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Breast Cancer Drives Detectable Reprogramming in White Blood Cells and Platelets

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Abstract

Background
Tumour-induced immune reprogramming enables cancer to evade surveillance. Our previous TNBC-THP1 co-culture studies showed activation of IL6 and proliferative signaling. Here, we assessed white blood cells (WBCs) and platelets from breast cancer patients to identify tumour-driven transcriptional changes.
Methods
Blood from IDBC study participants (NCT04495244) was collected before biopsy. WBCs and platelets were isolated, RNA-sequenced, and analyzed by Ingenuity Pathway Analysis (IPA) to identify differentially expressed genes (DEGs) and upstream regulators, integrated with co-culture transcriptomic data.
Results
Cancer WBCs showed 10,319 DEGs and 600 DEGs were found to be platelet-specific. Key regulators included TRAF2, BMP4, SMAD3, and ITGA11 (WBCs) and IFNG, STING1, CDK6, RIPK2, CD44, TGM2, and STAT1 (platelets). WBCs reflected TGF-β–driven immune tolerance, while platelets showed IFNG/STING1-mediated inflammation and adhesion.
Conclusions
WBCs represent chronic immune modulation, and platelets indicate acute activation. These tumour-educated blood signatures may enable early detection and therapeutic monitoring in breast cancer.

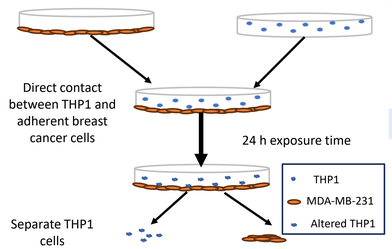
Objectives

To investigate the molecular mechanism of tumour education in immune cells by integrating upstream regulator analysis from clinical samples and *in vitro* models.

Methods

RNAseq in *in vitro* models

- Tumour-immune co-cultures to study tumour-education mechanism mimicking dynamic reprogramming of immune cells by the cancer microenvironment.
- Direct contact between human monocytic cell line (THP1) and breast cancer cells (MDA-231)



RNAseq

Bioinformatics/Global and pathway analysis/Gene expression changes/Upstream regulators

RNAseq in clinical samples

- RNAseq in clinical samples: isolated cancer WBCs or platelets vs. non-cancer

Fresh whole blood from IDBC clinical study participants was collected (cancer vs. non-cancer) prior to biopsy (NCT04495244)

Whole blood fractionation
(Fresh whole blood was separated into white blood cells and platelet fractions)

Platelets | White blood cells

RNAseq

- Changes of gene expression in cancer vs. heathy population
- Analysis: Cancer averaged vs. non-cancer
- Gene Expression Changes, canonical pathway analyses, upstream regulators

Comparative Analysis

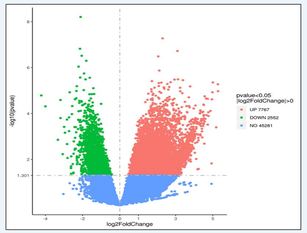
- Results from clinical WBCs and platelets were compared to THP1/TNBC co-culture model to identify shared DEGs, pathways, and upstream regulators
- Integration of differential expression, pathway analysis, and upstream regulators.
- Comparative transcriptomics to reveal conserved, actionable oncogenic programs.

Figure 1. Transcriptomic analysis methodology in cell models and blood samples

Results

Cancer-driven alteration of transcriptome in immune cells

Tumour education in cancer white blood cells leads to significant gene modulation



10,319 differentially expressed genes in cancer WBC vs. normal WBC

Figure 2A. Differential gene expression in Cancer WBC vs. normal WBC.

Tumour-Educated WBCs Exhibit Coordinated Regulatory and Functional Changes

WBC-specific regulators

Upstream regulator	Effect	Acivation Z score
PTPRR	Activated	5.46
ETV6-RUNX1	Activated	5.23
COPS5	Activated	5.00
NCOA1	Activated	3.16
KAT5	Activated	2.91
TNFRSF9	Activated	2.84
CD3E	Activated	2.67
PPP2R2A	Activated	2.65
PPP2R5A	Activated	2.65
TRAF2	Activated	2.60
PACS1	Activated	2.00
GRK2	Activated	2.00
SNHG15	Activated	1.97
HIBCH	Activated	1.96
MIAT	Activated	1.91
ETV3	Activated	1.81
DNMT1	Activated	1.78
SMAD3	Activated	1.58
RGS1	Activated	1.55
ITGA11	Activated	1.48
CRTC2	Inhibited	-1.99
SPI1	Inhibited	-2.23
SMAD4	Inhibited	-2.26
PIWIL4	Inhibited	-2.26
TGM2	Inhibited	-2.34
FOXO4	Inhibited	-2.61
NORAD	Inhibited	-2.67
PDGF-BB	Inhibited	-3.10
NTRK1	Inhibited	-5.43

Mechanistic Model of Upregulated Regulators in Cancer WBCs

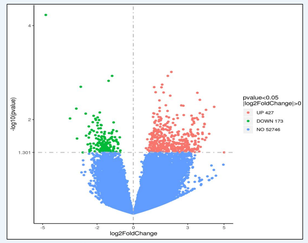
Tumour-derived factors activate interconnected upstream regulators:
- TRAF2 → NF-κB → IL6/TNF pathway → chronic inflammation
- SMAD3 → TGF-β signaling → immune tolerance
- BMP4 → SMAD-dependent transcription → stress adaptation
- ITGA11 → integrin signaling → cell adhesion and trafficking
- ZNF281 → transcriptional reprogramming → suppressed antigen presentation
- DNMT1, KAT5, NCOA1 → Epigenetic reprogramming

Functional Outcomes:

Immune tolerance; Reduced antigen presentation; Altered adhesion and trafficking; Chronic inflammation; Altered cytokine output

Tumour-promoting immune states favoring tumour progression

Tumour education in cancer platelets leads to significant gene modulation



600 differentially expressed genes in cancer platelet fraction vs. normal

Figure 2B. Differential gene expression in Cancer Platelets (CP) vs. Normal (NP).

