Disparate STING signalling as a link between bladder cancer cell innate immune response and the immunostimulatory state of the tumour microenvironment following genotoxic therapies

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Summary: Bladder cancer DNA damage-driven innate immune STING activity states are highly relevant to the mechanistic understanding of patient cytotoxic responses to chemotherapy and radiotherapy. As a T cell-inflamed immunostimulatory is required for successful cancer cell clearance knowledge of how cancer cell innate immune signalling modulates the tumour microenvironment is vital for understanding patient response or resistance to genotoxic therapy

Introduction

A T cell-inflamed immunostimulatory tumour microenvironment (TME) is required for successful cancer cell clearance and checkpoint inhibitor immunotherapies. Chemotherapy (CT) or radiotherapy (RT) direct both immunostimulatory and immunosuppressive states. DNA damage elicits cytosolic micronuclei, detectable by the DNA sensor cGAS and its adaptor STING. Nuclear DNA damage is also detected by cGAS-independent STING activity via PARP1, p53, and TRAF6 as a distinct pro-inflammatory cytokine profile (figure 1).

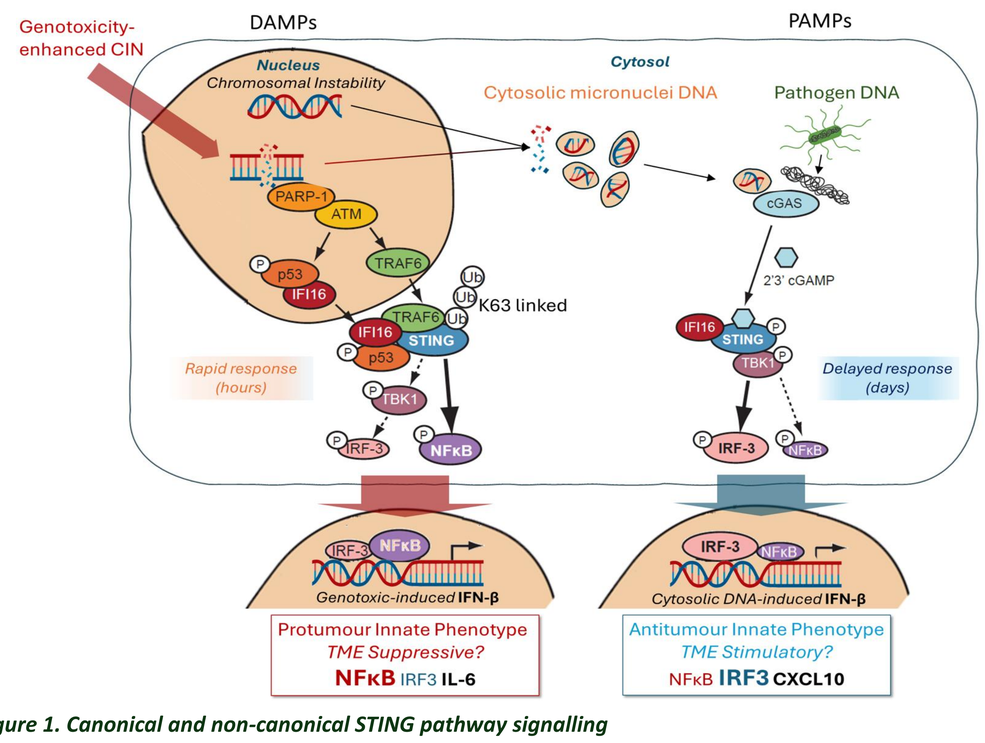

Figure 1. Canonical and non-canonical STING pathway signalling

We are investigating the role of DNA damage-driven innate immune responses in muscle-invasive bladder cancer (MIBC), the 11th common UK cancer with high recurrence and 22% stage IV survival after standard-of-care cystectomy. The high mutational load offers a favourable model system to study disparate STING signalling following CT/RT and for correlation with TME reactive states.

Methods

Three MIBC cell lines J82, T24, UMUC3 are under analysis for their mechanistic innate immune responses following treatment with four CT agents and Ionising Radiation. Use of **M**ethotrexate, **V**inblastine, **D**oxorubicin [**A**driamycin], **C**isplatin mirrors **UK NICE** bladder cancer **MVAC** management guidelines.

