DNA Damage Response Deficiency (DDRD) in Breast Cancer is associated with a STING-dependent Innate Immune Response

Eileen E. Parkes[1], Steven M. Walker[1,2], Nuala McCabe[1,2], Laura E. Taggart[1,2], Laura Hill[2], Karen D. McCloskey[1], Niamh E. Buckley[1], Manuel Salto-Tellez[1,3], Kienan I. Savage[1], Paul B. Mullan[1], D. Paul Harkin[1,2], and Richard D. Kennedy[1,2]

[1]Centre for Cancer Research and Cell Biology, Queens University Belfast, United Kingdom.[2]Almac Diagnostics, Craigavon, Northern Ireland, United Kingdom.[3]Northern Ireland Molecular Pathology Laboratory, Queens University Belfast, United Kingdom.

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Key Findings:

- DNA damage response deficient human breast cancers are associated with CD8+ and CD4+ lymphocytic infiltration.
- The epithelial component of DDRD tumors releases chemokines that can account for lymphocytic infiltration.
- Chemokine release is dependent on activation of the cGAS-STING-IRF3 pathway which is constitutively activated in DNA repair deficient cells, or by exogenous DNA damaging agents.
- Activation of the cGAS-STING-IRF3 pathway is cell cycle specific and is associated with an accumulation of cytosolic DNA in the S-phase of the cell cycle.
- Activation of the cGAS-STING-IRF3 pathway is associated with expression of the immune-checkpointing gene PD-L1 which may prevent immune-mediated tumor cell death.
- This may provide a therapeutic rationale for immune-checkpoint targeted therapies in the context of DNA damage response deficiency in cancer.

Migration of PBMCs is CXCL10 and CCL5 -dependent

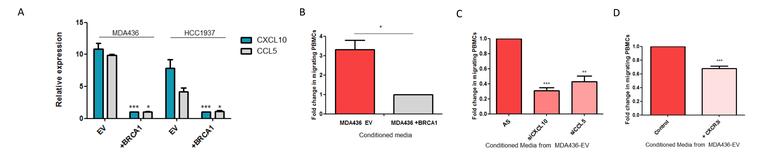


Figure 2 A) *BRCA1*-mutant cell lines MDA-MB-436-EV and HCC1937-EV express CXCL10 and CCL5. This expression is shut down when repair is corrected. B) Migration of peripheral blood mononuclear cells (PBMCs) is increased into media conditioned by *BRCA1*-mutant cells. C) siRNA mediated knockdown of CXCL10 and CCL5 reduces migration. D) CXCR3 inhibitor treatment of PBMCs reduces migration.

Expression of CXCL10 and CCL5 is associated with constitutive activation of the STING-TBK1-IRF3 innate immune pathway

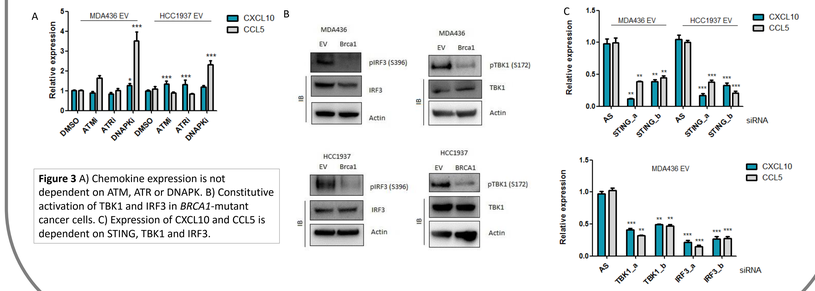


Figure 3 A) Chemokine expression is not dependent on ATM, ATR or DNAPK. B) Constitutive activation of TBK1 and IRF3 in *BRCA1*-mutant cancer cells. C) Expression of CXCL10 and CCL5 is dependent on STING, TBK1 and IRF3.

PD-L1 expression is increased in response to endogenous and exogenous DNA damage, cytosolic DNA, and dependent on STING

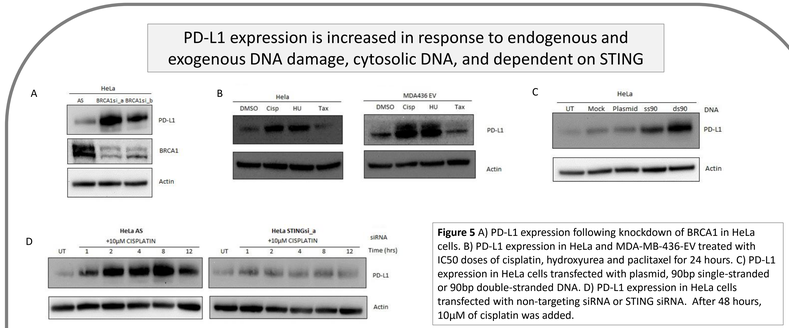
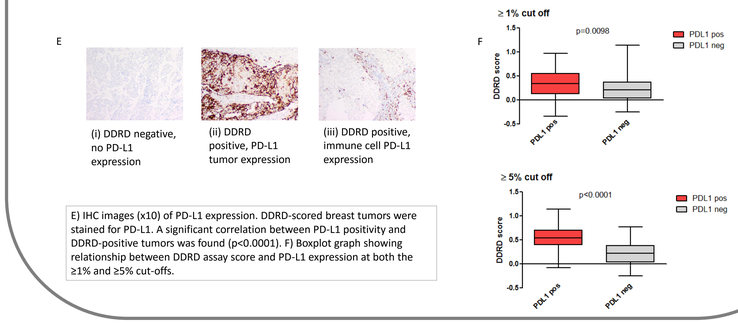


Figure 5 A) PD-L1 expression following knockdown of BRCA1 in HeLa cells. B) PD-L1 expression in HeLa and MDA-MB-436-EV treated with IC50 doses of cisplatin, hydroxyurea and paclitaxel for 24 hours. C) PD-L1 expression in HeLa cells transfected with plasmid, 90bp single-stranded or 90bp double-stranded DNA. D) PD-L1 expression in HeLa cells transfected with non-targeting siRNA or STING siRNA. After 48 hours, 10μM of cisplatin was added.

