Characterization of genomic alterations in radiation-related breast cancer using array-CGH (comparative genomic hybridization)

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Genetics
Second cancer

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Background:

Ionizing radiation is an established risk factor for breast cancer, and the risk increases linearly with dose (1). Breast cancer is among the most radiogenic tumors identified so far among the atomic-bomb survivors (2). The greatest radiation-related risk was observed for young-onset breast cancer among women who were exposed at a younger age (3,4). Similarly, breast cancer is the most common secondary cancer among childhood cancer survivors, other than basal cell carcinoma of the skin (5). Epidemiologic studies of radiation-exposed cohorts have been primarily descriptive; molecular events responsible for the development of radiation-induced breast cancer are undefined. A major cause of the under-exploration is the difficulty in conducting quantitative molecular assays, particularly array-based profiling analyses, using archival formalin-fixed paraffin-embedded (FFPE) tissues, which are the only tumor tissue resources available from most of these cohorts. Due to formalin fixation, RNA/DNA undergoes extensive degradation and therefore is poor substrate for amplification and quantification, particularly with the use of less ideal fixation agent and long storage time. It is only recently that the advance of technologies has made it possible to investigate the molecular genetic alterations in FFPE tissues.

Radiation-induced breast cancer is possibly a highly complex phenomenon, which likely involves accumulating genetic and epigenetic changes. DNA copy number changes are among the most common mechanisms that can lead to altered expression and function of genes residing within the affected region of the genome. Identifying regions with copy number aberrations and further finding genes that are involved will improve our understanding of the molecular basis of radiation-caused breast cancer. Over the past decade, the microarray-based comparative genomic hybridization (array-CGH) technology has been developed and used as a powerful tool enabling the genome-wide detection of DNA copy-number aberrations in various types of cancers. A number of regions that are frequently involved in gains and losses have been identified. In addition, genomic copy number profiles can also distinguish distinct tumor subtypes. For example, copy number patterns classified breast tumors into specific subtypes that correspond to those previously defined by mRNA expression profiling (6). In addition, CGH patterns of genomic aberrations were shown to identify specific profiles for BRCA1- and BRCA2-associated tumors, which are different from those of nonhereditary forms of breast cancer (7). These findings suggest that array-CGH data can provide a promising starting point for identifying genes associated with cancer pathogenesis. Recently, array-CGH has been successfully applied to amplified DNA from FFPE tissue sections. Johnson et al. demonstrated that as few as 2000 microdissected cells from archival FFPE tissues could be successfully used for array-CGH investigations when amplification was used (8). Protocols specifically for array-CGH analyses of FFPE tissues have been developed for some commonly used array-CGH platforms such as Illumina and Agilent.

The ongoing cohort study of the Japanese atomic bomb survivors (Life Span Study, LSS) and the Childhood Cancer Survival Study (CCSS) in the U.S. provide unique resources to study the etiology of radiation-induced breast cancer because of the large number of stored archival tissues (~500 from LSS and ~50 from CCSS), individual radiation dose estimates, family history and other risk factor information, and well defined cancer outcomes. Taking advantage of the recent advancement of array-CGH technologies, we therefore propose to systematically

characterize genomic alterations in breast cancer tissues and to relate them to radiation exposures.

Study Objectives:

1. To estimate the prevalence and patterns of DNA copy number changes in radiation-exposed breast tumor tissues obtained from participants in the LSS and CCSS studies using array-CGH.
2. To identify distinct genomic aberrations related to radiation exposure and doses by comparing CGH profiles among breast tumors exposed to high-dose, low-dose, and no radiation.
3. To examine the relationship between copy number changes and age at exposure and type of radiation exposures (acute vs. protracted).

Study design:

Pilot study:
Although recent studies have demonstrated promising results using array-CGH to amplify DNA samples extracted from FFPE tissues, it may still be technically challenging to apply this technology on old archived tissues, particularly with suboptimal fixation. Given how precious the tissue samples from both studies are, we propose to conduct a pilot study to test the feasibility of using these samples for a genome-wide analysis of gene copy number changes using array-CGH. For the pilot, we are planning to obtain FFPE tissue sections from 10 non-LSS Japanese breast cancer cases with similar age (storage time) and fixation conditions as LSS subjects. The rationale of this sample selection is to test the success rate under a worst case scenario, since tumor blocks from LSS cases are older and were fixed in suboptimal conditions compared to CCSS cases. We will extract DNA from 3-5 5-µm sections, perform WGA, and conduct array-CGH in the pilot study. The results from this pilot study, such as minimum number of tissue sections required, the quantity and quality of the DNA, the impact of whole genome amplification (WGA), and success of array-CGH will provide useful guidance for the actual study.

Actual study:

Study population: We propose to obtain archival FFPE blocks from 45 invasive breast cancer cases in the CCSS cohort. These cases will be selected because of their tissue availability and known radiation treatment status. The distribution of type of first cancer diagnosis and radiation to site of breast cancer are shown in Table 1 and 2. The majority of these cases had Hodgkin lymphoma as the first cancer and presumably had received high doses (> 40 Gy) of radiation to the breast area. Actual dose to the site of where the breast cancer is developed is available for most of these cases. Age at diagnosis of the first cancer for all CCSS cases in this study was before age 20; therefore, age at radiation exposure was young for all members of this cohort. Most of these cases (N=34) also had early onset breast cancer (before age 40). In addition to CCSS cases, we will select ~75 invasive breast cancer cases among LSS participants: 25 exposed at younger age (< 20), 25 exposed at older age (> 20), and 25 non-exposed LSS or non-LSS

cases. In each group, we will try to identify 15 early onset (< age 40) and 10 late onset (> age 40) breast cancer cases. This selection strategy will enrich cases with young age at exposure and early age at onset, which will likely increase the power to detect genetic changes that are associated with radiation exposures. The range of radiation doses among LSS participants is 0-5 Sv, much lower than those received by most CCSS cases.