Results

The MIBC cell lines J82, T24, and UMUC3 capture a range of genomic instability and gender of MIBC patients (figure 2).

Name	Sex	*TERT*	*PIK3CA*	*H-RAS*	*K-RAS*	*INK4A*	*RB1*	*PTEN*	*TP53*	Genome Instability	% gain	% lost	Chrom. alterations
J82	M	**Mut**	**Mut**	WT	WT	WT	**Mut**	**Mut**	**Mut**	MED	20.3	25.7	12
T24	F	**Mut**	WT	**Mut**	WT	**LOH**		**Mut**	**Mut**	LOW	11.6	19.4	6
UM-UC-3	M	**Mut**	WT	WT	**Mut**	**Mut**		**Mut**	**Mut**	HIGH	27.8	28.1	10

Figure 2. Muscle Invasive Bladder Cancer Cell lines: Key mutations and genomic instability

Micronuclei detection classifies cell line baseline burden. ≈50% cells harbour ≥1 micronuclei. Per-cell micronuclei number, size, compactness, cluster density, and closeness to nuclear envelope are attributes suited for downstream mechanistic analysis following genotoxic assault.

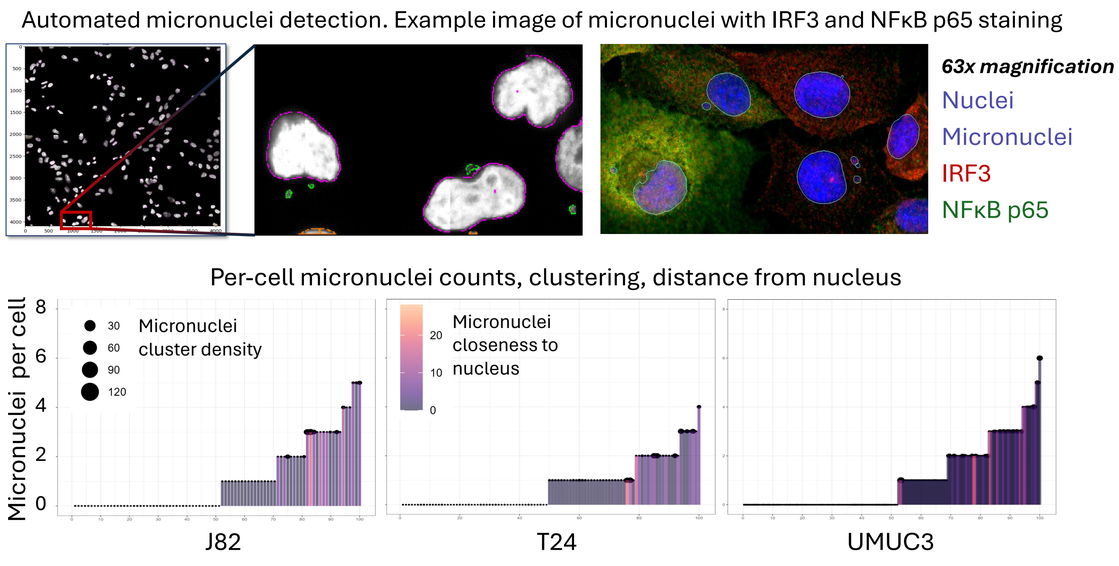

Figure 3. Micronuclei detection with masked and peripheral edge IRF3 and NFκB intensity analysis

Wide-ranging cell line-specific cytotoxic and cytostatic responses occur following Methotrexate, Vinblastine, Doxorubicin, Cisplatin treatment. 72-hour, 12-point cytotoxicity ranges of each drug with IC_{50}, EC_{50}, GR_{50} modelling exposed varied cytotoxic and cytostatic µM values (figure 4).

