., Devilee, P. and Fodde, R. (2001) A targeted mouse Brca1 mutation removing the last BRCT repeat results in apoptosis and embryonic lethality at the headfold stage. *Oncogene*, **20**, 2544–2450.
19. Xu, X., Qiao, W., Linke, S.P., Cao, L., Li, W.M., Furth, P.A., Harris, C.C. and Deng, C.X. (2001) Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. *Nat. Genet.*, **28**, 266–271.
20. Ludwig, T., Fisher, P., Ganesan, S. and Efstratiadis, A. (2001) Tumorigenesis in mice carrying a truncating Brca1 mutation. *Genes Dev.*, **15**, 1188–1193.
21. Shen, S.-X., Weaver, Z., Xu, X., Li, C., Weinstein, M., Chen, L., Guan, X.-Y., Ried, T. and Deng, C.-X. (1998) A targetted disruption of the murine *Brca1* gene causes γ-irradiation hypersensitivity and genetic instability. *Oncogene*, **17**, 3115–3124.
22. Gowen, L.C., Johnson, B.L., Latour, A.M., Sulik, K.K. and Koller, B.H. (1996) *Brca1* deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat. Genet.*, **12**, 191–194.
23. Huber, L.J., Yang, T.W., Sarkisian, C.J., Master, S.R., Deng, C.X. and Chodosh, L.A. (2001) Impaired DNA damage response in cells expressing an exon 11-deleted murine Brca1 variant that localizes to nuclear foci. *Mol. Cell. Biol.*, **21**, 4005–4015.
24. Welcsh, P.L., Owens, K.N. and King, M.C. (2000) Insights into the functions of BRCA1 and BRCA2. *Trends Genet.*, **16**, 69–74.
25. Venkitaraman, A.R. (2002) Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell*, **108**, 171–182.
26. Deng, C.-X. and Wang, R.-H. (2003) Roles of BRCA1 in DNA damage repair: a link between development and cancer. *Hum. Mol. Genet.*, **12**, 113R–123.
27. Bochar, D.A., Wang, L., Beniya, H., Kinev, A., Xue, Y., Lane, W.S., Wang, W., Kashanchi, F. and Shiekhattar, R. (2000) BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell*, **102**, 257–265.
28. Scully, R., Chen, J.J., Plug, A., Xiao, Y.H., Weaver, D., Feunteun, J., Ashley, T. and Livingston, D.M. (1997) Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell*, **88**, 265–275.
29. Zhong, Q., Chen, C.F., Li, S., Chen, Y.M., Wang, C.C., Xiao, J., Chen, P.L., Sharp, Z.D. and Lee, W.H. (1999) Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. *Science*, **285**, 747–750.
30. Folias, A., Matkovic, M., Bruun, D., Reid, S., Hejna, J., Grompe, M., D'Andrea, A. and Moses, R. (2002) BRCA1 interacts directly with the Fanconi anemia protein FANCA. *Hum. Mol. Genet.*, **11**, 2591–2597.
31. Wang, Q., Zhang, H., Guerrette, S., Chen, J., Mazurek, A., Wilson, T., Slupianek, A., Skorski, T., Fishel, R. and Greene, M.I. (2001) Adenosine nucleotide modulates the physical interaction between hMSH2 and BRCA1. *Oncogene*, **20**, 4640–4649.
32. Lee, J.S., Collins, K.M., Brown, A.L., Lee, C.H. and Chung, J.H. (2000) hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. *Nature*, **404**, 201–204.
33. Tibbetts, R.S., Cortez, D., Brumbaugh, K.M., Scully, R., Livingston, D., Elledge, S.J. and Abraham, R.T. (2000) Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. *Genes Dev.*, **14**, 2989–3002.
34. Cortez, D., Wang, Y., Qin, J. and Elledge, S.J. (1999) Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. *Science*, **286**, 1162–1166.
35. Chapman, M. and Verma, I.M. (1996) Transcriptional activation by BRCA1. *Nature*, **382**, 678–679.
36. Monteiro, A.N., August, A. and Hanafusa, H. (1996) Evidence for a transcriptional activation function of BRCA1 C terminal region. *Proc. Natl Acad. Sci. USA*, **93**, 13595–13599.
37. Ouchi, T., Monteiro, A.N., August, A., Aaronson, S.A. and Hanafusa, H. (1998) BRCA1 regulates p53-dependent gene expression. *Proc. Natl Acad. Sci. USA*, **95**, 2302–2306.
