Investigating the Potential Genotoxic Effects of Ionising Radiation in BRCA1 Heterozygous Mutant Cells

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Introduction

Ionising radiation, including X-rays, is a well-identified mutagenic agent in spite of its multiple clinical applications, ranging from diagnostic imaging to therapeutic anti-cancer strategies. X-rays damage our genetic material (DNA) and can cause cancer. Healthy and competent individuals have multiple DNA repair pathways to repair damage to DNA, but this may not be the case in individuals who have faulty copies of particular genes involving in DNA repair.

X-rays damage DNA by causing double strand breaks (DSBs). BRCA proteins, encoded by the two major tumour-suppressing genes, BRCA1 and BRCA2, maintain genomic stability by helping to repair DNA DSBs (see Figure 1). Individuals with heterozygous mutations in either BRCA genes have one normal copy and one faulty copy of the gene and this causes increased rates of breast and ovarian cancer in these people[1]. It is concerned that these individuals might have compromised DNA repair capacity as the normal copy of the gene may not be able to compensate for the loss of function of the faulty copy. Such an effect could lead to increased levels of DNA damage after exposure to medical X-rays, with potential health consequences.

Interestingly, even though BRCA1 and BRCA2 protect the DNA of all cell types, mutations in these genes are strongly associated with breast and ovarian cancers[2]. Given that these tissues have the highest levels of the hormone estrogen, it is possible that estrogen is somehow contributing to cancer formation.

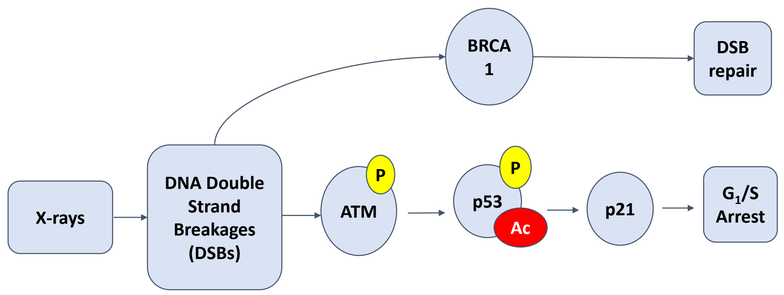

Figure 1. Simplified overview of DSB response[3]. X-rays damage DNA by causing DSBs. The ATM kinase is autophosphorylated and activated after DSBs, leading to the phosphorylation of multiple downstream substrates, e.g. BRCA1 and p53 that control cell cycle arrest and DNA repair. Other protein modifications, including acetylation also regulate these processes.

Aims

- Investigate whether heterozygous mutations in BRCA1 affect levels of DNA damage after X-rays. Normal breast cells will be compared with BRCA1 mutant.
- Investigate whether estrogen is able to cause DNA damage in breast cells
- As a separate project, DNA damage responses were measured in cells after X-rays. The aim was to identify a cellular change that could be used as a potential biomarker of X-ray exposure. Current biomarkers are not perfect.

Results

1. **These experiments measured DNA damage in normal and BRCA1 mutant cells after X-rays.**
   - **Micronucleus Assay:** DNA damage can cause small fragments of DNA ('micronuclei' or 'MN') to appear in cells. DNA damage = more cells with MN.

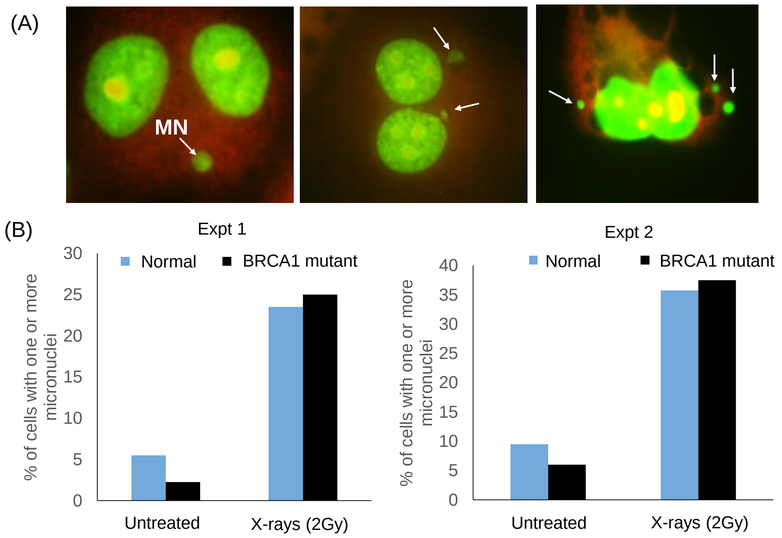

Figure 2. Micronucleus (MN) assay in normal and BRCA1 mutant breast epithelial cells.
(A) Representative images of MN assay demonstrating the presence of MN, as indicated by arrows. Cells were incubated with 4μg/ml Cytochalasin-B for 24 hours to prevent cytoplasm from dividing, thus allowing for MN to be scored in viable once-divided cells which appear as binucleated cells. Acridine orange dye stained the DNA of the cells green and the cytoplasm red. (B) Quantification of micronuclei in cells described above.

