Introduction

- The loss of function of several DNA-damage response (DDR) genes has been reported in breast cancer.
- A dysfunction in DDR is exploited by DNA-damage as well as novel targeted therapeutics such as PARP-1 inhibitors.
- Identification of those patients with DDR dysfunction could inform the selection of effective chemotherapeutic agents in the clinic.
- We report the identification of a novel molecular subgroup in breast cancer related to DDR deficiency (DDRD) that can be identified by a 44-gene signature (DDRD signature).
- The DDRD signature is a significant predictor of BRCA and Fanconi anemia (FA) mutational status as well as an independent predictor of response to neoadjuvant anthracycline-based chemotherapy.

Identification of DDRD molecular subgroup

- A cohort of 107 primary breast cancer FFPE samples enriched with 60 BRCA1 and BRCA2 mutant tumors was sourced from the Mayo Clinic.
- Unsupervised analysis of gene expression data was performed using the genes with the most variable expression.
- The DNA damage dataset of the 107 breast datasets were evaluated separately as the ER has a dominant effect on clustering which could prevent the identification of an ER-independent subgroup.
- Following pathway analysis the most significant biology associated with both the ER-positive (probeset cluster 6, Figure 1A) and ER-negative (probeset cluster 3, Figure 1B) datasets related to interferon and immune response signaling.
- Since immune signaling has been reported to be modulated in response to DNA-damage [Rodier et al., Nat. Cell Biol. 11, 973-979 (2009)], we combined samples displaying up-regulation of genes related to these pathways to form a putative DDRD molecular subgroup, which can be identified by a 44-gene signature.

Materials and Methods

- Tumor material
  - 107 microdissected breast cancer FFPE samples were sourced from the Mayo Clinic, Rochester.
- Gene expression profiling
  - RNA was extracted from FFPE tumor samples using the Roche High Pure RNA Paraffin Kit and amplified using the NuGEN WT-Ovation™ FFPE System. The amplified product was hybridized to the Almac Breast Cancer DSA.
- DNA damage repair assays
  - HCC1937-EV and HCC1937-BR cells were mock irradiated or treated with 20 Gy X-Rays. Cells were fixed and stained with anti-H2AX (γH2AX). Cells (100) were scored and those containing >6 γH2AX foci were scored positive.
- Assessment of PARP-1 inhibitor sensitivity
  - Cells were exposed to PARP-1 inhibitor for 12-14 days following which time colonies with more than 50 cells were counted.
- Assessment of cisplatin sensitivity
  - Cells were exposed to cisplatin for 96 hours and viability was assessed using a luminescent cell viability assay.

Identification of a novel breast cancer molecular subgroup associated with a deficiency in DNA-damage response

- The signature significantly enriched for BRCA1/2 mutational status within the training set, with an area under the receiver operator curve (AUC) of 0.68 (CI = 0.56-0.78, p = 0.0021), (Figure 2A).
- The DDRD signature was also found to be able to distinguish between FA mutant and normal samples [Vanderwal et al., Blood 114, 5290-5296 (2009)] with an AUC of 0.90 (CI = 0.76-1.00, P < 0.001) (Figure 2B) suggesting that the DDRD subgroup may encompass tumors with loss of the FA/BRCA pathway through multiple mechanisms.

Figure 1 Clustering analysis of BRCA1/2 mutant and sporadic wildtype control

- Hierarchical clustering analysis of ER-positive (A) and ER-negative (B) BRCA1/2 mutant and sporadic wildtype control breast samples.
- Probeset cluster groups are annotated on the right-hand side and pathway analysis of each probeset cluster group is annotated on the left-hand side of each image.

Figure 2 DDRD signature is predictive of BRCA (A) and Fanconi anemia (B) mutational status

- The DDRD signature was applied to BRCA1 mutant HCC1937 empty vector control cells (HCC1937-EV) and HCC1937 cells which BRCA1, and thus DDR functionality, was corrected (HCC1937-8R) (Figure 3A).
- DDRD signature scores were significantly higher within HCC1937-8R cells (Figure 3B).
- Consistent with this, HCC1937-8R cells were more sensitive to cisplatin (Figure 3C) and the PARP-1 inhibitor KU0058948 (Figure 3D) relative to HCC1937-8R cells.
- The signaling detected by the DDRD signature is thus intrinsic to the cell and not a feature of immune infiltrate as confirmed by a lack of association (p = 0.1433) with immune infiltrate in the TRANSBIG breast cancer dataset [DeSantis et al., Cancer Res. 13, 3207-3214 (2007)].

Figure 3 DDRD signature and therapeutic response in a BRCA1 isogenic cell-line model

- The DDRD signature is independent of clinical factors
- The DDRD signature's ability to predict response to DNA-damaging chemotherapeutics was assessed by application to data collected from 3 publicly available datasets (Tabuchi et al., Clin. Cancer Res. 16, 5351-5361 [2010]; Uramoto et al., J. Natl. Cancer Inst. 103, 264-273 [2011]; Bennekom et al., Lancet Oncol. 8, 1011-1017 [2007]).
- In each study, breast cancer patients were treated with neoadjuvant anthracycline-based regimens. Pathological complete response (pCR) or residual disease (RD) were used as clinical endpoints.
- The DDRD signature was shown to be significantly associated with response to anthracycline-based chemotherapy.

Conclusions

- We describe a novel subgroup in breast cancer, deficient in response to DNA-damage.
- The DDRD subgroup is defined by immune signaling previously reported to be activated in response to persistent DNA-damage.
- The DDRD signature is capable of significantly predicting both BRCA and FA mutational status.
- The DDRD subgroup demonstrates sensitivity to DNA-damaging chemotherapy in breast cancer.
- The DDRD signature is a significant predictor of response to chemotherapy independent of other clinical factors.