Radioactive PTT as Part of Screening Protocol for Prospecting Radiation Workers

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Abstract

Heterozygous mutations in *BRCA1* or *BRCA2* have been found to be associated with enhanced cellular radiosensitivity with impaired proliferative capacity after irradiation and could predispose increased risk of radiation-induced mutagenesis and carcinogenesis (1,2). Deficient repair mechanism exhibited by lymphocytes from breast cancer patients provides associated vulnerability to genotoxicity of ionizing radiation. Other genes may also play a role in terms of clinical radiation hypersensitivity needed in predicting response to radiotherapy. However, relaxation of cell cycle checkpoints, production of micronuclei, and loss of proliferative capacity which have been exhibited by impairment of irradiated cells lacking functional *BRCA1* and *BRCA2*, accentuate the notion that heterozygous women may respond differently to radiation. The radioactive protein truncation test (PTT), utilized as screening procedure to detect frameshift mutations, can be employed to clarify radiosensitivity of individuals carrying a mutated *BRCA1* gene. It can, therefore, be incorporated in the series of clinical assays used in standard screening protocols for prospective nuclear facility workers.

INTRODUCTION

Workers in nuclear facilities typically undergo medical screening prior to, and during employment, as part of routine personnel monitoring in their respective institutions. Information acquired therein is used as a contributory source of information on the health effects of radiation to address concerns about adequacy of radiation protection standards. Existing complete database of nuclear staff, including their quantitative radiation dose exposure estimates obtained through the substantial work and attention given by the health physics staff at these facilities, allow researchers to follow them over time for study purposes geared towards continually establishing appropriate radiation exposure limits for worker protection (3).

Quantitative evidence from these epidemiological researches provide basis for the current radiation protection standards used by the International Commission on Radiation Protection (4,5). However, there is a progressively growing concern on low-level occupational exposure of nuclear facility employees to ionizing radiation provoked by the significant increases in cancer deaths related to radiation they received on-the-job. Though radiation workers tend to be a particularly healthy group of people, several studies of nuclear workers "show significant positive trends in cancer mortality with cumulative radiation dose" particularly among older personnel despite that received radiation exposures were well below the limits defined by current standards. Studies indicate that the magnitude of radiation-cancer risk estimate is contentiously 10-folds the estimate from an A-bomb survivor (6,7,8,9). While a great deal about the human health effects of radiation exposure has been learned over the last 50 years, there is much more that is poorly understood including the large uncertainties about potential cancer and non-cancer effects, extent of genetic and reproductive effects, and the effects of interactions between ionizing radiation and other physicochemical exposures. Increasing susceptibility with age is consistent with studies that show declines with age in immune system efficiency and in body's ability to correctly repair DNA damage (10,11,12) but this may be aggravated by certain genetic inadequacies as in the case of individuals carrying germline mutations in radiosensitive tumor suppressor genes.

Like its other tumor suppressor gene cousins, *BRCA1*, interplays in various biochemical pathways to prevent proliferation of cancer cells in the presence of environmental mutagens and carcinogens. Certain mutations in these genes may impair their significant biological function to

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subdue tumor progression. In this paper, we tackle the effect of having a heterozygous *BRCA1* mutation on radiosensitivity of an individual and consider its application in screening radiation workers.

RADIOSENSITIVITY AND *BRCA1/2*

The **BR**east **CA**ncer susceptibility gene, type **1** (*BRCA1*) is a large gene of 22 coding exons spanning more than 70 kb of genomic DNA. It is located at chromosome 17q21 and isolated in 1994. Exon 11 corresponds to 61% of the total coding sequence of 5592 nucleotides (13,14,15). The corresponding protein (Brca1), composed of 1863 amino acids having a molecular weight of 220 kDa, is a tumor suppressor that seems to be consistently observed to have a role in DNA recombination and/or repair as well as in transcriptional regulation (16,17).

Like *BRCA1*, **BR**east **CA**ncer susceptibility gene, type **2** (*BRCA2*) spans more than 70 kb of genomic DNA. Located at chromosome 13q12-13 (17,18), the coding sequence comprises 26 exons (10,254 nucleotides) with exons 10, 11, and 27 being the 3 large ones (18, 19). The corresponding protein (Brca2) has 3418 amino acid residues (384 kDa) and is also believed to be a tumor suppressor (17).

