G2 checkpoint control and G2 chromosomal radiosensitivity in cancer survivors and their families

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Significant inter-individual variation in G2 chromosomal radiosensitivity, measured as radiation-induced chromatid-type aberrations in the subsequent metaphase, has been reported in peripheral blood lymphocytes of both healthy individuals and a range of cancer patients. One possible explanation for this variation is that it is driven, at least in part, by the efficiency of G2-M checkpoint control. The hypothesis tested in the current analysis is that increased G2 chromosomal radiosensitivity is facilitated by a less efficient G2-M checkpoint. The study groups comprised 23 childhood and adolescent cancer survivors, their 23 partners and 38 of their offspring (Group 1) and 29 childhood and young adult cancer survivors (Group 2). Following exposure to 0.5 Gy of 300 kV X-rays, lymphoblastoid derivatives, before and after G2 to mitosis (7, 8). A comparison of radiation-induced chromatid aberration levels in both normal and AT lymphocytes and their lymphoblastoid derivatives, before and after G2 to mitosis transition, provided direct evidence that activation of the ATM-dependent G2 checkpoint following irradiation is a key event in the repair of damage prior to progression to metaphase (7). Moreover, abrogation of the G2 checkpoint in normal cells by pre-irradiation treatment with caffeine (7) or the benzene metabolite hydroquinone (9) was found to result in enhanced levels of radiation-induced G2 chromosome damage progressing to metaphase.

Materials and methods

Sample collection
Peripheral blood samples were collected from Danish survivors of childhood and young adult cancer who had been treated with radiotherapy, their partners and their offspring. This work formed part of a large multinational study groups and test the hypothesis that an increase in G2 chromosomal radiosensitivity is facilitated by a less efficient G2-M checkpoint. We present previously unreported mitotic inhibition data for the cancer families of the initial study (15) and the results of applying the PCC methodology to study G2 checkpoint efficacy in survivors of cancer in the extended study (16).

Introduction
Ionising radiation delivered in the G2 phase of the cell cycle can cause a transient ataxia telangiectasia mutated (ATM)-dependent cell cycle arrest, which allows time for DNA repair and prevents the progression of damaged cells from the G2 phase into mitosis (1). Studies of radiation-induced mitotic inhibition have revealed that cell cycle arrest in G2 is much less pronounced in cells from patients with the inherited cancer-prone syndrome ataxia-telangiectasia (AT) than in normal cells (2, 3). Less G2 arrest has also been postulated to contribute towards the enhanced G2 chromosomal radiosensitivity seen in cells from some patients with sporadic cancer, e.g. breast cancer (4).

The premature chromosome condensation (PCC) technique enables categorisation of each cell cycle phase by the visualisation of distinct chromosome morphologies and has been applied to the study of cell cycle kinetics following irradiation. An early application of the PCC technique revealed that arrested cells in G2 repair many of their DNA breaks before mitosis (5), indicating that one purpose of G2 checkpoint delay is to allow additional time for the repair of DNA damage. More recently, the PCC technique has been improved (6) and combined with the G2 radiosensitivity assay (7,8). A comparison of radiation-induced chromatid aberration levels in both normal and AT lymphocytes and their lymphoblastoid derivatives, before and after G2 to mitosis transition, provided direct evidence that activation of the ATM-dependent G2 checkpoint following irradiation is a key event in the repair of damage prior to progression to metaphase (7). Moreover, abrogation of the G2 checkpoint in normal cells by pre-irradiation treatment with caffeine (7) or the benzene metabolite hydroquinone (9) was found to result in enhanced levels of radiation-induced G2 chromosome damage progressing to metaphase.

G2 chromosomal radiosensitivity has been postulated as a marker for cancer predisposition with many studies concluding that enhanced radiosensitivity may be a marker of low-penetrance cancer predisposition genes involved with the recognition and repair of DNA damage (10–14). Previously we reported on the relationship between G2 chromosomal radiosensitivity and early onset cancer in a population of Danish childhood and adolescent cancer survivors and their families (15) and this study has now been extended (16). Here, we examine the relationship between G2 chromosomal radiosensitivity and efficacy of the G2 checkpoint in the same study groups and test the hypothesis that an increase in G2 chromosomal radiosensitivity is facilitated by a less efficient G2-M checkpoint. We present previously unreported mitotic inhibition data for the cancer families of the initial study (15) and the results of applying the PCC methodology to study G2 checkpoint efficacy in survivors of cancer in the extended study (16).
collaboration between research groups in the USA, UK, Denmark and Finland utilising epidemiology and genetic techniques to study germ cell mutagenesis and cancer susceptibility (17) (http://www.gcct.org, last accessed October 20, 2010). Approval for the study was obtained from the Danish Scientific Ethical Committee and the Danish Data Protection Agency. All cancer patients were selected from the Danish survivor cohort, which was derived from the files of the nation-wide population-based Danish Cancer Registry. Full details of patient selection, blood sampling and cancer diagnosis have been reported (15,16). Two groups provided material for the analyses reported here. Group 1 comprised 28 cancer survivors and their families who provided blood samples between March 2002 and April 2003 (15). As described previously (15), only 23 of the 28 Danish cancer survivor families proved suitable for G2 chromosomal radiosensitivity analysis and these 23 survivors, their 23 partners, and 38 offspring were subsequently analysed for radiation-induced mitotic inhibition. Group 2 comprised 30 cancer survivors, who provided samples between June and December 2006 (16). One of these samples failed to culture successfully, leaving 29 samples, which were analysed for G2 chromosomal radiosensitivity (16) and also for radiation-induced G2 delay using the PCC technique.

