

Radiosensitivity and breast cancer susceptibility

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Summary

In this study, peripheral blood lymphocytes from *BRCA1*, *BRCA2* and *CHEK2* mutation carriers were found to have increased radiosensitivity when compared to controls. A correlation between telomere length and sensitivity to radiation was also identified. These results may have an impact on the diagnosis and treatment of breast cancer in these individuals in the future.

Introduction

Work carried out in Manchester identified through *in vitro* assays on peripheral blood lymphocytes that individuals diagnosed with breast cancer were less likely to repair chromosome breaks post irradiation. **This study examined if increased chromosome breaks are also present post irradiation in those with an inherited susceptibility to breast cancer.**

Individuals carrying mutations in breast cancer susceptibility genes were assessed for:

- a) increased chromosome radiosensitivity
- b) reduced apoptotic response to radiation

Recruitment

The following individuals were recruited:

- 4 breast cancer patients with a known *CHEK2* mutation
- 30 *BRCA1* mutation carriers and 20 *BRCA2* mutation carriers (without a malignancy)
- 50 age matched controls without a malignancy.

Individuals with known mutations come from very particular groups of breast cancer families and therefore must be matched carefully with controls. Additional individuals were also recruited, including those who had been treated for breast cancer, newly diagnosed patients unselected for family history as well as newly diagnosed patients with a strong family history with an uninformative *BRCA1*, *BRCA2* or *CHEK2* mutation tests.

Method

A blood sample was taken from each individual. This was irradiated and then incubated for 30mins / 3 hours / 10 hours to allow chromosome repair to take place. Cells were harvested and those in metaphase examined for chromosome breaks (larger than the width of a chromatid) and gaps (less than the width of a chromatid). Flow cytometry was used to detect cells undergoing apoptosis.

The cell cycle kinetics of *CHEK2*, *BRCA1* and *BRCA2* mutation carriers and controls were examined to ensure that there were no alterations in check points. No significant differences were found after 8 and 12 hours with or without radiation in any of the patient groups. Therefore the metaphase spreads examined for breaks after the incubation period came from cell populations irradiated at the same stage of the cell cycle. Cells examined in metaphase would have been i) in G₂ at the point of irradiation if treated 3 hours before harvest and ii) in S phase if irradiated 10 hours before.

A S phase enrichment assay was carried out in order to increase the number of cells that were in S phase at the point of irradiation by approximately 3 fold. Irradiation at this point would put the maximum stress on *brca1*, *brca2* and *chek2* function.

Results

a) Chromosome radiosensitivity

BRCA1 mutation carriers (and to a lesser extent *BRCA2*) had a significant increase in the number of chromosome breaks per cell post radiation compared to controls. The same effect was not seen for gaps, which may suggest that gaps are repaired by *BRCA1/2* independent mechanisms.

RNA expression in lymphocytes was examined pre and post radiation to identify which genes are switched on and off following irradiation. **In both *BRCA1* and *BRCA2* mutation carriers there was a significant reduction in *BRCA1/2* expression compared to the controls post irradiation (with no obvious difference pre radiation) suggesting haploinsufficiency.** Several other genes were found to have a mean fold change of greater than 1.5 fold in mutation carriers including some relevant to DNA repair pathways.

***CHEK2* mutation carriers also had an increased number of breaks post irradiation compared with the control groups. This was observed for both cells irradiated during the G_2 and in the S phase enrichment assay. The control group with the highest number of breaks was individuals from the same clinic with a strong family history but uninformative mutation testing. This suggests that unidentified familial factors may be implicated in repairing DNA breaks.**

To investigate whether breast cancer patients have a higher number of chromosome breaks post radiation because of *CHEK2* mutations, radiosensitivity was measured in 100 newly diagnosed individuals. The most common *CHEK2* mutations were searched for in the 25 most radiosensitive patients. However no mutations were identified in this sub-group.

This result may indicate the importance of identifying *Chek2* mutation carriers prior to mammography and radiotherapy. However it is difficult to extrapolate *in vitro* results from peripheral blood lymphocytes into possible effects on live breast tissue.

b) Apoptotic response

Cells undergo apoptosis when the amount of DNA damage in the cell is beyond repair. There was no significant difference in the number of cells undergoing apoptosis post radiation between *BRCA1* and *BRCA2* mutation carriers who have not had cancer and controls.

However, it was observed that if the apoptotic response was measured in breast cancer patients before treatment and one year after treatment there was a reduction in apoptotic response much greater than that predicted due to decreasing response with age (a 15% reduction rather than the normal 0.5% reduction). It is not known the reason for this change and how long the effect remains post treatment.

Telomere Length

Telomere shortening in breast tissue is thought to be a factor in the cause of structural defects that contribute to the development of breast cancer. Telomere length was identified in 250 individuals to see if a correlation was present between telomere length and breast cancer susceptibility. Older individuals had shorter telomeres in both cancer patients and controls as expected. No association was demonstrated between telomere length and breast cancer susceptibility.

A link between radiosensitivity (chromosome breakage and apoptosis) and telomere length was then investigated. Individuals with shorter telomeres were found to have a higher number of chromosome breaks per cell post irradiation. However, when the results were adjusted for age, this effect was no longer significant. In addition, those with shorter telomeres had a reduced apoptotic response. This relationship was still significant after age adjustment of the data. Therefore telomere length does appear to correlate with sensitivity of cells to radiation.

Conclusions

These results may be particularly relevant in the treatment of breast cancer in individuals with an inherited susceptibility to breast cancer, who are known to have a higher risk of future secondary tumours.