Linkage studies suggest that mutations in *BRCA1/2* are responsible for ~80% of families containing multiple cases of early-onset breast cancer and are associated with increased risk of cancers at other sites including the ovary, prostate, and pancreas (19,20). Other breast cancer-related genes include: *HRAS, KRAS, NRAS, P53, ERBB2, CCND1, FGFR1, PTEN, ATM,* and *E-cadherin* (21).

Since Brca1 and Brca2 are thought to be significantly involved in correctly repairing DNA damage, a study assessed the association of heterozygous mutations in *BRCA1/2* with cellular radiosensitivity through *in vitro* radiation clonogenic survival assay on dermal fibroblasts obtained from *BRCA* heterozygotes and radiation-induced chromatic break assay on lymphocytes from *BRCA* heterozygotes. Results from both assays suggested that cells having heterozygous mutation in *BRCA1* or *BRCA2* were more radiosensitive than controls (1). This corroborates a former study by the same author suggesting that radiation-induced chromatid breaks can be used as predictor of breast cancer risk (22).

Examination of the death pathways of human cells with various *BRCA1* and *BRCA2* genotypes after exposure to gamma-rays indicated defective repair of DNA double-strand breaks in irradiated cells lacking functional *BRCA1* and *BRCA2*. This impairment resulted in cell cycle checkpoints relaxation, micronuclei production, and loss of proliferative capacity. Moreover, heterozygous *BRCA1* and *BRCA2* mutations led to enhanced radiosensitivity coupled with impaired proliferative capacity after irradiation (2). An earlier study noted that hypersensitivity of human cancer cells containing mutated *BRCA1* can be reversed by the expression of forms of *BRCA1* that are not growth suppressing and requires the ring finger of *BRCA1* (BARD domain), its transactivation domain, and its BRCT domain. However, unlike *BRCA2*, *BRCA1* does not function in the repair of double-stranded DNA breaks but instead with transcription-coupled DNA repair (TCR). TCR ability correlated with radioresistance as cells containing *BRCA1* showed increased TCR and radioresistance; whereas, cells without *BRCA1* showed decreased TCR and radiosensitivity. This finding indicates physiologic significance to interaction of *BRCA1* with the basal transcription machinery (23). All of these suggest that *BRCA1/2* germline mutations could possibly predispose to increased risk of radiation-induced mutagenesis and carcinogenesis (1).

PROTEIN TRUNCATION TEST

Cancer predisposing genes commonly exhibit mutations that result to truncated protein products. Approximately eighty-six percent (86%) of detected *BRCA1* mutations (24) as well as 20 of the first 21 (95%) reported *BRCA2* mutations (17,18,25) generate a truncated protein. Other recent detected mutations described in the BIC online database strengthen this contention (26).

Protein truncation test (PTT), also referred as the *in vitro* synthesized protein assay (IVSP), exploits the principle of *in vitro* protein synthesis. PTT has been utilized in at least 25 studies to screen a portion or the entire *BRCA1* and/or *BRCA2* coding sequences. The general strategy utilized in these studies entails cDNA to be used as PCR template for the 22 smaller exons containing 39% of the coding sequence, while exon 11 is amplified from genomic DNA.

In PTT, the coding region of a gene is screened for presence of translation terminating mutations using *de novo* protein synthesis from amplified copy. This procedure includes three important steps. First step involves isolation of genomic DNA and amplification of the target gene coding sequences using PCR, or alternatively, isolation of RNA and amplification of the target sequence using RT-PCR. The resulting PCR products (Figure 1) are used as template for the *in vitro* synthesis of RNA, which is subsequently translated into protein. Final step involves the SDS-PAGE of the synthesized protein (Figure 2). Shorter protein products of mutated alleles are easily distinguished from full length protein products of normal alleles. A schematic diagram of PTT is shown in Figure 3 (27).

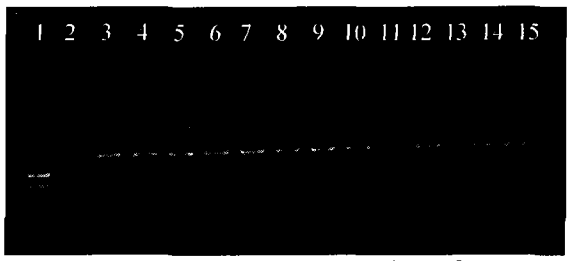

FIGURE 1: PCR products (~3.5kbp) of genomic DNA at exon 11 from Filipino early-onset and/or familial breast cancer patients using *BRCA1* primers described elsewhere (28). Lane 1 (500 bp ladder); lanes 2 and 11 (negative controls), lanes 3-10, 12-15 (PCR products).