38. Zhang, H., Somasundaram, K., Peng, Y., Tian, H., Bi, D., Weber, B.L. and El-Deiry, W.S. (1998) BRCA1 physically associates with p53 and stimulates its transcriptional activity. *Oncogene*, **16**, 1713–1721.
39. Ouchi, T., Lee, S.W., Ouchi, M., Aaronson, S.A. and Horvath, C.M. (2000) Collaboration of signal transducer and activator of transcription 1 (STAT1) and BRCA1 in differential regulation of IFN-gamma target genes. *Proc. Natl Acad. Sci. USA*, **97**, 5208–5213.
40. Pao, G.M., Janknecht, R., Ruffner, H., Hunter, T. and Verma, I.M. (2000) CBP/p300 interact with and function as transcriptional coactivators of BRCA1. *Proc. Natl Acad. Sci. USA*, **97**, 1020–1025.
41. Yu, X., Wu, L.J.C., Bowcock, A.M., Aronheim, A. and Baer, R. (1998) The C-terminal (BRCT) domains of BRCA1 interact *in vivo* with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. *J. Biol. Chem.*, **273**, 25388–25392.
42. Wong, A.K., Ormonde, P.A., Pero, R., Chen, Y., Lian, L., Salada, G., Berry, S., Lawrence, Q., Dayananth, P., Ha, P. *et al.* (1998) Characterization of a carboxy-terminal BRCA1 interacting protein. *Oncogene*, **17**, 2279–2285.
43. Li, S., Chen, P.L., Subramanian, T., Chinnadurai, G., Tomlinson, G., Osborne, C.K., Sharp, Z.D. and Lee, W.H. (1999) Binding of CtIP to the BRCT repeats of BRCA1 involved in the transcription regulation of p21 is disrupted upon DNA damage. *J. Biol. Chem.*, **274**, 11334–11338.
44. Scully, R., Anderson, S.F., Chao, D.M., Wei, W.J., Ye, L.Y., Young, R.A., Livingston, D.M. and Parvin, J.D. (1997) BRCA1 is a component of the RNA polymerase II holoenzyme. *Proc. Natl Acad. Sci. USA*, **94**, 5605–5610.
45. Anderson, S.E., Schlegel, B.P., Nakajima, T., Wolpin, E.S. and Parvin, J.D. (1998) BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase a. *Nat. Genet.*, **19**, 254–256.
46. Fan, S., Wang, J.A., Yuan, R., Ma, Y., Meng, Q., Erdos, M.R., Pestell, R.G., Yuan, F., Auborn, K.J., Goldberg, I.D. *et al.* (1999) BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science*, **284**, 1354–1356.
47. Fan, S., Ma, Y.X., Wang, C., Yuan, R.Q., Meng, Q., Wang, J.A., Erdos, M., Goldberg, I.D., Webb, P., Kushner, P.J. *et al.* (2001) Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. *Oncogene*, **20**, 77–87.
48. Kawai, H., Li, H., Chun, P., Avraham, S. and Avraham, H.K. (2002) Direct interaction between BRCA1 and the estrogen receptor regulates vascular endothelial growth factor (VEGF) transcription and secretion in breast cancer cells. *Oncogene*, **21**, 7730–7739.
49. Fan, S., Ma, Y.X., Wang, C., Yuan, R.Q., Meng, Q., Wang, J.A., Erdos, M., Goldberg, I.D., Webb, P., Kushner, P.J. *et al.* (2002) p300 Modulates the BRCA1 inhibition of estrogen receptor activity. *Cancer Res.*, **62**, 141–151.
50. Lee, D., Kim, J.W., Seo, T., Hwang, S.G., Choi, E.J. and Choe, J. (2002) SWI/SNF complex interacts with tumor suppressor p53 and is necessary

for the activation of p53-mediated transcription. *J. Biol. Chem.*, **277**, 22330–22337.
51. Otsuki, T., Furukawa, Y., Ikeda, K., Endo, H., Yamashita, T., Shinohara, A., Iwamatsu, A., Ozawa, K. and Liu, J.M. (2001) Fanconi anemia protein, FANCA, associates with BRG1, a component of the human SWI/SNF complex. *Hum. Mol. Genet.*, **10**, 2651–2660.
52. Garcia-Higuera, I., Taniguchi, T., Ganesan, S., Meyn, M.S., Timmers, C., Hejna, J., Grompe, M. and D'Andrea, A.D. (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell*, **7**, 249–262.
53. Lim, D.S. and Hasty, P. (1996) A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol. Cell. Biol.*, **16**, 7133–7143.