Array-CGH: Genomic DNA will be extracted from microdissected tumor cells and amplified according to protocols recommended by Agilent. Agilent 244K CGH arrays will be used to detect genome-wide chromosome aberrations in tumor samples. This oligonucleotide-based array-CGH platform has the ability to accurately measure copy number changes with high resolution (<50kb). Validated methods have been developed to measure DNA copy number changes in FFPE tissues Agilent arrays.

Data analyses: The main objective of this proposed study is to find genome-wide effects of radiation exposure on DNA copy number changes. We will compare the frequency of: 1) overall number of changes; and 2) specific regions that are recurrent (occurred in multiple individuals within a specific tumor group), between exposed and non-exposed breast cases to examine the impact of radiation exposure on global and regional genomic rearrangement. Further, we will identify the relationship of these radiation-related alterations with radiation doses, age at radiation exposure (using age at diagnosis of first cancer as a surrogate for CCSS cases), type of radiation exposure, and age at diagnosis of breast cancer. Since the study populations (Japanese and U.S.) and range of radiation doses received by CCSS and LSS cases are quite different, we will conduct separate analyses for these two populations, but we will compare radiation-related chromosomal regions identified in the two study populations. We will organize these cases into several main groups:

1. Exposed vs. non-exposed groups: The exposed group will contain ~35 CCSS cases who had radiation exposure in the breast area as treatment for their first cancer and 50 exposed LSS cases. The non-exposed group includes ~10 non-exposed CCSS cases and 25 non-exposed LSS cases.
2. High doses vs. low doses: We anticipate that we will have actual breast dose data available for most CCSS cases. For the CCSS cases without actual dose estimates, we will use combined information from first cancer diagnosis and radiation to the breast area (Tables 1 & 2) to estimate breast doses. For example, cases with Hodgkin lymphoma who did not use breast shielding were presumably exposed to high radiation doses. For both LSS and CCSS cases, we will use a cutoff to define high-dose and low-dose groups.
3. Age at exposure, young vs. old: Most CCSS cases were diagnosed with the first cancer between age 11 and 20; only 7 cases were diagnosed before age 10. Therefore, the power to examine the relationship between copy number changes and age at exposure within the CCSS population is limited. Within the LSS dataset, we will compare copy number changes between the 25 early-exposed (< age 20) and 25 late-exposed (> age 20) cases.

4. Early-onset vs. late-onset breast cancer: Early-onset group will include CCSS and LSS exposed cases who were diagnosed with breast cancer before age 40 (N~60), and late-onset will be exposed cases who were diagnosed after age 40 (N~30).

This proposed study is by far the largest study of exploring genome-wide DNA copy number changes in breast tumors that are related to radiation exposure. Given the high radiation exposure particularly among CCSS cases, we expect to see different CGH profiles between exposed and non-exposed cases. Based on previously published studies, we know spontaneous (radiation unrelated) breast cancer has well characterized copy number changes. In addition, different molecular subtypes defined by global gene expression tend to have distinct array-CGH profiles. The goal of our analysis is two fold: 1) we will identify additional altered regions that are specifically related to radiation exposure; and 2) if radiation-related regions are among previously characterized ones, we will associate them with molecular subtypes by comparing to published data for subtype-specific changes. The power calculations for detecting differences in prevalence of DNA gains and losses between exposed (N~85) and non-exposed (N~35) samples with α=0.05 are shown in Table 3. Based on power calculations, our study should have sufficient power ($\geq$80%) in detecting a 20% difference in the prevalence of a recurrent region between exposed and non-exposed cases in the CCSS study alone. We will have substantially more power to detect common genetic alterations occurring among both LSS and CCSS cases.

Budget:

DNA extraction & WGA	$50/sample
Agilent 244K microarray	$450/sample
Labeling and hybridization reagents	$150/sample
Total/per sample	$650/sample
Total for the pilot study (N=10)	$6,500 (already available)
Total for the actual study (N=120) + shipping	$78,500

Table 1. Type of first cancer among the 45 CCSS cases with tissue available.

Diagnosis	Frequency	Percent
Leukemia	5	10.87
HD	29	63.04
NHL	1	2.17
Kidney (Wilms)	2	4.35
Neuroblastoma	1	2.17
Soft tissue sarcoma	4	8.70
Bone cancer	4	8.70

Table 2. Radiation sites among the 45 CCSS cases with tissue available.

Radiation site	Frequency	Percent
Received RT to breast	17	36.96
Received RT near breast	5	10.87
Received RT, breast shielded	10	21.74
Received RT to chest(or TBI), no RT to breast	1	2.17
Received RT to other area, no RT to breast	6	13.04
Received RT, unknown sites	1	2.17
No RT	5	10.87

Table 3. Power calculations for detecting differences in the prevalence of a recurrent region between 85 exposed and 35 non-exposed cases at an alpha-level of 0.05.

N (%) among non-exposed cases	N (%) among exposed cases	Power
0 (0%)	9 (10%)	67.3%
1 (3%)	9 (10%)	32.8%
1 (3%)	18 (20%)	84%
1 (3%)	27 (30%)	99%
9 (10%)	27 (30%)	79.5%
9 (10%)	45 (50%)	99.9%

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