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14. ABSTRACT How <i>Brcal</i> and <i>p53</i> collaborate in tumorigenesis and how the <i>Brcal</i> mutation affects breast cancer response to chemotherapeutics are not well understood. Mice carrying somatic mutations of <i>Brcal</i> and <i>p53</i> in the mammary epithelium cells that lead to accelerated tumor formation. The inactivation of <i>Brcal</i> and <i>p53</i> under a constitutive active MMTVCre or WAPCre results in complete penetrance of mammary tumors with a median tumor latency of 15.1 months and 6.6 months, respectively. There is a significant acceleration compared to the median tumor latency of 17.5 months and 10.5, respectively, in mice carrying mutated <i>p53</i>. Acceleration of tumorigenesis in the <i>Brcal</i> and <i>p53</i> floxed mice is correlated with a rapid progression from hyperplastic to carcinoma. WAPCre targeted <i>p53</i>-deficient cells developed both ERα-positive and -negative tumors, while <i>Brcal</i> mutation resulted in a greater frequency of ERα-negative tumors with high rates of lung and liver metastasis. The effects of doxorubicin and cisplatin treatment on spontaneous tumors and tumor transplantations were examined. Although the absence of <i>Brcal</i> and <i>p53</i> accelerate tumor growth, CDDP but not doxorubicin was effective in slowing tumor growth. Neither treatment was effective for tumors with a <i>p53</i> deficiency.					
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INTRODUCTION

BRCA1 and *BRCA2* are breast cancer susceptibility genes where mutations lead to cancer phenotypes that account for the hereditary breast and ovarian cancer syndrome¹⁻³. Hereditary breast cancer accounts for 5-10% of all breast cancers diagnosed each year⁴ and of these, *BRCA1* mutations are seen in 45-50% of these cases⁵. In sporadic breast and ovarian cancer, *BRCA1* mutations are rare⁶⁻⁷; however, 30-40% of sporadic cancers show reduced or absent expression of *BRCA1*⁸. *BRCA1* is a nuclear protein that contains multiple functional domains interacting with numerous molecules, which include products of tumor-suppressor genes, oncogenes, DNA damage-repair proteins, cell cycle regulators and transcriptional activators and repressors⁹⁻¹⁰.

Using predictive biomarkers for response to DNA-damaging chemotherapy in breast and ovarian cancer has been investigated by looking at the DNA damage response gene *p53* and the results obtained from these studies have been contradictory¹¹⁻¹². Since many chemotherapeutic agents function by damaging DNA directly or indirectly, the role of *BRCA1* after chemotherapy-induced DNA damage and as a predictive biomarker of response to these drugs has been the subject of several *in vitro* and *in vivo* studies⁹. Given that one of *BRCA1*'s functions is to help repair damaged DNA, *BRCA1*-disrupted mouse embryonic stem cells and mouse embryonic fibroblasts (MEFs) were more sensitive to the alkylating agent mitomycin C, the platinum compounds cisplatin, carboplatin and oxaliplatin and various topoisomerase I & II poisons¹³⁻¹⁵. Clinical trials that address the role of *BRCA1* in response to chemotherapy have been conducted⁹. Unfortunately, all trials have been retrospective and no trial was designed to study the role of *BRCA1* in response to chemotherapy. Therefore, to better study the role of *BRCA1* in response to chemotherapy, we have generated mice carrying somatic mutations of *Brcal* and *p53* in the mammary epithelium cells which have lead to accelerated tumor formation.