54. Bender, C.F., Sikes, M.L., Sullivan, R., Huye, L.E., Le Beau, M.M., Roth, D.B., Mirzoeva, O.K., Oltz, E.M. and Petrini, J.H. (2002) Cancer predisposition and hematopoietic failure in Rad50(S/S) mice. *Genes Dev.*, **16**, 2237–2251.
55. Heyer, B.S., MacAuley, A., Behrendtsen, O. and Werb, Z. (2000) Hypersensitivity to DNA damage leads to increased apoptosis during early mouse development. *Genes Dev.*, **14**, 2072–2084.
56. Hakem, R., de la Pompa, J.L., Elia, A., Potter, J. and Mak, T.W. (1997) Partial rescue of Brca1 (5-6) early embryonic lethality by p53 or p21 null mutation. *Nat. Genet.*, **16**, 298–302.
57. Moynahan, M.E., Chiu, J.W., Koller, B.H. and Jasin, M. (1999) Brca1 controls homology-directed DNA repair. *Mol. Cell*, **4**, 511–518.
58. Crook, T., Crossland, S., Crompton, M.R., Osin, P. and Gusterson, B.A. (1997) p53 mutations in BRCA1-associated familial breast cancer. *Lancet*, **350**, 638–639.
59. Tait, D.L., Obermiller, P.S. and Holt, J.T. (2000) Preclinical studies of a new generation retroviral vector for ovarian cancer BRCA1 gene therapy. *Gynecol. Oncol.*, **79**, 471–476.
60. Randrianarison, V., Marot, D., Foray, N., Cabannes, J., Meret, V., Connault, E., Vitrat, N., Opolon, P., Perricaudet, M. and Feunteun, J. (2001) BRCA1 carries tumor suppressor activity distinct from that of p53 and p21. *Cancer Gene Ther*, **8**, 759–770.
61. Fan, S., Yuan, R., Ma, Y.X., Meng, Q., Goldberg, I.D. and Rosen, E.M. (2001) Mutant BRCA1 genes antagonize phenotype of wild-type BRCA1. *Oncogene*, **20**, 8215–8235.
62. Sylvain, V., Lafarge, S. and Bignon, Y.J. (2002) Dominant-negative activity of a Brca1 truncation mutant: effects on proliferation, tumorigenicity *in vivo*, and chemosensitivity in a mouse ovarian cancer cell line. *Int. J. Oncol.*, **20**, 845–853.
63. Brown, M.A., Nicolai, H., Howe, K., Katagiri, T., Lalani el, N., Simpson, K.J., Manning, N.W., Deans, A., Chen, P., Khanna, K.K. *et al.* (2002) Expression of a truncated Brca1 protein delays lactational mammary development in transgenic mice. *Transgenic Res.*, **11**, 467–478.
64. Cornelis, R.S., Neuhausen, S.L., Johansson, O., Arason, A., Kelsell, D., Ponder, B.A., Tonin, P., Hamann, U., Lindblom, A., Lalle, P. *et al.* (1995) High allele loss rates at 17q12-q21 in breast and ovarian tumors from BRCAl-linked families. The Breast Cancer Linkage Consortium. *Genes Chromosomes Cancer*, **13**, 203–210.
65. Albuquerque, C., Breukel, C., van der Luijt, R., Fidalgo, P., Lage, P., Slors, F.J., Leitao, C.N., Fodde, R. and Smits, R. (2002) The 'just-right' signaling model: APC somatic mutations are selected based on a specific level of activation of the beta-catenin signaling cascade. *Hum. Mol. Genet.*, **11**, 1549–1560.
66. Smits, R., Hofland, N., Edelmann, W., Geugien, M., Jagmohan-Changur, S., Albuquerque, C., Breukel, C., Kucherlapati, R., Kielman, M.F. and Fodde, R. (2000) Somatic *Apc* mutations are selected upon their capacity to inactivate the β-catenin downregulating activity. *Genes Chromosomes Cancer*, **29**, 229–239.
67. Lamlum, H., Ilyas, M., Rowan, A., Clark, S., Johnson, V., Bell, J., Frayling, I., Efstathiou, J., Pack, K., Payne, S. *et al.* (1999) The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: a new facet to Knudson's 'two-hit' hypothesis. *Nat. Med.*, **5**, 1071–1075.
68. Fodde, R. and Smits, R. (2002) Cancer biology. A matter of dosage. *Science*, **298**, 761–763.
69. Yang. Y., Swaminathan, S., Martin, B.K. and Sharan, S.K. (2003) Aberrant splicing induced by missense mutations in *BRCA1*: clues from a humanized mouse model. *Hum. Mol. Genet.*, **12**, 2121–2131.