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Gender based radiosensitivity

Gender bias in individual radiosensitivity and the association with genetic polymorphic variations

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ABSTRACT

Purpose: To assess the extent of variation in radiosensitivity between individuals, gender-related dissimilarity and impact on the association with single nucleotide polymorphisms (SNPs).

Materials and methods: Survival curves of 152 fibroblast cell strains derived from both gender were generated. Individual radiosensitivity was characterized by the surviving fraction at 2 Gy (SF2). SNPs in 10 radiation responsive genes were genotyped by direct sequencing.

Results: The wide variation in SF2 (0.12–0.50; mean = 0.33) was significantly associated with 3 SNPs: *TP53* G72C ($P = 0.007$), *XRCC1* G399A ($P = 0.002$) and *ATM* G1853A ($P = 0.01$). Females and males differed significantly in radiosensitivity ($P = 0.004$) that impacted genetic association where only *XRCC1* remained significant in both gender ($P < 0.05$). Meanwhile, discordant association was observed for *TP53* that was significant in females ($P = 0.012$) and *ATM* that was significant in males ($P = 0.0006$). When gender-specific SF2-mean (0.31 and 0.35 for females and males; respectively) was considered, further discordance was observed where *XRCC1* turned out not to be associated with radiosensitivity in males ($P > 0.05$).

Conclusions: Although the variation in individual radiosensitivity was associated with certain SNPs, gender bias for both endpoints was evident. Therefore, assessing the risk of radiation exposure in females and males should be considered separately in order to achieve the ultimate goal of personalized radiation medicine.

Ionizing radiation (IR) is ubiquitous in nature and living organisms are continuously exposed to variable level of low radiation doses from natural radioactive background and escalating doses from medical practices and industrial applications [1]. Although IR has many beneficial applications in modern life, it might cause deleterious effects particularly if it has been misused [2–4].

Individuals, however, do not respond equally to similar doses of IR. Human population heterogeneity in radiosensitivity is illustrated by rare genetic disorders such as ataxia-telangiectasia (A-T), Nijmegen breakage syndrome (NBS), NBS-like, ligase IV deficiency (LIG4 syndrome) and ataxia-telangiectasia like disorder (ATLD). Cells derived from those patients are hypersensitive to IR due to mutations impacting DNA double-strand break (DSB) recognition, signaling, and repair capacity [5,6]. However mutations are rare and present only in a small percentage of hypersensitive individuals [7].

To explain the wide range of radiosensitivity, attention is focused on the more common genetic polymorphic variations between individuals. Unlike genetic mutations that disrupt the function of the encoded protein, single nucleotide polymorphisms (SNPs) may only cause subtle changes that can influence the rate of mRNA transcription, mRNA stability, its rate of translation to protein and/or the protein–protein interactions resulting in sub-optimum protein function leading to different degrees of susceptibility to IR, environmental factors, infectious agents, diseases and individual response to pharmacological agents [8,9]. Furthermore, there are variations between human populations, so a SNP allele that is common in one geographical or ethnic group may be infrequent in another [10].

The association between SNPs and radiosensitivity in the general population has not been systematically studied. This is important because IR poses accentuated health hazard particularly with the continuous increase in the applications of radiation

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technologies in various aspects of health and diseases. The consequent increase in the collective doses received by the population particularly in radiosensitive individuals [11] may cause an increase in the cumulative deleterious effects in humans which in its turn may be translated to increase in the long term appearance of certain types of complications and cancers [12]. The main deterministic and stochastic health effects of radiation exposure are the induction of toxicity in organs and tissues, neoplastic transformation in addition to potential hereditary consequences [4].

The term "radiogenomics" has initially been applied to identify candidate genetic biomarkers to individualize risk of developing morbidity in radiotherapy patients [13], which gained momentum with the advent of genome wide association studies [14,15]. Similarly, it seems tempting to hypothesize that "radiogenomics" can also apply to individual variations in radiosensitivity in the populace. Currently health protection policies do not take into account any contribution of genetic variations to individual risk of radiation exposure [16]. Such contribution would help to develop more refined approaches to assess radiation health risk in humans.

In this study, we have explored this hypothesis using 152 fibroblast cell cultures established from normal individuals. Cellular radiosensitivity was measured by the gold-standard clonogenic survival assays. Genetic variations were determined by direct genotyping of 10 selected SNPs in genes known to be involved in radiation response (*CDKN1A (p21)* C31A (Ser/Arg) rs1801270, *TP53 (p53)* codon G72C (Arg/Pro) rs1042522, *HDM2 (MDM2)* promoter T309G rs2279744, *ATM* G1853A (Asp/Asn) rs1801516, *XRCC1* G399A (Arg/Gln) rs25487, *XRCC3* G241A (-strand C/T) (Thr/Met) rs861539, *LIG4 (DNA-Ligase 4)* C9T (Thr/Ile) rs1805388, *PRKDC (DNA-PKcs)* T3434C (-strand A/G) (Ile/Thr) rs7830743, *TGFB1* C10T (Lue/Pro) rs1982073 and *XRCC5 (KU80)* A2790G 3′ UTR rs1051685).

