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TITLE: Rendering DNA Repair Defective by Targeting Wild-type BRCA1 Nuclear Shuttling in Sporadic Breast Cancer as a Therapeutic Agent

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**14. ABSTRACT**

Agents targeting DNA double strand break repair (DSBR) deficiency, such as PARP1 inhibitors, are highly selective in killing BRCA1-mutated breast tumors, and the toxicity is minimal in mouse models. However, more than 90% of breast cancers are sporadic, which carry wild-type (wt) BRCA1 and are proficient in DSBR. BRCA1 is a nuclear shuttling protein, which regulates homologues recombination (HR)-mediated DSBR when nuclear and enhances apoptosis when cytoplasmic. BARD1 retains BRCA1 in the nucleus, whereas ionizing radiation (IR) induces cell cycle-in dependent export of BRCA1 to the cytosol. Both BARD1 and IR in duce BRCA1 shuttling via the CRM1/exportin pathway. It is hypothesized that targeting BRCA1 from the nucleus to the cytoplasm will render cells defective in DSBR and enhance apoptosis. The combination of induced repair deficiency and augmented apoptosis will render sporadic breast cancers highly susceptible to selective killing by agents targeting DNA DSB lesions.

This study will determine if targeting BRCA1 from the nucleus to the cytosol using IR and tr-BRCA1, which releases BRCA1 from BARD1 in nuclear, will compromise DSBR and result in a pro-apoptotic environment which renders tumor cells susceptible to PARP1 inhibitor-induced cytotoxicity. Both BRCA1-proficient human breast cancer cell lines and mouse breast tumor models will be used. DSBR will be measured *in vivo* using a bioluminescence/GFP reporter system. Cytotoxic response to PARP-1 inhibitor will be determined by colony formation *in vitro*, tumor growth delay *in vivo*, and cleaved caspase-3 and annexin-V staining for apoptosis *in vivo* and *in vitro*.

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## INTRODUCTION

This study will determine if targeting BRCA1 from the nucleus to the cytosol using IR and tr-BRCA1, which releases BRCA1 from BARD1 in nuclear, will compromise DSBR and result in a pro-apoptotic environment which renders tumor cells susceptible to PARP1 inhibitor-induced cytotoxicity. Both BRCA1-proficient human breast cancer cell lines and mouse breast tumor models will be used. DSBR will be measured *in vivo* using a bioluminescence/GFP reporter system. Cytotoxic response to PARP-1 inhibitor will be determined by colony formation *in vitro*, tumor growth delay *in vivo*, and cleaved caspase-3 and annexin-V staining for apoptosis *in vivo* and *in vitro*. However, the proposed research project can not initiated due to the delayed arrival of the postdoc research fellow, who was retained in foreign country for the background surveillance procedure required by for the visa application at American embassy. Until May 2009, the research fellow finally arrived and started to work in the laboratory. Due to this delay, limited progress has been made.

## BODY

***Work Tasks:***

*Task 1*. To determine if IR-induced BRCA1 cytosolic accumulation will inhibit DNA double strand break repair (DSBR), promote apoptosis, and sensitize breast cancer cell to PARP1 inhibitor.

- To confirm IR-induced BRCA1 cytosolic accumulation in the human breast cancer cell line MCF7/DR-GFP in culture (completed) and in mouse tumor model (month 1-3).

  Animal model work has been delayed due to the recruited postdoc can not arrive on time due to the visa can not issued by American embassy.
- To determine ionizing radiation (IR)-induced suppression of DSBR using a bioluminescence/GFP reporter system in MCF7/DR-GFP (month 1-6).

Since the arrive of the postdoc couple month ago, we performed the experiments to determine the IR induced BRCA1 NE and the functional correlation of BRCA1 location and the HR capacity, as summarized below.

- To compare PARP1 inhibitor induced cytotoxicity in cell culture model and tumor response in animal breast tumor model, with or without pre-treatment with IR (month 6-10)

This experiments have to be delayed due to the same reason above.

- The DSB repair deficient and BRCA1-mutated HCC1937 human breast cancer cell line will be used as a positive control for PARP1 inhibitor. 5 mice will be used for each treatment group

This experiments have to be delayed due to the same reason above.

