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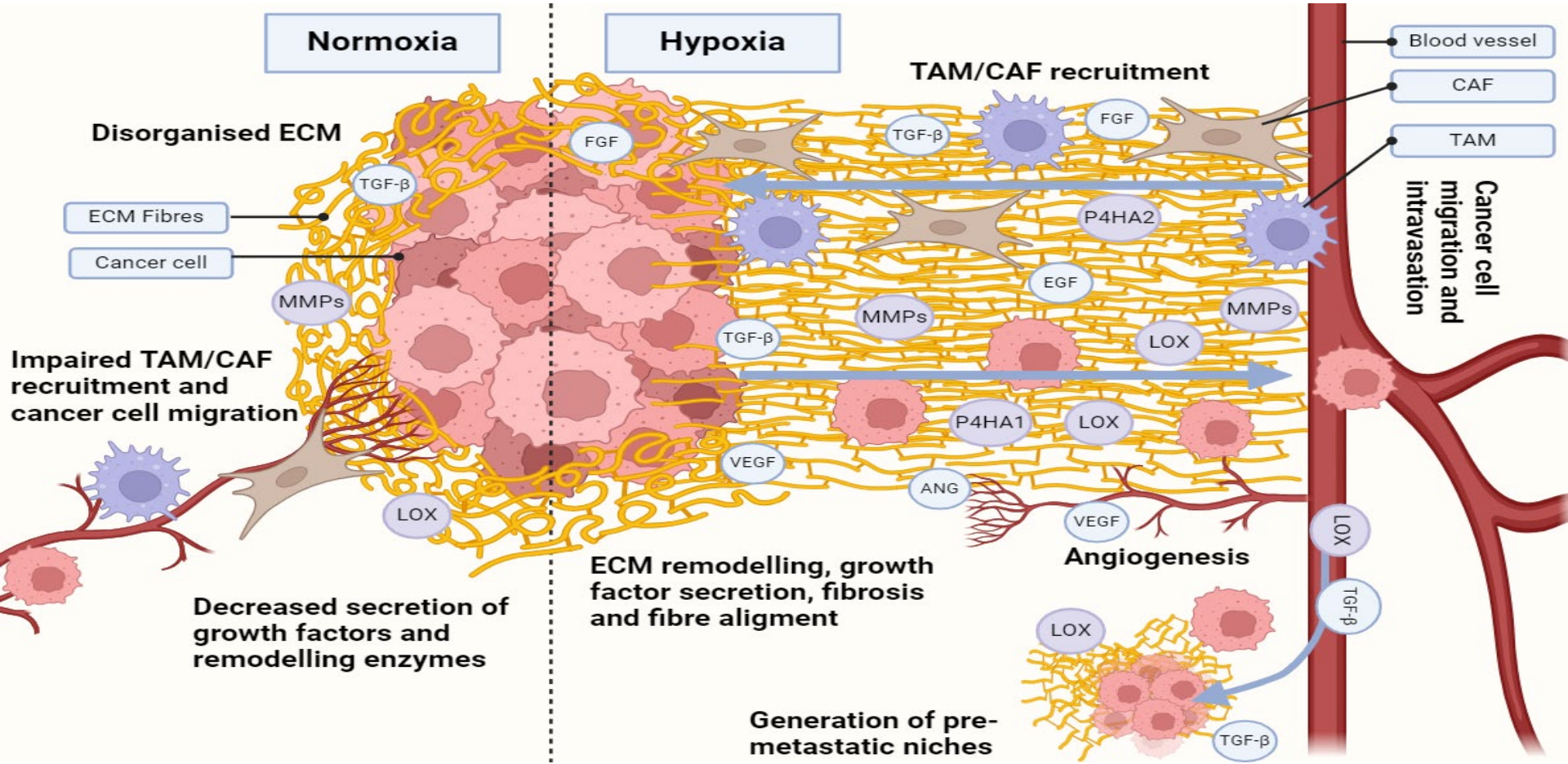
A pan-cancer characterisation of the hypoxic ECM identifies a gene signature predictive of radiotherapy benefit