KEY RESEARCH ACCOMPLISHMENTS

Here, we have established an animal model that closely mimics human *BRCA1*-mediated breast tumorigenesis. Our mouse model represents many of the same features seen in its human counterpart. For example, *BRCA1*-mutant breast cancers are characterized by high nuclear grade, *p53* mutation, ER α and PR negativity and *myb* and *c-myc* amplification. Sporadic breast cancer cases often show amplification of *ErbB-2* and overexpression of cyclin D1 and Bcl-2. However, in *BRCA1*-mutant breast tumors, the expression levels of *ErbB-2*, cyclin D1 and Bcl-2 are rarely amplified or overexpressed. We established the mouse model by using the Cre/loxP system in which mice, we previously established, carrying the floxed *p53* gene were crossed to mice carrying the *Brcal* floxed gene to generate, double floxed mice. In order to get deletion of *p53* and *Brcal* we then further crossed the mice to MMTV-Cre or WAP-Cre. After deletion of these genes, the rate of tumor formation was greatly enhanced in double mutant mice (*p53/Brcal*) compared to that of single mutant mice (*p53*; Figure 1). While the tumor rate was ongoing, characterization of the tumors was carried and these tumors revealed many similar features to the one described above. Our tumors were highly ER α and PR negative, HER2 negative and basal-cell like. The next step was to examine how these tumors responded to various chemotherapeutic agents. The agents chosen were doxorubicin, cisplatin and carboplatin (this was added later because it is less toxic to patients and the animals). *In vitro* data comparing *p53* tumor to *p53/Brcal* tumors showed no difference between the different chemotherapeutic agents. Reasons for this are still ongoing. However, the *in vivo* studies carried using spontaneous or transplanted tumors showed different results. Although the absence of *Brcal* and *p53* accelerate tumor growth, cisplatin and carboplatin but not doxorubicin was effective in slowing tumor growth. Neither treatment was effective for tumors with a *p53* deficiency. Currently, why these tumors are so responsive to cisplatin and carboplatin but not doxorubicin are being investigated along with reasons why we see an accelerated tumor formation in *p53/Brcal* mutant mice. In

completing the in vivo studies, we noticed that after a few months, the tumors began to reoccur. Once this occurred, we confirmed the results and we have established a cisplatin-resistance model for Brca1 mediated tumorigenesis. Resistance always occurs in patients and to find a way around this will provide a treatment outcome. We plan to identify gene involved in cisplatin resistance through array CGH or DNA microarray. Once we have identified possible gene, we will study them in more detail and try to identify potential small molecules that can inhibit these genes.

REPORTABLE OUTCOMES

Currently, we are in the process of understanding why Brca1-mediated tumors are so responsive to cisplatin. Once understand, the manuscript will be sent out for publication.

CONCLUSIONS

This study here took the approach of pharmacogenetics by looking at what mutations are found then applying the correct chemotherapeutic agent to treat these tumors. We know the function of Brca1 therefore, we examined a common chemotherapeutic agent, doxorubicin and one that targets the DNA double strand break repair protein, cisplatin and carboplatin. Our results indicate that if you understand which mutations the patients then a more favorable outcome can occur. We also hope in the future to provide some new insights into why are patients resistance to chemotherapeutics agents like cisplatin.

REFERENCES

1. Hall, J.M., Lee, M.K., Newman, B., Morrow, J.E., Anderson, L.A., Huey, B. and King, M.C. (1990). Linkage of early-onset familial breast cancer to chromosome 17q21. *Science*, **250**: 1684-1689.
2. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P.A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L.M., Ding, W., et al. (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*. **266**:66-71.
3. Wooster, R., Neuhausen, S.L., Mangion, J., Quirk, Y., Ford, D., Collins, N., Nguyen, K., Seal, S., Tran, T., Averill, D., et al. (1994). Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science*. **265**:2088-90.
4. Rosen E.M., Fan, S., Pestell, R.G. & Goldberg, I.D. (2003). BRCA1 in hormone-responsive cancers. *Trends Endocr. Meta.*, **14**: 378-385.
5. Nathanson, K.N., Wooster, R. & Weber, B.L. (2001). Breast cancer genetics: what we know and what we need. *Nat. Med.*, **7**: 552-556.
6. Lambie, H., Miremadi, A., Pinder, S.E., Bell, J.A., Wencyk, P., Paish, E.C., Macmillan, R.D. & Ellis, I.O. (2003). Prognostic significance of BRCA1 expression in sporadic breast carcinomas. *J Pathol.* **200**:207-13.
7. Futreal, P.A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L.M., Haugen-Strano, A., Swensen, J., Miki, Y., et al. (1994). BRCA1 mutations in primary breast and ovarian carcinomas. *Science*. **266**:120-2.
8. Wilson, C.A., Ramos, L., Villasenor, M.R., Anders, K.H., Press, M.F., Clarke, K., Karlan, B., Chen, J.J., Scully, R., Livingston, D., Zuch, R.H., Kanter, M.H., Cohen, S., Calzone, F.J. & Slamon D.J. (1999). Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. *Nat Genet.* **21**:236-40.