The G2 chromosomal radiosensitivity assay

The G2 chromosomal radiosensitivity results for both study groups have been reported (15,16). The assay was performed according to the method previously described (18), which was based on modified versions of earlier published techniques (12,19). Briefly, whole blood samples were cultured for 72 h and irradiated with 0.5 Gy of 300 kV X-rays. Following a 30-min recovery period, 0.2 ml colcemid (10 μg/ml) was added for 60 min prior to plunging into ice and harvesting. Metaphase slides were prepared according to standard procedures and stained with Giemsa. One hundred well-spread metaphases were analysed and the total number of chromatid gaps and breaks was determined to give the G2 aberration yield. Studies using the G2 assay in our laboratory have shown that G2 aberration yield in Group 1. Mitotic inhibition is calculated as the percentage reduction in mitotic index in irradiated compared with non-irradiated samples. The relative decrease in the mitotic index following irradiation represents the extent of G2 checkpoint delay. A scatter plot showing the correlation between the previously derived radiation-induced G2 aberration yields (15) and the corresponding percentage mitotic inhibition is illustrated in Figure 1. Spearman’s rank correlation analysis revealed that there were no statistically significant relationships between mitotic inhibition and G2 aberration yield in cancer survivors (P = 0.751), partner controls (P = 0.634), offspring (P = 0.824) or when all three groups were considered together (P = 0.379).

For Group 2, which comprised only cancer survivors, G2 checkpoint delay is presented in Table II. A scatter plot of the G2 aberration yields observed at metaphase and the corresponding G2 checkpoint delay measured by PCC analysis is presented in Figure 2. No statistically significant relationship between the G2 chromosomal radiosensitivity assay and G2 checkpoint delay was observed. G2 aberration yield in Group 1. Mitotic inhibition is calculated as the percentage reduction in mitotic index in irradiated compared with non-irradiated samples.

Results

For Group 1, mean and median mitotic inhibition data are presented in Table I. One-way analysis of variance of the mitotic inhibition values for cancer survivors, partners and offspring revealed no statistically significant differences (P = 0.212). Comparison of median values using the Mann–Whitney U-test also revealed no statistically significant differences between the cancer survivors (P = 0.660) or offspring (P = 0.171) and the partner control group (Table I). When making gender comparisons, no statistically significant differences in mitotic inhibition values were observed between male and female survivors (P = 0.560), partners (P = 0.065) or offspring (P = 0.193). In addition, there was no statistically significant correlation between mitotic inhibition and age when survivors (P = 0.559), partners (P = 0.237) and offspring (P = 0.510) were considered as separate groups. A scatter plot showing the correlation between the previously derived radiation-induced G2 aberration yields (15) and the corresponding percentage mitotic inhibition is illustrated in Figure 1. Spearman’s rank correlation analysis revealed that there were no statistically significant relationships between mitotic inhibition and G2 aberration yield in cancer survivors (P = 0.751), partner controls (P = 0.634), offspring (P = 0.824) or when all three groups were considered together (P = 0.379).

For Group 2, which comprised only cancer survivors, G2 checkpoint delay is presented in Table II. A scatter plot of the G2 aberration yields observed at metaphase and the corresponding G2 checkpoint delay measured by PCC analysis is presented in Figure 2. No statistically significant relationship between the G2 chromosomal radiosensitivity assay and G2 checkpoint delay was observed. G2 aberration yield in Group 1. Mitotic inhibition is calculated as the percentage reduction in mitotic index in irradiated compared with non-irradiated samples.

Table I. Mitotic inhibition in 23 childhood and adolescent cancer survivors, their 23 partners and 38 offspring (Group 1)

<table>
<thead>
<tr>
<th>Donor group</th>
<th>Number</th>
<th>Mean age (years) (range)</th>
<th>Mean mitotic inhibition (%) ± SD</th>
<th>Median mitotic inhibition (%) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>8</td>
<td>33.5 (31.0–36.0)</td>
<td>81.1 ± 19.7 (86.3 (33.3–94.7)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>15</td>
<td>32.9 (25.0–37.0)</td>
<td>79.0 ± 17.5 (83.3 (33.3–100.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>33.1 (25.0–37.0)</td>
<td>79.7 ± 17.9 (84.6 (33.3–100.0)</td>
<td></td>
</tr>
<tr>
<td>Partners</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>15</td>
<td>34.6 (26.0–43.0)</td>
<td>84.1 ± 9.1 (87.5 (66.7–93.1)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>34 (31.0–36.0)</td>
<td>66.0 ± 29.8 (78.2 (0.0–91.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>34.4 (26.0–43.0)</td>
<td>77.8 ± 20.3 (82.4 (0.0–93.1)</td>
<td></td>
</tr>
<tr>
<td>Offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>22</td>
<td>4.7 (0.3–10)</td>
<td>86.7 ± 11.3 (87.9 (60.0–100.0)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>16</td>
<td>6.7 (0.6–14)</td>
<td>82.3 ± 10.4 (82.8 (55.6–96.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>5.5 (0.3–14)</td>
<td>84.8 ± 11.0 (86.1 (55.6–100.0)</td>
<td></td>
</tr>
</tbody>
</table>