Background: Extracellular matrix and hypoxia



Bigos *et al*. Front Oncol 2024

Hypothesis and aims

Hypothesis

- Hypoxia alters the ECM composition in cancer, influencing radioresistance

Aims

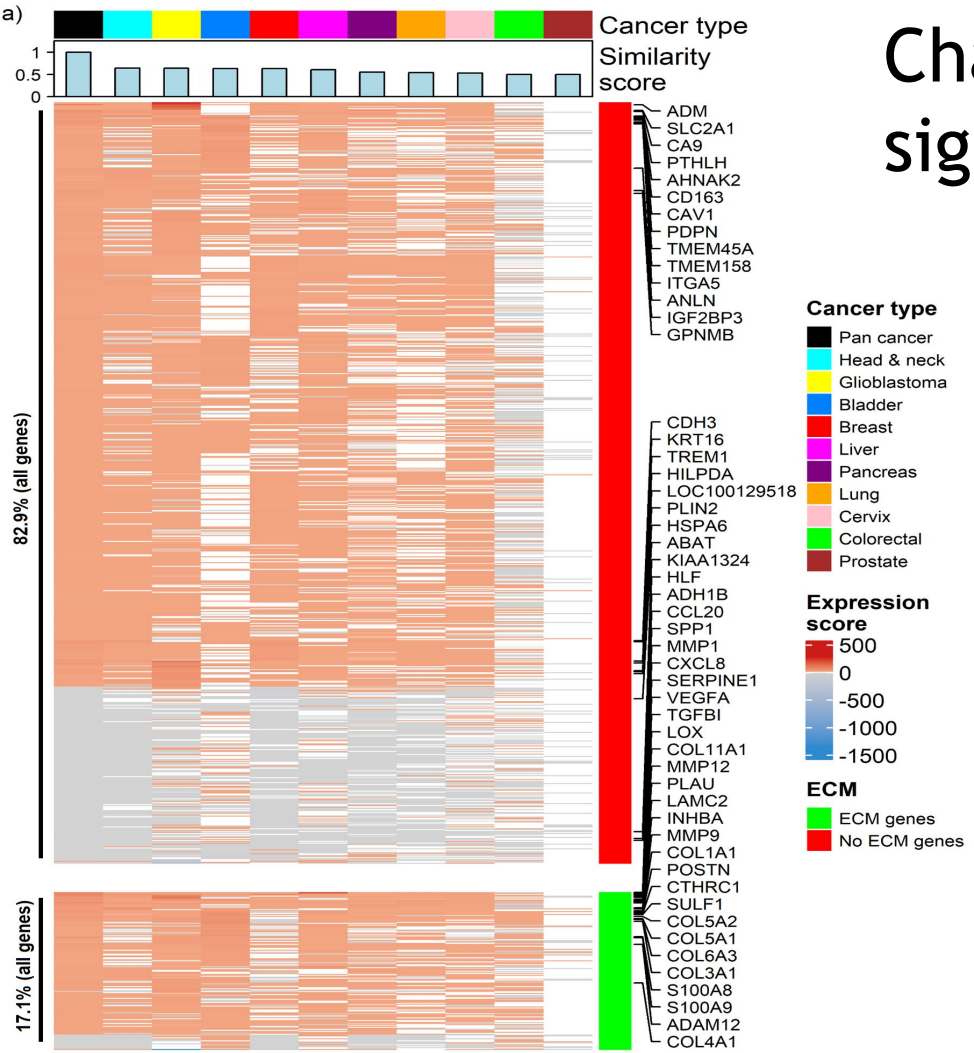
- To characterise the hypoxic ECM composition in cancer
- To identify a signature associated with radiotherapy benefit

Characterising hypoxia signalling: meta-analysis

- **Hypoxia-scores:** Retrospectively validated cancer-specific hypoxia signatures
- **Differential gene expression (DEGs):** *limma* analysis for each cohort independently
- **Random-effects model (REM):** Integrate differential gene expression data
- **Significance:** FDR<0.05, fold change >1 or <-1, frequency > 30%