- ➢ DNA damage is greatly increased in both control and mutant cells after X-rays
- ➢ No significant difference in DNA damage between control and BRCA1 mutant cells after X-rays.

2. **These experiments investigated whether estrogen (or its breakdown products/metabolites) can cause DNA damage.**

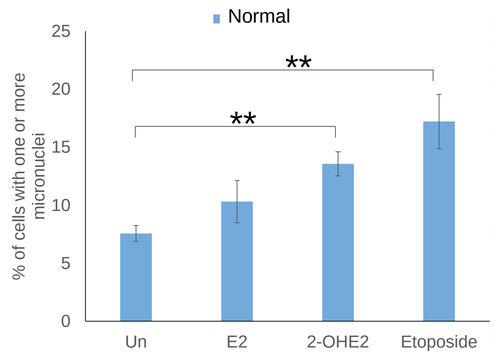

Figure 3. MN assay after estradiol and its metabolite treatment in normal breast cells.
Cells were treated with 10 nM E2, 1 μM 2-OHE2 and 5μM etoposide (positive control) then the levels of DNA damage was assessed with MN assay after 3 hours of incubation. Bars, represent average percentage of binucleated cells with one or more micronuclei and SEM combined from four separate experiments (>400 cells counted per cell line per experiment). Significance of changes was assessed using two-tailed t-test, **, $p<0.01$.

- ➢ Both estradiol (E2) and its metabolite, 2-hydroxyestradiol (2-OHE2) can cause DNA damage. Etoposide is a DNA damaging agent, used as a positive control.
- ➢ 2-OHE2 causes higher level of DNA damage than its parent hormone E2.

3. **These experiments measured DNA damage responses (e.g. protein levels/ protein modification) in cells after X-rays.**
   - There is current interest in measuring DNA damage response proteins to use as potential biomarkers of exposure to X-rays.
   - Responses in proliferating cells were compared to non-proliferating cells
   - The role of the ATM kinase in controlling these responses were investigated using an ATM inhibitor.

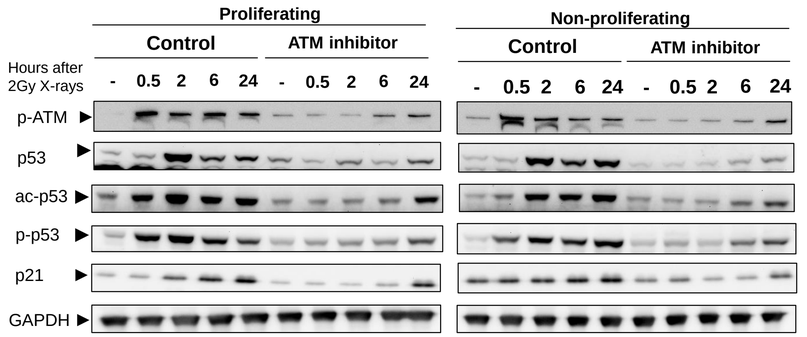

Figure 4. Western blot analysis of levels of DNA damage response proteins after X-rays, e.g. phospho-ATM (p-ATM), p53, acetyl-p53, phospho-p53 (Ser15), p21 and GAPDH (loading control) in human lymphoblastoid cells at different time points following X-rays exposure. Data representative of three independent experiments.

- ➢ Levels of P-ATM, Ac-p53, P-p53 and p21 remained relatively stable even at 24 hours after X-ray exposure in the absence of ATM inhibitor.
- ➢ ATM inhibition blocks the activation of DNA damage responses after X-rays.

Conclusion

- Radiobiological concerns in BRCA mutation carriers due to increased DNA damage in breast epithelial cells observed after IR exposure.
- Potential roles of estrogen in causing DNA damage or impairing DNA damage repair mechanisms.
- Given that the gamma-H2AX, current X-rays biomarker, disappears rapidly after X-rays exposure, we consider that pATM, acetyl-p53, p53(Ser15) and p21 warrant further investigation as biomarkers of X-rays exposure because their levels seemed to last longer after X-rays exposure.

Future Work

- Study levels of DNA damage caused by other BRCA1 mutations[4]
- Investigate how estrogen damages DNA and whether estrogen can enhance DNA damage after X-rays

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1. Sedic, Maja et al. "Haploinsufficiency for BRCA1 Leads to Cell-Type-Specific Genomic Instability and Premature Senescence." *Nature Communications* 6 (2015): 7505.
2. Savage K. I. et al. "BRCA1 Deficiency Exacerbates Estrogen-Induced DNA Damage and Genomic Instability." *Cancer Res 2014;* (74) (10) 2773-2784
3. Deng C-X. BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. *Nucleic Acids Research* 2006; 34(5):1416-1426
4. Cochran, Rory L. et al. "Functional Isogenic Modeling of *BRCA1* Alleles Reveals Distinct Carrier Phenotypes." *Oncotarget* 6.28 (2015): 25240–25251.

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