9. Kennedy, R.D., Quinn, J.E., Mullan, P.B., Johnston, P.G. & Harkin, D.P. (2004). The role of BRCA1 in the cellular response to chemotherapy. *J. Natl. Can. Inst.*, **96**: 1659-1668.
10. Yoshida, K. & Miki, Y. (2004). Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci.*, **95**: 866-871.
11. Chakravarthy B, Pietenpol JA. (2003). Combined modality management of breast cancer: development of predictive markers through proteomics. *Semin Oncol.* 30:23-36.
12. Cleator S, Parton M, Dowsett M. (2002). The biology of neoadjuvant chemotherapy for breast cancer. *Endocr Relat Cancer.* 9:183-95.
13. Moynahan, M.E., Cui, T.Y. & Jasin, M. (2001). Homology-directed DNA repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. *Cancer Res* 61:4842-50.
14. Bhattacharyya, A., Ear, U.S., Koller, B.H., Weichselbaum, R.R. & Bishop, D.K. (2000). The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. *J Biol Chem.* 275:23899-903.
15. Fedier, A., Steiner, R.A., Schwarz, V.A., Lenherr, L., Haller, U. & Fink, D. (2003). The effect of loss of Brca1 on the sensitivity to anticancer agents in p53-deficient cells. *Int J Oncol.*, 22:1169-73.

APPENDICES

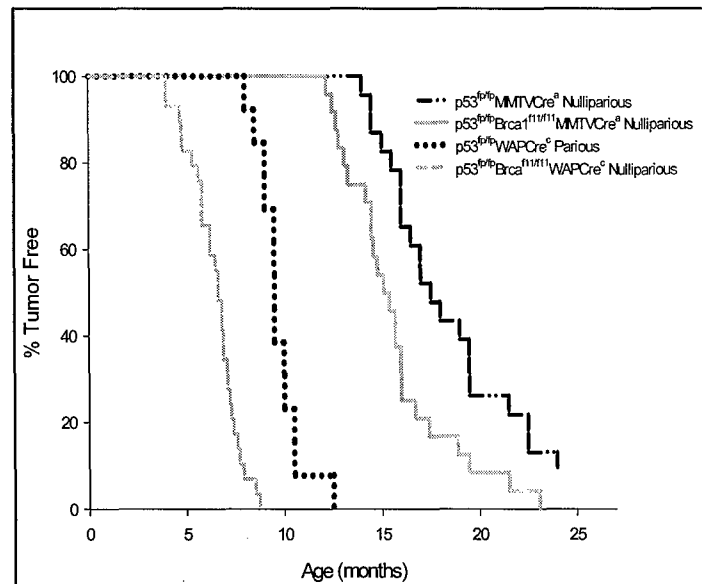


Figure 1- Kaplan Meier plot comparing p53 mutated mice (black) to p53/Brca1 mutated mice (gray). This shows the accelerated rate of tumorigenesis in the p53/Brca1 mice.