Cancer type	Total patients	Total cohorts
Bladder	1,257	8
Breast	3,766	12
Colorectal	1,937	14
Glioblastoma	3,064	18
Liver	1,698	13
Head & neck	1,492	15
Lung	1,446	7
Pancreas	1,430	13
Prostate	4,587	21
Cervix	568	8
All	21,	129

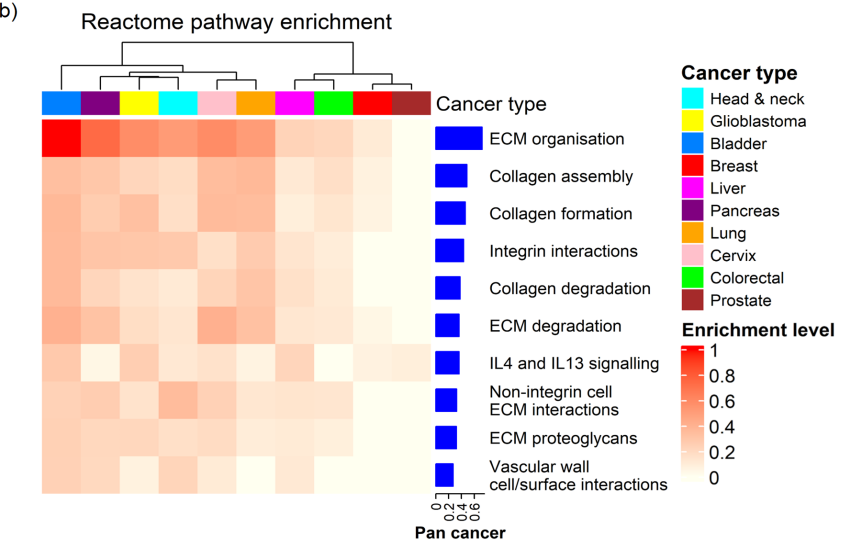
Characterising hypoxia signalling: meta-analysis



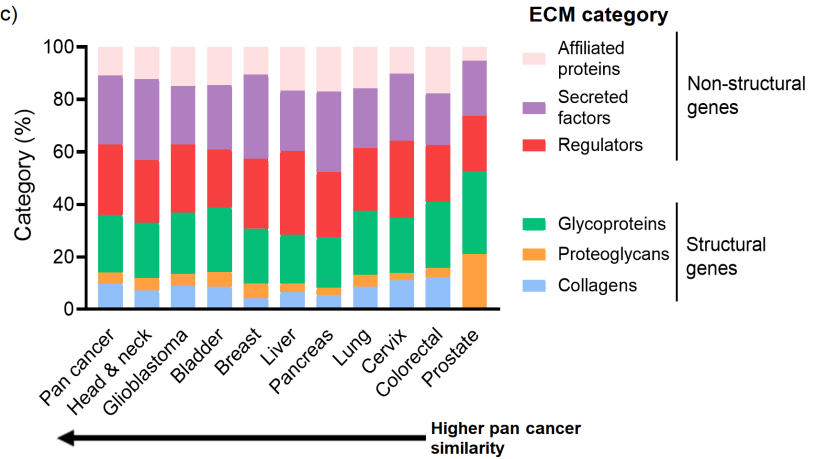
Hypoxia induces pan-cancer gene expression changes

>17% of hypoxia-regulated genes are extracellular matrix (ECM)

Characterising hypoxia signalling: meta-analysis



Hypoxia regulates ECM pathways at pan-cancer level



Hypoxia changes in ECM gene expression are prevalent

Identifying suitable models

Cell culture:
- Hypoxia (0.2% O_2)
- Normoxia (20.9% O_2)