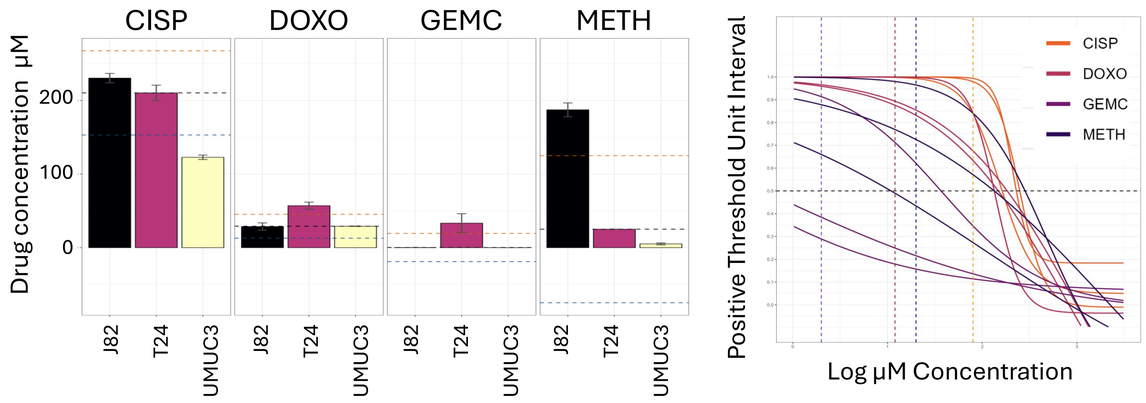

Figure 4. Cytotoxicity growth rate-corrected IC_{50} assays (GR_{50}): Cisplatin, Doxorubicin, Gemcitabine, Methotrexate

Sub-lethal CT dosages in bladder cancer cells elicit a range of pro-inflammatory anti-cancer or pro-cancer cytokine profiles. Cisplatin 5-point dosage, 6-point 72-hour time course: J82 cells secrete **IL-6** and **CXCL10** after sub-lethal treatment. T24 cells secrete **IL-6** *but not CXCL10.* UMUC3 cells *do not secrete either* (figure 5).

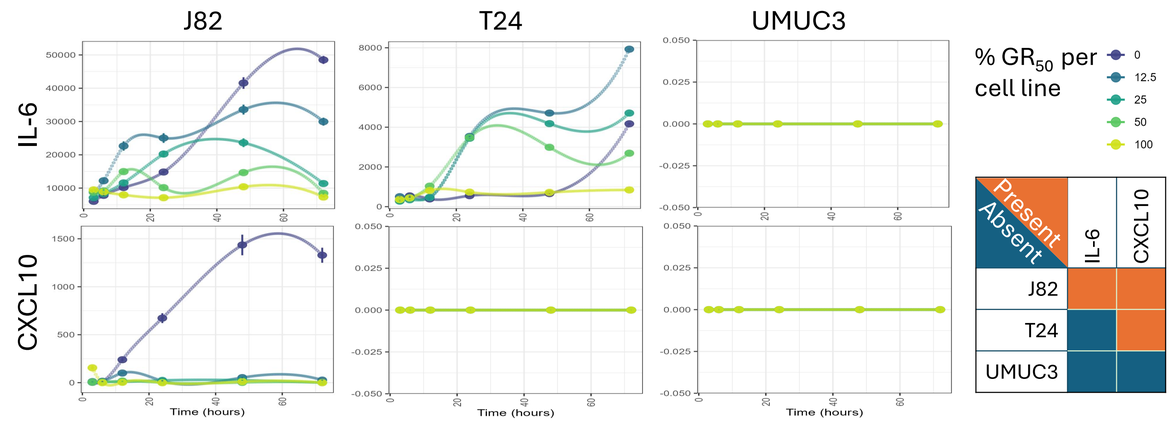

Figure 5. 72-hour cytokine profiles for IL-6 & CXCL10 following 0%, 12.5%, 25%, 50%, 100% of cell line-drug GR_{50}

High-resolution quantitative confocal analysis of 1000s of cells reveals cell-specific IRF3 & NFκB p65 responses to cytosolic HT-DNA. **J82**: IRF3 & p65 translocate to nuclei. **T24:** IRF3 & p65 translocate to cytosol. **UMUC3:** No DNA response (figure 6).