Work completed for Aim 1 is summarized as follow,

***Aim 1:*** *To determine if IR-induced BRCA1 cytosolic accumulation will inhibit DSBR, promote apoptosis, and sensitize breast cancer cell to PARP1 inhibitor. DSBR will be measured using a bioluminescence/GFP reporter system in both cultured breast cancer cells and tumors in vivo. PARP1 inhibitor induced cytotoxicity and tumor response will also be determined.*

**Result from Aim 1:**

We found that DNA damage induces Rad51 foci, an *in vivo* marker of HR activity, is seen predominantly in cells with nuclear BRCA1 but rarely in cells with cytosolic BRCA1**(Fig. 1a and 1b).** This suggests that HR activity is suppressed when BRCA1 is sequestered in the cytosol. Consistent with this, γ-H2AX foci which is an *in situ* marker of DSBs is seen predominantly in cells with cytosolic BRCA1 but rarely in cells with nuclear BRCA1 (**Fig. 1c and 1d**). These data suggest that the function of BRCA1 in the repair of DSBs is dependent on its nuclear localization. Nuclear export of BRCA1 protein is sufficient to inhibit its function and induce HR-mediated DSB repair deficiency which results in an accumulation of DSBs.

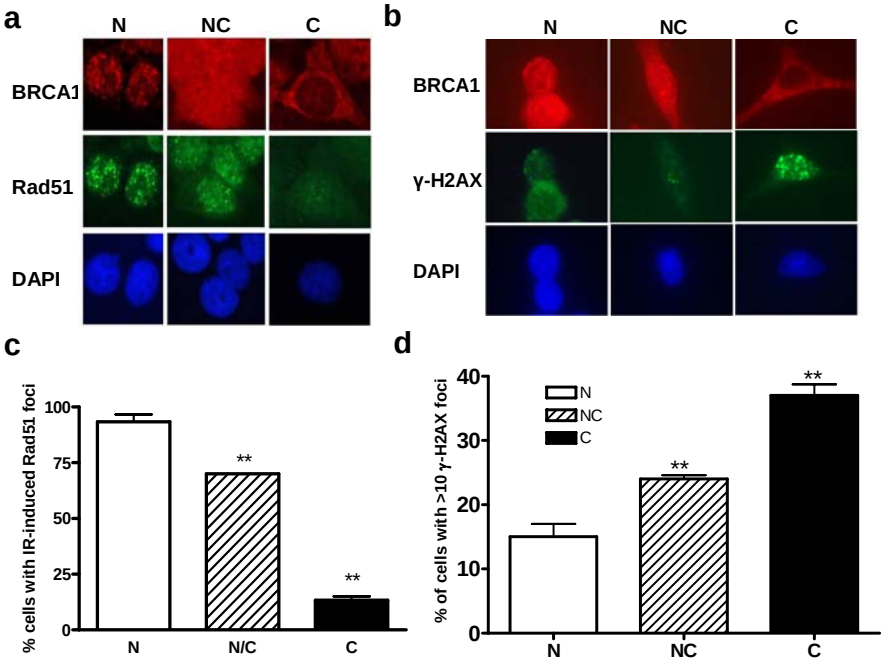


**Fig. 1.** Homologous recombination (HR)-mediated repair of DSB requires nuclear BRCA1. Rad51 foci, an *in vivo* functional marker of HR activity, and γ-H2AX foci, an *in situ* marker of DSBs, in relation to BRCA1 subcellular localization are analyzed in MCF7 human breast cancer cells following 3Gy radiation. Representative immunocytochemistry staining of BRCA1 (Red) localization which are categorized by nuclear only (N), cytosolic only(C), or nuclear/cytosolic (N/C) in (**a**) and **(b);** Rad51 foci (green); (**a**) γ-H2AX foci (green) (**b**); nucleus (blue) in (**a**) and **(b)**.
(**c**) Percentage of cells expressing Rad51 foci as a function of BRCA1 localization. IR-induced Rad51 foci are found predominantly in cells with BRCA1 present in the nucleus (76%), suggesting that HR is strongly associated with nuclear BRCA1. (**d**) Percentage of cells expressing γ-H2AX foci as a function of BRCA1 localization. Radiation-induced γ-H2AX foci are predominantly in cells with BRCA1 present in the cytosol (38%), suggesting that the defect of DSB repair is strongly associated with cytosolic BRCA1. **p<0.001

*Task 2.* To determine if targeting BRCA1 from the nucleus to the cytosol by disrupting BARD1 function will result in sensitization of breast cancer cell to PARP1 inhibitor.

- To establish a inducible expression of the tr-BRCA1 peptide in MCF7/DR-GFP using the tet-off system: MCF7/DR-GFP/tr (month1-4)

This experiment is in progress. We have not established stable cell lines with tet-off inducible tr-BRCA1.

- To determined if expression of the small peptide tr-BRCA1can effectively release wt BRCA1 from the nucleus to the cytosol (Month 3-5)

See summary below.

- To determined if expression of the small peptide tr-BRCA1can efficiently suppress DSBR in MCF7/DR-GFP/tr cells (month 5-6)

See summary below.

- To establish mouse breast tumor model using MCF7/DR-GFP/tr, in which BRCA1 cytoplasmic translocation is inducible in vivo (month 6-10)

These experiments in on going.

