Biological Ageing Effect of Radiotherapy in Breast Cancer


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The ability of peripheral blood lymphocytes to undergo radiation-induced apoptosis in vitro usually falls by only 0.5% per year. In a recent study, it has been found that in vitro apoptotic response to ionising radiation in peripheral blood lymphocytes fell by 15% in 12 breast cancer patients when repeated one year post radiotherapy (Docherty et al., 2007). This is equivalent to 30 years of biological ageing over a one year period post irradiation. Our hypothesis is that the decrease in peripheral blood lymphocytes ability to undergo apoptosis is a result of irradiation-induced biological ageing. This project aims to validate proposed in vitro markers of biological ageing in peripheral blood lymphocytes (including global genomic Methylation by High Performance Liquid Chromatography analysis and Telomere Lengths using Single Telomere Length Analysis and Telomere Restriction Fragment analysis) and radiosensitivity assays (including radiation-induced apoptotic response via Sub-G1 and Annexin V/FITC assays, and DNA Single Strand Break & Double Strand Break formation and repair via Comet and gamma-H2AX assays). We plan to investigate these observations within a larger breast cancer patient and control cohort to investigate the cause of biological ageing. It is hoped that these assays could be used to predict response to treatment/complications or prognosis, or to determine the effectiveness of bio-preventative agents in the future.
Biological Ageing Effect of Breast Cancer Radiotherapy (Pilot Study)

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Is there a link between in vitro biological ageing, radiosensitivity and cancer susceptibility?

BACKGROUND

Normally apoptotic response (AR) is reduced by 0.9% per year. Docherty et al. (2007) has found declined AR by 15% in breast cancer patients after one year follow up post radiotherapy.

This is equivalent to 30 years biological ageing effect that was found in one year.

OBJECTIVE

Does biological ageing occur post radiotherapy?

Could be measured in PBL by AR & DNA Single / Double Strand Breaks Repair

METHODS

Sub-G1 peak assay

Annexin V-FITC assay

DNA repair

SSB – Comet assay

DNA SSB Repair

Telomere Length – STELA & TRF

Global Methylation – HPLC

Plan of Action

Third trial:

? Radiation dose asymmetry

? Flask’s position

? Radiator

DNA DSB repair: y H2AX assay

Phosphorylated H2AX: proteinase repair foci.

DNA SSB repair: Early step in response to DSB

Time course of DNA SSB repair (mean PI signal measurements)

- Irradiated PBL (4 Gy, blue ⬤) were fixed after 0, 2, 4 or 24 h incubation.
- Mock-irradiated PBL (0 Gy, red ⬤) were only fixed after 0 and 24 h incubation.
- Similar DNA SSB repair capacity between the two samples.

AR: 2. Annexin V-FITC/PI assay

Annexin V: Anti-coagulant, high affinity to Phosphatidylserine (PS) binding (Ca++ dependent)

Apoptosis: Caspase cascade activation...

Inhibition of Aminophospholipid translocase (APT) ... activation of Phospholipid scramblase (PLSCR) ... exposure of PS

Cells (%)

First Trial

Second Trial

Third Trial

Apoptosis (%)

Sample - A

Sample - B

0 Gy 4 Gy

0 Gy 4 Gy

Decreased % apoptosis (24 h after irradiation):

3rd Trial: % Induced Apoptosis (0 Gy – 4 Gy) A

UL: 0.3

UR: 28

B

UL: -0.4

UR: 15.5

Sub-G1 assay vs Annexin V-FITC/PI assay

AR by Annexin V assay < AR by Sub-G1 assay

? Low PI concentration (Annexin V assay) – false negative.

? Higher rate of apoptosis earlier than 24 h – Include positive control

Conclusions:

Established collaboration at a new centre.

Age variation between donors A & B (~ 15 years) was confirmed by AR (A higher than B) and DNA SSB repair capacity (more efficient repair at younger age)

Suggests:

- Reproducibility of the last trial results
- PI conc. & reproducible results
- Include more controls
- Include more controls
- Include more controls

For additional information please contact:

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DNA ID

DNA DSB Repair

DNA DSB Repair

Cell Cycle Analysis by Flow Cytometry

Non-apoptotic cell

Apoptotic cell

DNA content (cell cycle phases): G0 < 2n; G1 = 2n; S = 2-4n; G2, M = 4n

Apoptosis – DNA fragmentation (i.e. n = 2, sub-G1 peak)

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