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

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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-damaging 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.

Materials and Methods

- *Tumor material*

107 macrodissected 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 2Gy X-Rays. Cells were fixed and stained with anti-γ-H2AX. Cells (100) were scored and those containing >6 γ-H2AX 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 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.
- Estrogen receptor (ER)-positive and ER-negative cohorts were analyzed 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.

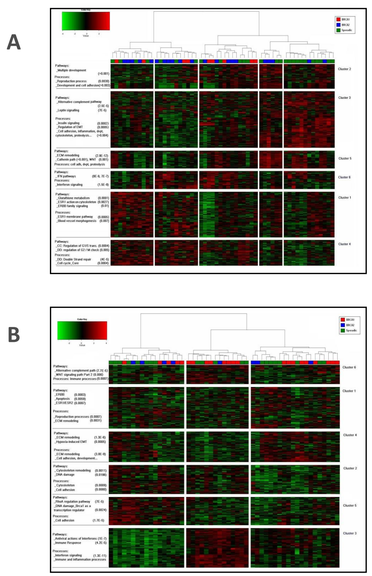

Figure 1
Clustering analysis of BRCA1/2 mutant and sporadic wildtype control
- Hierarchical clustering analysis of ER-positive (A) and ER-negative (B) BRCA1/2 mutant and sporadic wildtype control breast samples.
- Probeset cluster groups are annotated on the right-hand side and pathway analysis of each probeset cluster group is annotated on the left-hand side of each image.

DDRD detects dysfunction in BRCA/FA pathway

- 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 [Vanderwerf *et al., Blood* 114, 5290-5298 (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.

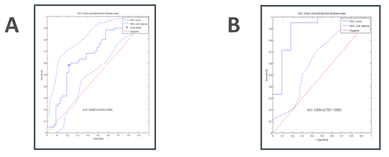

Figure 2
DDRD signature is predictive of BRCA (A) and Fanconi anemia (B) mutational status

DDRD signaling is intrinsic to the cell

- The DDRD signature was applied to BRCA1 mutant HCC1937 empty vector control cells (HCC1937-EV) and HCC1937 cells in which BRCA1, and thus DDR functionality, was corrected (HCC1937-BR) (Figure 3A).
- DDRD signature scores were significantly higher within HCC1937-EV relative to HCC1937-BR cells (Figure 3B).
- Consistent with this, HCC1937-EV cells were more sensitive to cisplatin (Figure 3C) and the PARP-1 inhibitor KU0058948 (Figure 3D) relative to HCC1937-BR 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 [Desmedt *et al., Clin. Cancer Res.* 13, 3207-3214 (2007)].

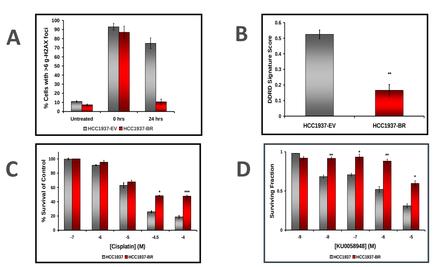

Figure 3
DDRD signature and therapeutic response in a BRCA1 isogenic cell-line model

DDRD predictive of response to chemotherapy

- The DDRD signature's ability to predict response to DNA-damaging chemotherapeutics was assessed by application to data combined from 3 publicly available datasets [Tabchy *et al., Clin. Cancer Res.* 16, 5351-5361 (2010); Iwamoto *et al., J. Natl. Cancer Inst.* 103, 264-272 (2011); Bonnefoi *et al., Lancet Oncol.* 8, 1071-1078 (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.

Prediction of pCR using DDRD signature									
Treatment	Sample Number	Clinical Outcome	AUC (CI)	ACC (CI)	SENS (CI)	SPEC (CI)	PPV (CI)	NPV (CI)	RR (CI)
FAC/FEC	203	pCR V RD	0.78 (0.7-0.85)	0.76 (0.64-0.83)	0.82 (0.69-0.92)	0.58 (0.52-0.62)	0.44 (0.36-0.48)	0.90 (0.81-0.95)	4.13 (1.94-9.87)

DDRD signature is independent of clinical factors

FAC/ FEC	Univariate	Multivariate
Variable	P value	P value
DDRD signature	0.0000	0.0014
ER	0.0004	0.0249
Stage	0.0459	0.0492
Grade	0.0010	0.0468

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.