- To compare PARP1 inhibitor induced cytotoxicity and tumor response with or without induction of tr-BRCA1 expression (month 4-11)

Experiments are on going.

***Aim 2:*** *To determine if targeting BRCA1 from the nucleus to the cytosol by disrupting BARD1 function will result in sensitization of breast cancer cell to PARP1 inhibitor. BARD1 prevents BRCA1 export to the cytosol through its binding to the N-terminal region of BRCA1, thereby masking the BRCA1 nuclear export signal (NES) and blocking BRCA1 interaction with CRM1/exportin.. It will be determined if expression of the small peptide tr-BRCA1, a truncated form (1-301aa) of BRCA1 that contains the NES and BARD1 binding activity, can effectively release wt BRCA1 from BARD1. It is proposed that this will drive BRCA1 to the cytosol. BRCA1 cytosolic accumulation will be determined by immunohistochemistry staining of BRCA1. DSB repair and cytotoxic response to PARP1 inhibitors will be determined as in aim2. Transient expression of the small peptide tr-BRCA1, a truncated form (1-301aa) of BRCA1 that contains the NES and BARD1 binding activity, can release wt-BRCA1 from BARD1 and effectively shift BRCA1 to the cytosol.*

**Results from Aim 2:**

Transient expression of the small peptide tr-BRCA1, a truncated form (1-301aa) of BRCA1 that contains the NES and BARD1 binding activity, can release wt-BRCA1 from BARD1 and effectively shift BRCA1 to the cytosol[14] (see **Fig. 2**).

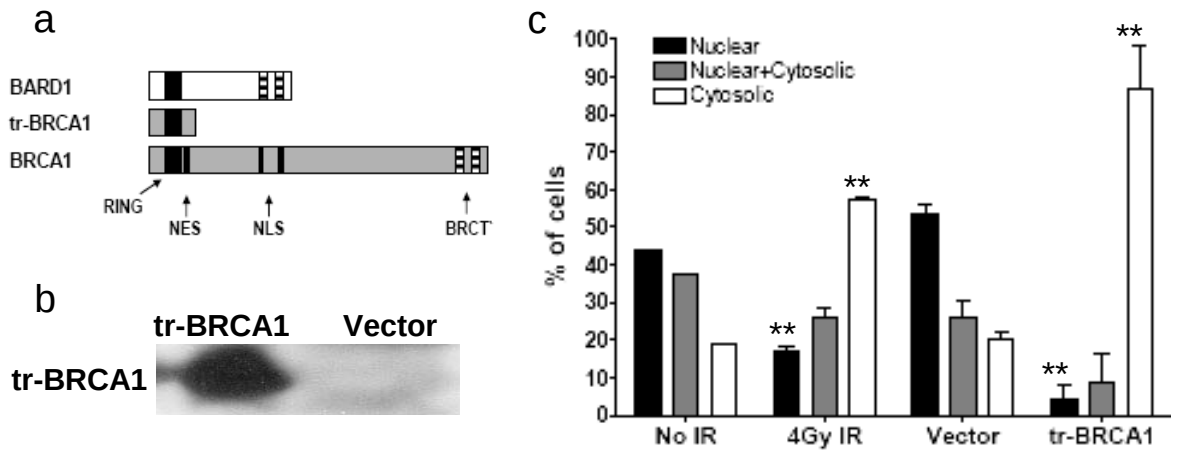


**Fig. 2.** (**a**) Schematic of BRCA1, BARD1, and tr-BRCA1. (**b**) Transient expression of tr-BRCA1in MCF7. **(c)** Cells were treated with either 3Gy radiation or transiently transfected with tr-BRCA1 or vector control. Four hrs after radiation or 24 hrs after transfection, cells were subjected to immunofluorescence staining for BRCA1 location. **p<0.001

## KEY RESEARCH ACCOMPLISHMENTS

The proposed research project can not initiated due to the delayed arrival of the postdoc research fellow, who was retained in foreign country for the background surveillance procedure required by for the visa application at American embassy. Until May 2009, the research fellow finally arrived and started to work in the laboratory. Due to this delay, limited progress has been made.

## REPORTABLE OUTCOMES

The proposed research project can not initiated due to the delayed arrival of the postdoc research fellow, who was retained in foreign country for the background surveillance procedure required by for the visa application at American embassy. Until May 2009, the research fellow finally arrived and started to work in the laboratory. Due to this delay, limited progress has been made.

## CONCLUSION

The proposed research project can not initiated due to the delayed arrival of the postdoc research fellow, who was retained in foreign country for the background surveillance procedure required by for the visa application at American embassy. Until May 2009, the research fellow finally arrived and started to work in the laboratory. Due to this delay, limited progress has been made.

We are putting 100% effort to carry on the study proposed and looking forward a productive and exciting research year.

## REFERENCES

**N/A**

## APPENDICES

**N/A**