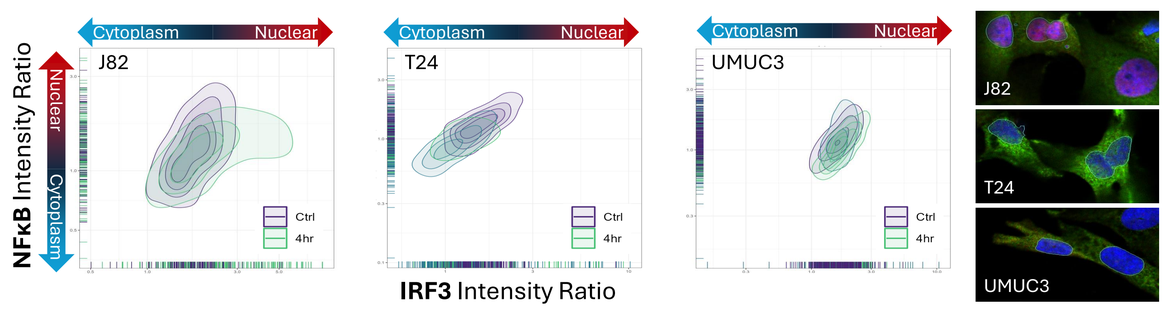

Figure 6. Quantitative confocal analysis of IRF3 and NFκB nuclear-cytoplasm ratios after HT-DNA transfection

J82 response to cytosolic HT-DNA: cytoplasm translocation of STING (0.5hr) then IFI16 (1hr). Nuclear STING intensities reduce at greater rates c.f. cytosol (figure 7).

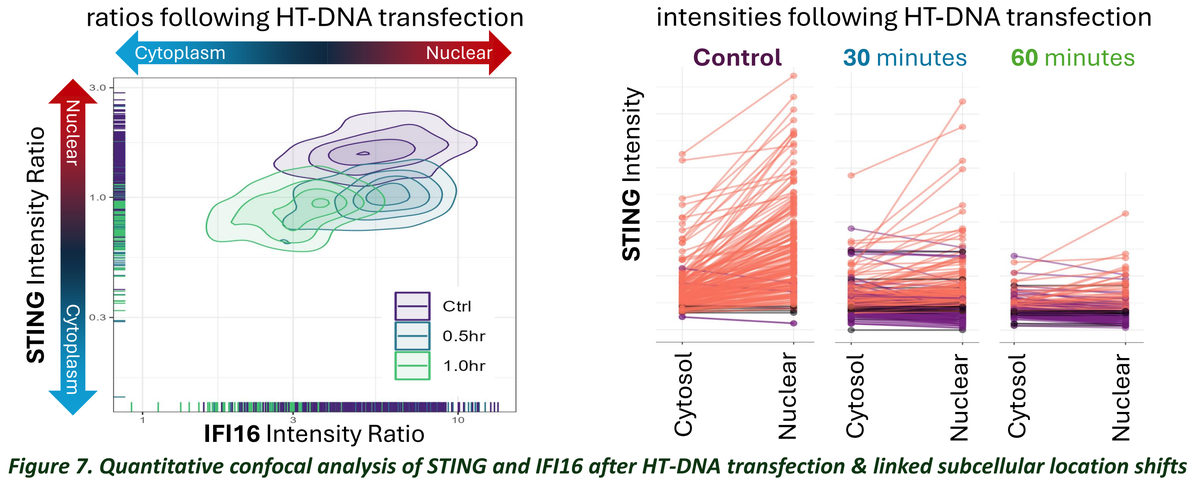

Figure 7. Quantitative confocal analysis of STING and IFI16 after HT-DNA transfection & linked subcellular location shifts

Conclusions and next steps

Bladder cancer cytotoxicity and STING activity states are highly relevant to the mechanistic understanding of responses to CT/RT. Assays to study sub-lethal MVAC doses following STING inhibition are underway to determine pathway fates and expose disparate cytokine response profiles.

Future work will integrate innate immune signatures with tumour tissue analysis to offer relevance for triaging patients who could benefit from bladder-sparing CT/RT and reveal favourable immunotherapy eligibility.