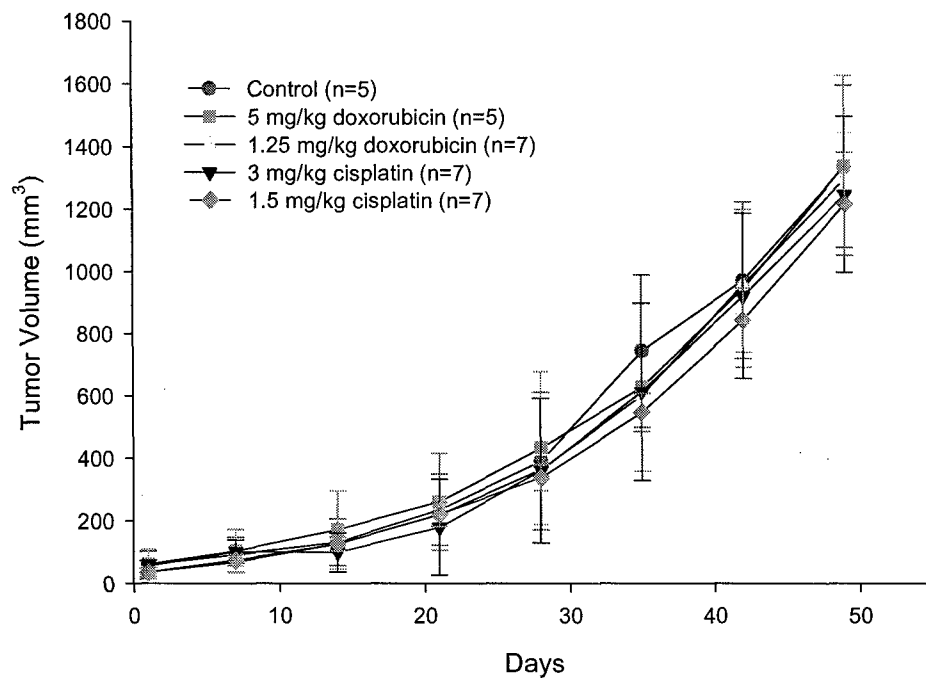


Figure 2- Lack of response of *p53* tumors to Doxorubicin & CDDP *in vivo*.

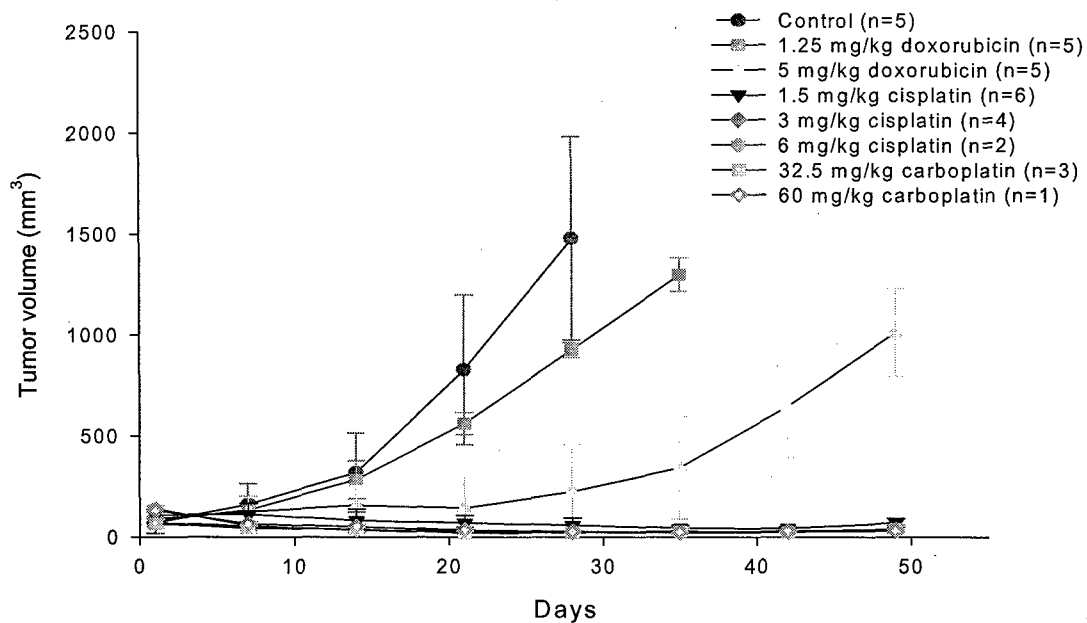


Figure 3- Response of *p53*Δ5&6*Brca1*Δ11 mammary tumors to doxorubicin, CDDP or carboplatin *in vivo*