Materials and methods

Cell strains and culture conditions

A total of 152 non-transformed fibroblast cell strains were used from our cell strain collections established from phenotypically normal individuals. The institutional review board (IRB) has approved the study. Donors have voluntarily participated and signed an informed consent. The method of establishing the fibroblast cell strains was described elsewhere [17]. Cells were maintained in DMEM culture medium supplemented with 15% fetal bovine serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin and incubated at 37 °C in 5% CO2 humidified atmosphere.

Cellular radiosensitivity measurements

Experiments were carried out using previously described methodology with minor modifications [18]. Briefly, to minimize experimental variations due to cell cycle differences, contact-inhibited cultures were used. Clonogenic survival was assessed using fixed number of seeded cells (tested + feeder) of 1000 cells/cm^2. Feeder cells, from the same cell strain tested, were irradiated with a single irradiation dose of 30 Gy (to prevent any cell division) and seeded in appropriate numbers 24 h before receiving the tested cells. The tested confluent fibroblast cultures were trypsinized, counted, diluted and seeded in an appropriate number to yield at least 50 colonies in each of 3 replicated flasks. Irradiation, with a single dose that ranged between 0 and 4 Gy, was delayed for 4–6 h after plating to allow the cells to attach to the surface of the flasks. The cells were incubated for 2–3 weeks, then they were fixed and stained using crystal violet. Colonies of at least 50 cells were scored as survivors. Three to five independent experiments were carried out for each cell strain.

DNA extraction, amplification and sequencing

DNA was extracted from cultured fibroblasts using Puregene DNA Purification Kit (Gentra System, Qiagen, Minneapolis, MN, USA) according to the manufacturer's instruction. PCR primers of the selected SNPs are available upon request. Relevant segments of DNA were amplified by thermal cycling as described previously [19]. The amplified fragment was directly sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instruction, and were run on the MegaBase 1000 sequencer (Applied Biosystems, Waltham, MA, USA). Sequencing results were aligned to the corresponding reference sequence and the SNPs were genotyped using SeqManII sequence analysis software (DNASTAR Inc., Madison, WI, USA).

Irradiation

Irradiation was performed using X-RAD 320 (Precision X-ray, CT, USA) biological irradiator at a maximum energy of 320 keV filtered with 2 mm Al, and a dose rate of 1.33 Gy/min. In addition to ionizing chamber (PTW, Freiburg, Germany), the absorbed dose was also measured using a GAFCHROMIC film, EBT2 model (International Specialty Products, Wayne, NJ, USA) as described previously [20].

Data analysis

Survival data from replicate experiments were pooled and fitted to the linear quadratic model of cell killing [SF = exp $(-\alpha D - \beta D^2)$, where α and β are constant and D is the dose], to generate cellular survival curves. The well-established parameter of the surviving fraction at 2 Gy (SF2) was used to characterize the radiosensitivity of each cell strain [21]. SF2 was computed from the whole survival curve and used as a unique measure of cellular sensitivity to radiation. The mean SF2 of the 152 cell strains was used to separate cell strains to 2 groups, radiosensitive (cases) and normal (controls).

The association between radiosensitivity groups (SF2), SNPs genotype and allelic frequency were measured by the odds ratio (OR) with its 95% confidence interval (95% CI). Significance of OR was assessed by the Chi-square (χ^2) test. A P-value of 0.05 or less is considered statistically significant. The alleles showing statistically significant ($P \leqslant 0.05$) association with increased radiosensitivity (decreased SF2) were considered as risk allele and given a score of 1. Therefore, cell strains homozygous for a risk allele have a score of 2, heterozygous have a score of 1, while those which do not harbor the risk allele have a score of zero. The number of risk alleles for each individual was calculated by summing the scores of the different SNPs significantly associated with radiosensitivity. Difference between groups was assessed by the non-parametric Mann–Whitney Rank Sum test. Correction for multiple comparisons was carried out using the Bonferroni method, which indicates statistical significance when the P-value is lower than the type I error (0.05) divided by the number of comparisons declared significant. Statistical analysis was carried out using the SigmaPlot platform (Version 12.5, Systat Software, Inc., San Jose, CA, USA) and the free online software, *Case Control Studies*, Institute of Human Genetics, Helmholtz Center Munich, Germany (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Results

Subjects and cellular radiosensitivity

The age of the 152 subjects included in this study ranged between 18 and 79 (median = 48) years old. There were 63 males and 89 females. The survival curves of the 152 fibroblast cell