

TITLE

Researchers

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Aim

1. To determine whether the level of topoisomerase II α (topo II α) expression in stimulated T-lymphocytes from peripheral bloods of a group of sporadic breast cancer patients is elevated, as compared with a group of non-cancer controls.
2. To determine whether topo II α expression level correlates with chromosomal radiosensitivity as measured by the G2 assay of chromatid breaks in T-lymphocytes from sporadic breast cancer patients.

Project Outline/Methodology

Blood samples were obtained from breast cancer patients, prior to treatment, and from a similar sized group of non-cancer controls. Blood was diluted in growth medium and incubated for 72 hours at 37°C. Following a small dose of gamma-rays cells were incubated with colcemid and calyculin A to block cells at metaphase. Following cell fixation, 50 chromosome spreads from each individual were stained and analysed for radiation-induced chromatid breaks. In parallel separated white cells from bloods of the same patients and controls were cultured for 72 hours before lysis in a detergent solution. These samples were analysed for the amount of topo II α present using an immuno-assay (A490nm), and for the amount of protein per sample (A570nm) using hrp/colorimetric assays. Topo II α expression was calculated as the ratio of sample A490nm/sample protein content.

Key Results

1. We devised a new technique for blocking and analysing chromatid breaks in metaphase cells.
2. We have so far analysed radiation-induced chromatid break frequencies in PHA stimulated T-lymphocytes from 51 breast cancer cases and 48 non-cancer control individuals.
3. We optimised a method for T-lymphocyte separation (ficoll and magnetic beads) from whole blood, PHA stimulation and cell lysis and dialysis procedures for sample preparation. We also developed a DAS-ELISA technique for measurement of topo II α , and a sensitive protein analysis (QP-BCA) method for breast cancer and control samples.

Conclusions

1. Topo II α expression was found to be significantly increased in lymphocytes from a group of breast cancer cases as compared with controls.
2. Chromatid break frequencies were elevated in 50% of breast cancer patients as compared to only 10% of controls.
3. Chromatid break frequencies showed a positive, but (based on the number of samples analysed) not a significant correlation with topo II α expression level.

What does this study add to the field?

This pilot study has increased our knowledge of the formation of chromatid breaks (i.e. shown the involvement of topo II α in chromatid breakage, and leads the way for future studies in larger groups of individuals.

Implications for Practice or Policy

Although the results of the project will not immediately benefit NHS patients, an understanding of the relationship between elevated chromatid radiosensitivity, topo II α expression and breast cancer susceptibility could help develop a future marker for susceptibility.

Where to next?

The results are sufficiently encouraging to warrant instigation of a larger study to confirm that topo II α expression underlies chromatid radiosensitivity.

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