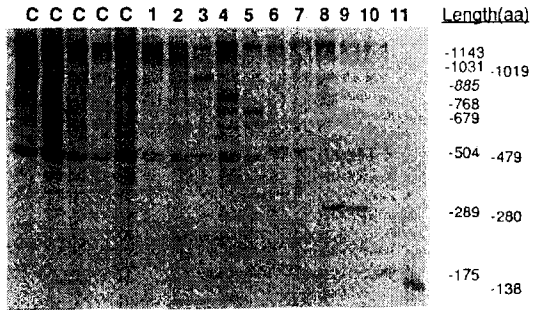

FIGURE 2: An example of PTT products separated by SDS-PAGE and visualized by autoradiography (28). Notice the truncated protein products in lanes 1-11 corresponding to germline *BRCA1* mutations at exon 11.

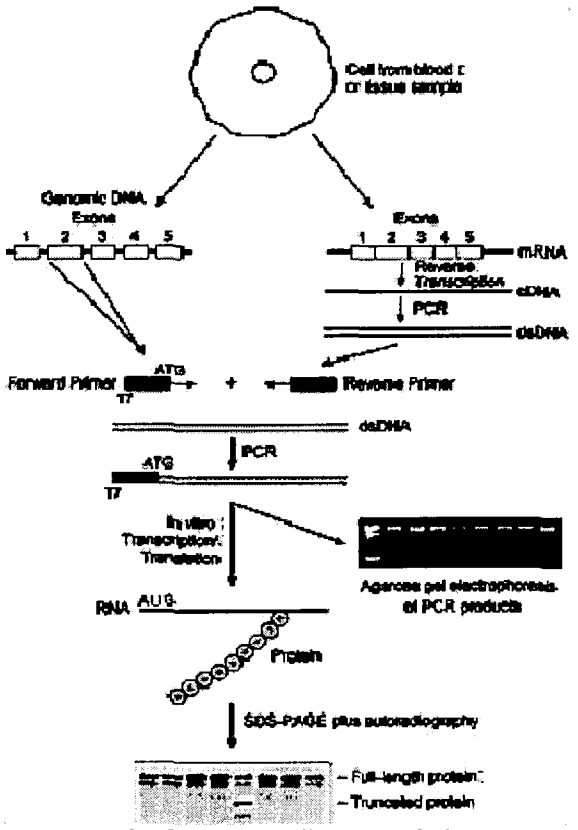

FIGURE 3: Schematic diagram of the protein truncation test (27).

MEDICAL SCREENING PROTOCOL AUGMENTATION

Increased risk of mutagenesis and carcinogenesis due to radiation exposure, as manifested by irradiated cells lacking functional *BRCA1/2*, intensifies the possibility that of women carrying a heterozygous *BRCA1/2* mutation will respond differently to radiation.

The Agency for Toxic Substances and Disease Registry (ATSDR) established seven criteria that have to be met before a medical monitoring program was recommended when persons were exposed to a radionuclide released from the Hanford nuclear facility making them significantly at increased risk for disease. These include: (a) evidence of exposure at a sufficient level of risk is documented, (b) well-defined population is at risk, (c), scientific basis exists for an association between exposure and health effects, (d) health effects are detectable and amenable to prevention/ intervention, (e) medical screening requirements should be satisfied, (f) accepted treatment/ intervention exists and a referral system is available, and (g) logistics must be resolved prior to program implementation (29). Such criteria can be adapted for medical monitoring programs in nuclear facilities. Detection of *BRCA1/2* germline mutations using PTT coupled with direct DNA sequencing can be incorporated in the medical screening to strengthen this criterion and serve as basis for computation of various risks.

With the integration of *BRCA1* PTT and knowledge on the risks of cancer lethality by radiation in the working population (4×10^{-2} per Sv for low-dose exposures), we suggest that dose limits for prospective radiation workers having heterozygous *BRCA1* mutations should be significantly reduced to take into account increased risk of radiation-induced carcinogenesis associated with these mutations (1,4).

A recent study reported that no mutations were detected in 22 cancer patients who developed severe normal tissue reactions after radiotherapy) that were tested for the majority of commonly described types of *BRCA1/2* mutations using PTT, direct DNA sequencing and exon 13 duplication test. This may indicate that genes other than *BRCA1/2* probably account for most cases of clinical radiation hypersensitivity but further mutation screening on radiation-sensitive individuals is still considered necessary (30). We therefore recommend that corroborating studies be performed to specifically determine dose limits for heterozygous yet highly competitive individuals seeking a position in a nuclear facility.

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