Author's response to reviews

**Title:** Haploinsufficiency for BRCA1 is associated with normal levels of DNA nucleotide excision repair in breast tissue and blood lymphocytes

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**Author's response to reviews:** see over
We would like to thank the reviewers for their careful and insightful reviews. It was clear from their comments, especially Dr. Sgambato’s suggestion that the clinical data was superfluous, that we had overwritten the NER aspect of the manuscript (our scientific area of interest) and underwritten the other aspects of the study. The revised manuscript better introduces and discusses the non-NER aspects of the work, while reducing the main text of the manuscript by 25% and the number of references from 56 to 40. In addition, we have reformatted the manuscript as a Case Report (this was suggested as an option in the cover letter accompanying the original submission, but the authors instructions gave the scientific article format as the default). To reiterate, the paper does 3 things:

1) provides a case report on a hereditary breast cancer patient whose tumor was detected only through the new screening technology of MRI. This patient participated in a study designed to determine the sensitivity and feasibility of screening of “high risk” (as determined by several breast cancer risk assessment models, not simply familial breast cancer) individuals with a low power MRI that, for various reasons, is more appropriate for use in community hospitals than the standard higher power machine. We stress that even after her tumor was found through MRI it was not detectable by conventional mammography. This patient and her tumor are therefore paradigms for a new group of patients and cancers that will impact upon the medical system if MRI, ultrasound, or any of several new options for breast cancer screening are widely adopted. …

2) demonstrates the application of our unique human mammary epithelial culture system to evaluate the in vitro growth and differentiation capacity of clinically-
derived breast epithelial samples. Several of the non-genetic risk factors associated with breast cancer seem to involve differentiation potential, and we were therefore prepared to see a reduced capacity for differentiation in this patient, which would have demonstrated a possible link between the known hereditary and "lifestyle" risk factors for breast cancer. Our results suggest that haploinsufficiency for BRCA1, the production of a tumor undetectable by mammography or the production of a tumor from dense normal breast tissue are NOT intrinsically linked to a change in in vitro differentiation capacity. If so, ALL such patients would exhibit this characteristic. We are still optimizing our in vitro culture system, however, so we are not as sanguine about making this a major point of the paper (i.e. putting it in the title).

3) since we submitted this manuscript a third paper has appeared that presents evidence that blood samples from sporadic breast cancer patients are deficient in NER (Kennedy et al., 2005, J. Natl. Cancer Inst. 97:127) (the other two reports are references 10 and 11 in the original manuscript, references 14 and 15 in the revised manuscript). This is a very important finding from the point of view of our research, because we have found NER deficiency in breast tumors themselves, in the normal breast tissue adjacent to the tumor and in blood lymphocytes from newly diagnosed (chemotherapy naïve) breast cancer patients, all by the UDS assay. It has been suggested, however, that the functional loss of NER observed in all three published studies and in our hands is not due to an effect on the NER pathway itself, but a pleiotropic effect of the known breast cancer-associated genes BRCA1 and BRCA2. Of course, this should only affect
10-15% of the “sporadic” population. The work of Hartman and Ford (ref 19) and Takimoto et al. (ref 20) suggest that modulation of BRCA1 expression alters NER capacity and expression of genes in the NER pathway. Since the NER deficiency has been observed in the blood (Kovacs et al., 1986; Xiong et al., 2001; Kennedy et al., 2005), however, it would have to be associated with haploinsufficiency for BRCA1, rather than the complete loss of BRCA1 activity that occurs in the tumor after the locus undergoes loss of heterozygosity. Thus, the potential link can be evaluated in the blood lymphocytes of BRCA1 carriers, such as our patient; moreover, we have the unique ability to also analyze the breast epithelium itself, in case there is tissue specificity for the effect (since BRCA1 patients develop cancer of the breast). Please note that the hypothesis requires that ALL BRCA1 mutation carriers be NER deficient, such that it is essentially impossible to prove, but very simple to disprove. Thus, the negative result in this study, demonstrated in the blood and tissue from both breasts, is definitive in demonstrating that BRCA1 haploinsufficiency is not a simple way of explaining the constitutive NER deficiency observed in breast cancer patients. We hope that the above discussion addresses Dr. Cleaver’s uncertainty on the significance of the findings and the length of the manuscript.

Dr. Sgambato – Major points

1-2) We hope that a case has been made for the definitiveness of our negative result for NER deficiency in the blood and breast tissue of our BRCA1 carrier patient. Should we analyze other BRCA1 carriers and find mixed results, it would just
indicate that whatever mechanism is at work to reduce the NER capacity of our sporadic cancer patients is not necessarily excluded from occurring in hereditary cancer patients as well. Indeed, there must be undetected familial patients among the ostensibly “sporadic” breast cancer populations studied in the three published reports. Thus, screening our present UDS-characterized breast cancer population for BRCA1 (and BRCA2) mutations would probably identify several previously unknown carrier individuals, but since our population has been most intensively characterized in the actual tumor tissue, we still could not conclude anything specifically about haploinsufficiency and NER deficiency.

3-4) The demonstration of NER deficiency in blood samples from breast cancer patients is not unrelated to our work in the tumors themselves, but we ourselves have analyzed relatively few blood samples (7 to date) versus 50+ tumors, so we are really investigating the possible genetic basis of the blood-based results reported by others. Some of the premature citations of our own work have therefore been removed. The 33 normal blood lymphocyte samples have been published and are described and cited (refs 18 and 54) in the Materials and Methods.

5) We are NOT suggesting that the UDS assay (or the HMEC tissue engineering system) be used as a screening technique, especially for the detection of BRCA1 mutation carriers (since we have just proven the two are unrelated). The UDS assay, as performed in our laboratory, is a fastidious and labor-intensive technique (thus, we do not believe that our results on multiple samples from this patient “might not be reliable”), and it would therefore be difficult to suggest using it for
widespread population screening. However, the Kovacs et al, (ref 10) paper used a simpler version of this assay involving quantitation by liquid scintillation counting that might be more appropriate for widespread application (we generally discourage the use of this technique because it has additional sources of error that cannot be monitored, but the accuracy of the grain counting assay may not be necessary for screening, or the loss in accuracy [usually consisting of false positives] may be tolerated in a screening application). The two more recent studies utilize “mutagen sensitivity” assays that are adaptations of long-standing clinical cytogenetic tests for chromosome breakage syndromes; they may also be appropriate for population screening, but population screening for low NER capacity as an independent risk factor for breast cancer, unrelated to the BRCA1 gene. To summarize, our method of measuring NER is the most accurate method available, but it is arduous and expensive. Once we have confirmed proof of principle with this assay, however, it is possible, even hopeful, that simplified versions may be applied for population screening.

6) The referenced discussion did make a leap: since we have found NER deficiency in ALL (17/17) stage I breast tumors (the same stage as that of our patient), we thought we had good reason to believe that such tumors in BRCA1 carriers would also be NER deficient. We agree that this discussion was too speculative (and unnecessarily lengthened the paper), and have removed it.

Minor points

1) Repetitive text has been removed.

2) We have removed all typos.
3) These Legends have been expanded.

4) The derivation of the samples pictured in Figure 3 has been described more clearly.

5) The patient was actually 35.7 years old at diagnosis. This more accurate age is now used consistently in the text.

Discretionary revisions:

The discretionary removal of this material was based on the view of the paper as a pure research article on NER activity. We hope that we have now justified its inclusion to the reviewer’s satisfaction.