Reproducibility of homologous recombination deficiency (HRD) scores in biopsies of triple negative breast cancer (TNBC) tumors

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BACKGROUND

- Tumors with defects in homologous recombination (HR) repair, including BRCA1 and BRCA2 mutations, show increased sensitivity to DNA damaging agents.
- The HRD score is significantly associated with both tumor BRCA1/2 mutations and response to DNA damaging agents in TNBC.
- Intranuclear heterogeneity is the presence of subpopulations of cells with unique genomes.
- Intranuclear heterogeneity may negatively impact response to therapy, and may contribute to the development of clinical resistance.

OBJECTIVE

- The goal of this study was to assess the impact of intranuclear heterogeneity on the HRD score by testing the HRD score on three distinct biopsies from different areas of the same tumor.

METHODS

Tumor Samples

- Tumors from 32 Stage II-III breast cancers were included.
- Biopsies were collected from 3 distinct regions of the surgically resected tumor.

Tumor DNA Analysis

- All tumors were analyzed using the HRD assay, a next generation sequencing assay performed using DNA extracted from FFPE tumor tissue [1].
- Each sample received an HRD score, which is the unweighted sum of three independent measures of HRD:
  - TAI (number of telomeric allelic imbalances) [2]
  - LOH (loss of heterozygosity) [3]
  - LST (long segment transitions) [4]
- All tumors were tested for BRCA1/2 mutations and BRCA1 promoter methylation.
- Tumor samples were characterized as having high (≥42) or low (<42) BRCA1/2 deficiency.

Statistical Analysis

- The mean standard deviation of the HRD scores was calculated for samples with more than one replicate. The mean standard deviation was calculated as the square root of the mean variance.

RESULTS

- High HRD scores were observed in all BRCA1/2 mutant (3 triplet; 1 single) and BRCA1 methylated (2 triplets) tumors. 4/24 BRCA1/2 wild-type tumors had high HRD scores (1 triplet; 3 pairs) (Table 1).
- HRD scores were highly consistent between independent biopsies of the same tumor reflecting the low degree of biological variation and the technical reproducibility of the assay (Figure 1). HRD scores from multiple biopsies of the same tumor showed more variation at higher levels of HR deficiency. There was a significant relationship between standard deviation and score (p-value = 0.026, correlation coefficient = 0.46).
- The mean standard deviation of HRD scores from the same tumor was 2.2 for samples with low HRD and 3.0 for samples with high HRD.
- The range of scores from the sample tumor was less than 5% of the samples with low HRD, versus 44% of the samples with high HRD.
- HR deficiency (high HRD score or BRCA1/2 deficiency) was the same in different biopsies from each of the 31 tumors (Figure 1), despite the presence of different genomic rearrangements observed in some of the matched tumor sets (Figure 2).

CONCLUSIONS

- High HRD scores were observed in all BRCA1/2 deficient tumors, and were more prevalent than either BRCA1/2 mutations or BRCA1/2 deficiency (32% vs. 13% or 19%).
- All markers of HRD examined, including BRCA1/2 mutation, BRCA1 promoter methylation, and HRD score were maintained between different samples from the same tumor.
- These results suggest that tumor heterogeneity should not result in discordant HRD scores depending on the tumor sample analyzed.

REFERENCES


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Table 1. Tumor Characteristics

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Triplet</th>
<th>Duplicate</th>
<th>Single</th>
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<tbody>
<tr>
<td>Total tumor biopsies</td>
<td>96</td>
<td>32</td>
<td>--</td>
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<tr>
<td>Sufficient DNA for sequencing</td>
<td>84% (81/96)</td>
<td>21</td>
<td>7</td>
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<tr>
<td>Evaluable HRD scores</td>
<td>86% (78/91)</td>
<td>16</td>
<td>7</td>
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<td>High HRD scorea</td>
<td>8% (6/76)</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>BRCA1 mutationb</td>
<td>4% (3/81)</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>Evaluable BRCA1 promoter methylation</td>
<td>94% (76/81)</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>BRCA1 promoter methylationc</td>
<td>8% (6/76)</td>
<td>11%</td>
<td>14%</td>
</tr>
<tr>
<td>BRCA2 mutation</td>
<td>11% (9/81)</td>
<td>14%</td>
<td>3%</td>
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Figure 1. HRD scores in multiple biopsies from the same tumor.

Figure 2. Genomic profiles of tumor samples at 3 biopsy sites. Examples are shown for tumors with similar (left) and divergent (right) biopsy triplets from the same tumor.