**Title:** A functional assay for homologous recombination (HR) DNA repair and whole exome sequencing reveal that HR-defective sporadic breast cancers are enriched for genetic alterations in DNA repair genes


**Body:**

**Background:** Germline mutations in the **BRCA1** and **BRCA2** genes lead to hereditary breast cancers that are defective in homologous recombination (HR) repair and sensitive to DNA damaging agents. HR deficiency (HRD) also occurs in sporadic breast cancers, but its incidence and etiology are unclear. Genomic signatures of HRD recently were employed as biomarkers in clinical trials with modest success. We posited that sporadic breast cancers displaying functional HR deficiency would harbor genetic alterations affecting HR DNA repair genes. To test this hypothesis, we applied a functional assay to define lack of competent HR DNA repair and sequenced the exomes of consecutive sporadic breast cancers.

**Methods:** We developed an assay to assess the ability of cancer cells to localize RAD51 into sub nuclear foci in response to ex-vivo irradiation (IR) in fresh sporadic breast cancer tissue specimens from 60 patients. RAD51 focus formation was compared between mock and IR conditions to determine relative fold induction. Twenty-nine tumors with sufficient DNA underwent whole-exome sequencing. Structural genomic signatures of HRD (i.e. large state transitions (LST), telomeric imbalance (NtAI), loss-of-heterozygositiy (LOH)) and a previously reported mutational signature related to BRCA1/2 hereditary breast cancers were assessed. HR deficient tumors were defined as those with both RAD51 foci defects and a genomic signature of HR deficiency (LST>15 or presence of a BRCA mutational signature). Somatic, germ-line, and copy number changes in HR genes were investigated.

**Results:** Seventeen of 60 (28%) tumors displayed defective RAD51 recruitment following ex-vivo IR (RAD51-DEF). RAD51-DEF was seen in all breast cancer subtypes, including 7 of 33 (21%) ER+/HER2-, 4 of 14 (29%) HER2+, and 6 of 13 (48%) triple-negative cases. Of the 29 sequenced tumors, 13 (45%) were RAD51-DEF and 16 (55%) were competent for inducing RAD51 foci. LST was elevated in 10 tumors (LST >15) and associated with RAD51-DEF (p=0.02), whereas NtAI (p=0.10) and LOH (p=0.052) did not show a significant association with RAD51-DEF. The BRCA1/2 mutational signature was evident in 4 tumors, all were RAD51-DEF (p=0.03) and 2 were BRCA2 mutated. Nine of 29 (31%) sequenced tumors were determined to have HRD by the RAD51 assay and presence of a genomic scar. Eight of these 9 (88%) cases with HRD had a genetic alteration of both alleles of a bona fide HR gene due to a pathogenic mutation (somatic or germline) coupled with loss of heterozygositiy or a homozygous deletion compared to 1 (5%) tumor without HRD (p<0.001). Both alleles of a gene were affected for BRCA2 (n=4), FAAP100 (n=2), CHEK2 (n=1), [italic]TP53BP1 (n=1) and BRCA1 (n=1). BRCA1 gene promoter methylation was found not to be significantly associated with HRD.

**Conclusion:** Combined functional and genomic analyses of breast tumors demonstrated that genetic loss of an HR gene may underpin HRD in sporadic breast cancers. Our findings warrant further comprehensive genetic assessment (somatic, germline, and copy number) of HR genes as potential biomarker for HR-directed therapies.