SD, Standard deviation.

Fig. 1. Correlation between radiation-induced mitotic inhibition and G2 aberration yield in Group 1. Mitotic inhibition is calculated as the percentage reduction in mitotic index in irradiated compared with non-irradiated samples.
Table II. G2 checkpoint delay in 29 childhood and young adult cancer survivors (Group 2)

<table>
<thead>
<tr>
<th>Donor group</th>
<th>Number</th>
<th>Mean age (years) (range)</th>
<th>G2 delay* (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>17</td>
<td>50.5 (38.0–68.0)</td>
<td>0.038 (−0.29 to 0.39)</td>
</tr>
<tr>
<td>Females</td>
<td>12</td>
<td>47.0 (32.0–62.0)</td>
<td>0.075 (−0.20 to 0.33)</td>
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<tr>
<td>Total</td>
<td>29</td>
<td>49.0 (32.0–68.0)</td>
<td>0.053 (−0.29 to 0.39)</td>
</tr>
</tbody>
</table>

\*See Materials and Methods for derivation.

Discussion

A relatively low radiation-induced mitotic inhibition value is thought to represent a deficient checkpoint in which less time is allowed for the repair of chromosome damage before the onset of mitosis and, thus, higher aberration yields would be observed at metaphase (2,4,21). In lymphocytes from normal donors, an increased G2 chromosomal radiosensitivity has been associated with a decrease in mitotic delay induced by 0.02 Gy X-rays, although no correlation was found when a dose of 0.3 Gy was used (22). The authors speculated that a saturation effect may occur at the higher dose suggesting that the 0.4–0.5 Gy X-ray dose, generally utilised in the G2 chromosomal radiosensitivity assay, may be too high to uncover subtle differences in G2 checkpoint delay. However, in a mixed population of breast cancer patients and normal females, an increase in mitotic delay correlated with a decrease in the level of chromatid aberration frequencies following exposure in G2 to an acute dose of 0.5 Gy (4). In addition, this study revealed less G2 arrest in breast cancer patients in comparison to normal females suggesting a putative G2 checkpoint defect that may contribute to the enhanced G2 chromosomal radiosensitivity seen in 40% of cases (4). The authors, however, suggested that only a very small proportion of radiosensitive patients have a putative G2 checkpoint deficiency and concluded that radiation-induced mitotic inhibition and G2-induced chromatid aberration frequency observed at the subsequent metaphase may not be causally related.

In the current study, the distributions and the median mitotic inhibition values reported for the cancer survivors, their partners and their offspring in Group 1 are broadly in line with those reported for breast cancer (4), prostate cancer (23), benign prostatic hyperplasia (23) and AT heterozygotes (24). However, Group 1 showed no association between mitotic inhibition and cancer status (Table I) nor any correlation between radiation-induced mitotic inhibition and G2 aberration yield (Figure 1). Unlike a report by Scott et al. (4), we found no age or gender effects on mitotic inhibition in the cancer survivors or in the corresponding partner control group.

The PCC technique has revealed that elevated chromosomal radiosensitivity observed at metaphase following G2 irradiation is directly linked to a less efficient G2 checkpoint in AT lymphocytes (7). However, application of the PCC technique has also demonstrated that despite an increased radiation-induced G2 delay, BRCA1 heterozygotes have an increase in chromosome aberrations at metaphase when compared to normal controls, thus suggesting that mitotic delay does not necessarily reduce the radiation-induced chromosome damage (20). In the present study, application of the PCC technique to evaluate G2–M checkpoint efficacy in the 29 childhood and young adult cancer patients of Group 2 revealed no correlation between the extent of radiation-induced G2 checkpoint delay and chromosomal radiosensitivity when G2-irradiated cells were examined for aberrations at the subsequent metaphase (Figure 2). In addition, there was no suggestion that gender or age influenced the extent of the radiation-induced delay (Table II).

The reasons for the large inter-individual variation observed when applying the G2 chromosomal radiosensitivity assay have not yet been elucidated. However, this study, using two different assays, has found no support for the suggestion that a longer G2 delay results in less radiation-induced chromosome damage progressing to metaphase, thus indicating that inter-individual variation in G2 chromosomal radiosensitivity is more likely to be driven by other factors, such as variation in DNA repair capability, rather than being influenced by G2 checkpoint differences.

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References