DDRD assay positive tumors are significantly associated with PD-L1 positivity on IHC



E) IHC images (x10) of PD-L1 expression. DDRD-scored breast tumors were stained for PD-L1. A significant correlation between PD-L1 positivity and DDRD-positive tumors was found ($p<0.0001$). F) Boxplot graph showing relationship between DDRD assay score and PD-L1 expression at both the ≥1% and ≥5% cut-offs.

DDRD assay predicts response to DNA damaging chemotherapy

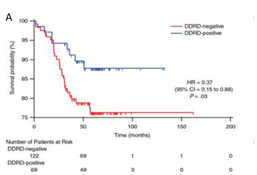
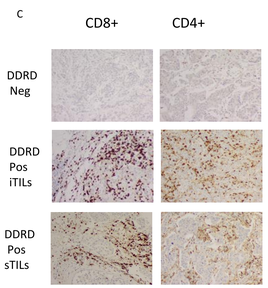


Figure 1 A) Overall survival following adjuvant FEC chemotherapy for DDRD positive and DDRD negative scored tumors.
B) Table of CD8+ and CD4+ intratumoral scores.
C) IHC images (x10) of T-cell infiltration in DDRD tumors, iTILs = intratumoral, sTILS = stromal.
D) siRNA mediated knockdown of components of FA/BRCA repair pathway results in upregulation of CXCL10 and CCL5 in HeLa cells.

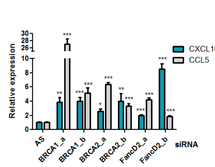
DDRD assay positive breast tumors are associated with CD8+ and CD4+ T-cell infiltration

B

Intratumoral	**CD8+ score**	*DDRD pos* (n=65)	*DDRD neg* (n=117)	*Fisher's exact test*
	3	6 (9.23%)	0 (0.00%)	p<0.0001
	2	29 (44.62%)	19 (16.24%)	
	1	25 (38.46%)	68 (58.12%)	
	0	5 (7.69%)	30 (25.64%)	
Intratumoral	**CD4+ score**	*DDRD pos* (n=65)	*DDRD neg* (n=119)	*Fisher's exact test*
	3	3 (4.62%)	1 (0.84%)	p<0.0001
	2	13 (20.00%)	3 (2.52%)	
	1	43 (66.15%)	75 (63.03%)	
	0	6 (9.23%)	40 (33.61%)	



Chemokines CXCL10 and CCL5 are expressed by cancer cells in response to endogenous DNA damage



Cytosolic DNA detected by cGAS is associated with both endogenous and exogenous S-phase DNA damage

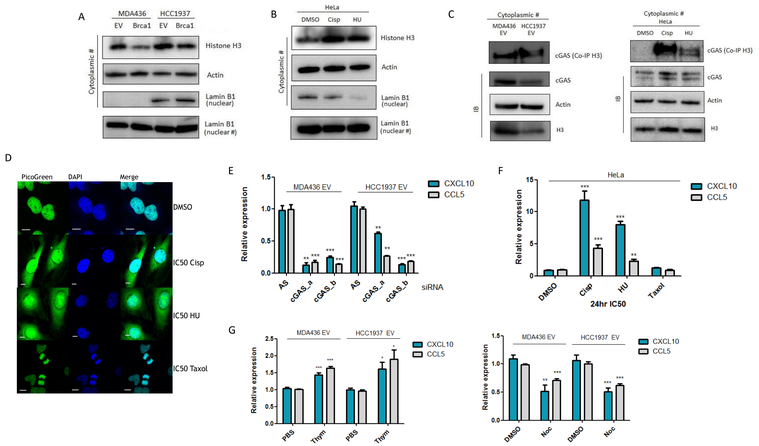
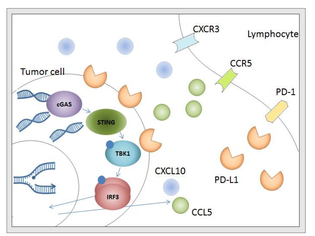


Figure 4 A) Cytosolic fractions of *BRCA1*-mutant and *BRCA1*-corrected cells were probed for Histone H3 (a marker of cytosolic DNA). B) Cytosolic fractions of HeLa cells treated with S-phase DNA damaging agents cisplatin and hydroxyurea were probed for Histone H3. C) Co-IP of Histone H3 reveals cGAS as the sensor of cytosolic DNA. D) PicoGreen staining of HeLa cells treated with IC50 doses of cisplatin, hydroxyurea and paclitaxel. E) cGAS-dependent expression of CXCL10 and CCL5. F) S-phase DNA damaging agents drive expression of CXCL10 and CCL5. G) Expression of CXCL10 and CCL5 is increased in S-phase and reduced in M-phase in the context of endogenous DNA damage response deficiency.

Model of innate immune activation in response to endogenous DNA damage response deficiency



Conclusions

- We propose a novel mechanism of immune infiltration in DDRD tumors, dependent on epithelial production of chemokines.
- Activation of this pathway and associated PD-L1 expression may explain the paradoxical lack of T-cell mediated cytotoxicity observed in DDRD tumors.
- We provide a rationale for exploration of DDRD in the stratification of patients for immune-checkpoint based therapies.

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Contact: e.parkes@qub.ac.uk