Tumour Education of Platelets Drives Inflammatory and Pro-metastatic Reprogramming

Platelet specific regulators

Upstream Regulator	Effect	Activation z-score
IFNG	Activated	2.48
STING1	Activated	2.22
IL17A	Activated	2.13
RIPK2	Activated	2.00
CD44	Activated	1.98
TGM2	Activated	1.91
STAT1	Activated	1.39
IL17F	Activated	1.34
FOXO3	Activated	1.19
IRF4	Inhibited	-1.52

Mechanistic Model of Upregulated Regulators in Cancer Platelets

IFNG / STING → JAK/STAT1 activation
↓
↑ RIPK2 → NF-κB / MAPK
↓
↑ CD44 (adhesion receptor)
↑ TGM2 (matrix remodeling)
↑ CDK6 (cytoskeletal remodeling)
↓
- Inflammatory cytokine release
- Microparticle shedding
- Platelet/tumour & platelet/immune adhesion
- Angiogenesis and immune modulation

Functional Outcomes:

Increased inflammatory cytokine release; ECM remodeling; Tumour cell protection; Immune evasion; Immunosuppression; Chronic inflammation

Proinflammatory, adhesive, and pro-metastatic platelet phenotype

Tumour education nodes shared between WBCs, platelets and *in vitro* models

Upstream Regulators	Functional outcome	Cancer WBC vs. non-cancer	THP1+ MDA 231
		Z score	Z score
TRAF2	Activates NFκB and MAPK pathways, promoting inflammatory signaling and survival.	2.60	2.45
SMAD3	Drive TGFβ/BMP signaling, contributing to epigenetic remodeling, differentiation arrest, and immune tolerance.	1.58	2.75
BMP4		1.28	3.30
ITGA11	Enhances integrin-mediated adhesion and migration, facilitating platelet-WBC-tumour interactions.	1.48	1.67
ZNF281	Transcriptional reprogramming toward pro-tumour gene expression, including immune suppression	1.41	2.65

Outcome: WBCs and THP1 cells acquire a partially activated but functionally suppressed phenotype, poised to support tumour survival and invasion.

Upstream Regulators	Functional Outcome	Cancer platelets vs. non-cancer	THP1+ MDA-231
		Z score	Z score
IFNG	Activate interferon-stimulated genes, creating an inflammatory yet tumour-permissive platelet phenotype.	2.48	6.77
STING1		2.24	6.66
CDK6	Supports cell cycle-related signaling, possibly sustaining platelet activation and secretion capacity.	2.00	2.35
RIPK2	Amplifies NF-κB-dependent inflammatory signaling, cytokine release, innate immune activation	2.00	5.30
CD44	Facilitate adhesion, extracellular matrix remodeling, and interaction with tumour cells.	1.98	3.06
TGM2		1.91	5.75
STAT1	Increased secretion of inflammatory cytokines and chemokines	1.39	5.67

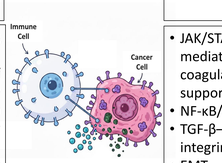
Outcome: Platelets become hyperactive, immune-interacting, and pro-metastatic, supporting circulating tumour cell survival.

Summary

Tumour-Driven Reprogramming in WBCs

- Persistent NF-κB and STAT signaling maintains pro-inflammatory state
- TGF-β and SMAD3-driven suppression of antigen presentation and cytotoxic activity
- Integrin (ITGA11)-mediated adhesion enhances trafficking to tumour microenvironment
- BMP4 and ZNF281 reprogram transcription → immune exhaustion phenotype
- Altered cytokine output

Outcome: immune-altered phenotypes that sustain tumour growth, chronic inflammation, and immune evasion



Tumour education of immune cells through cell contact of secreted molecules

Tumour-Driven Reprogramming in Platelets

- JAK/STAT1, cGAS/STING & NF-κB-mediated inflammation & pro-coagulation, sustaining a tumour-supportive state
- NF-κB/MAPK & SMAD3-driven survival
- TGF-β–driven immune tolerance and integrin-mediated trafficking
- EMT
- Immunosuppression

Outcome: Proinflammatory, adhesive, and pro-metastatic platelet phenotype. A shift toward innate immune sensing, inflammatory signaling, cell cycle activity and adhesion dynamics

Integrated Outcome:
Immune evasion, angiogenesis, platelet activation/aggregation, metastatic seeding, chronic inflammation leading to tumour-promoting states

Conclusions

- Breast cancer induces distinct transcriptional reprogramming in WBCs and platelets, reflecting chronic immune modulation and acute activation, respectively.
- Key upstream regulators (e.g., TRAF2, SMAD3, ITGA11 in WBCs; IFNG, STING1, CD44 in platelets) mirror those seen in the TNBC-THP1 co-culture model, confirming translational relevance.
- WBCs exhibit TGF-β-driven immune tolerance and integrin-mediated trafficking, while platelets emphasize innate sensing, inflammation, and vascular interaction.
- Tumours "educate" immune cells to suppress anti-tumour immunity and sustain chronic inflammation that supports tumour progression.
- These tumour-educated immune changes are detectable non-invasively in blood, supporting their potential as early detection biomarkers and a foundation for therapeutic strategies targeting tumour–immune crosstalk.

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