Four bladder cancer cell lines:
- T24, UMUC3, J82, RT4

Proteomics
- Secretome
- ECM

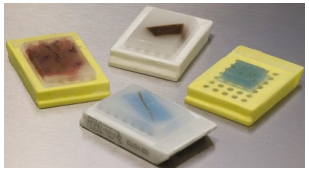
Transcriptomics

ChIP-Seq

MS

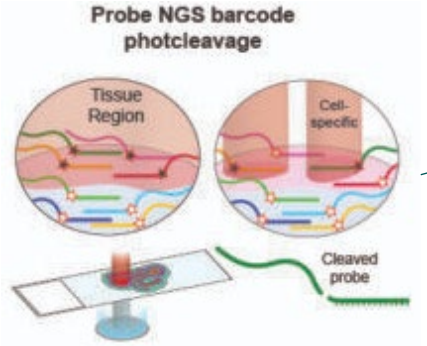
NGS

FFPE (n = 32) from bladder cancer patients

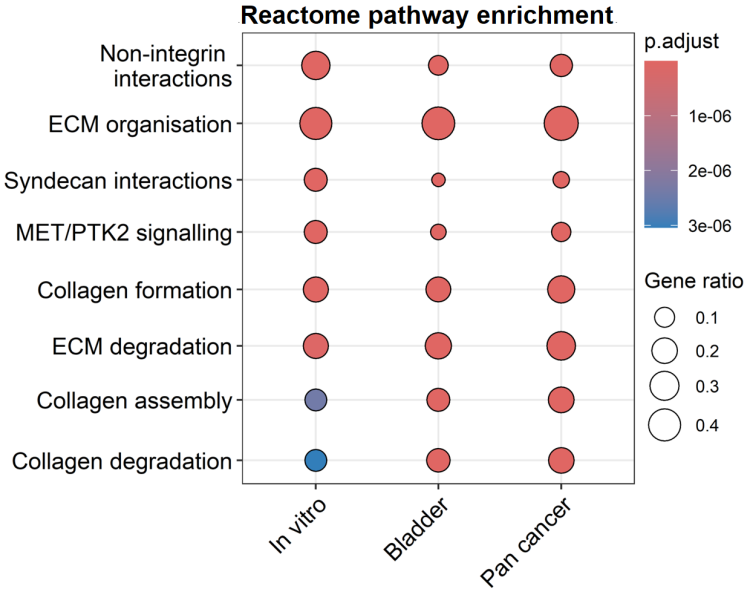


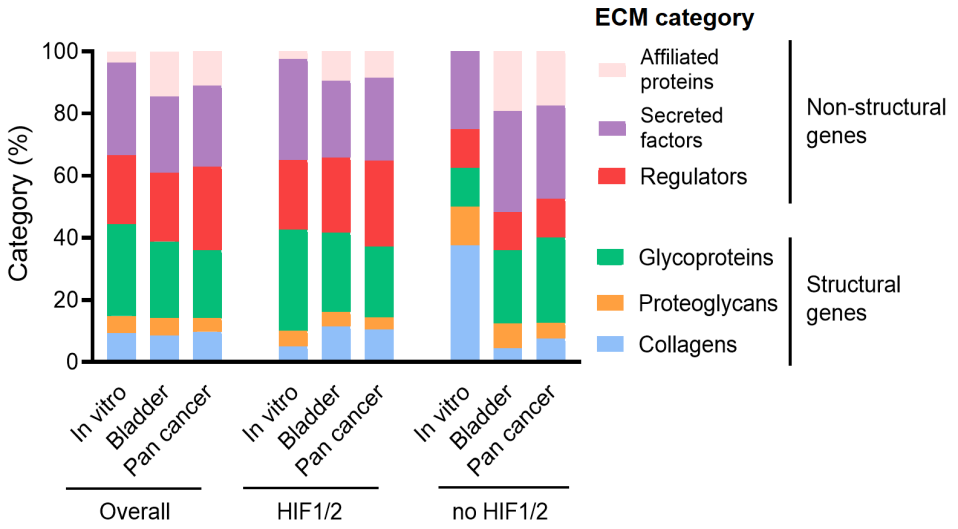
In situ hybridisation and staining

CA9 to stain hypoxic areas



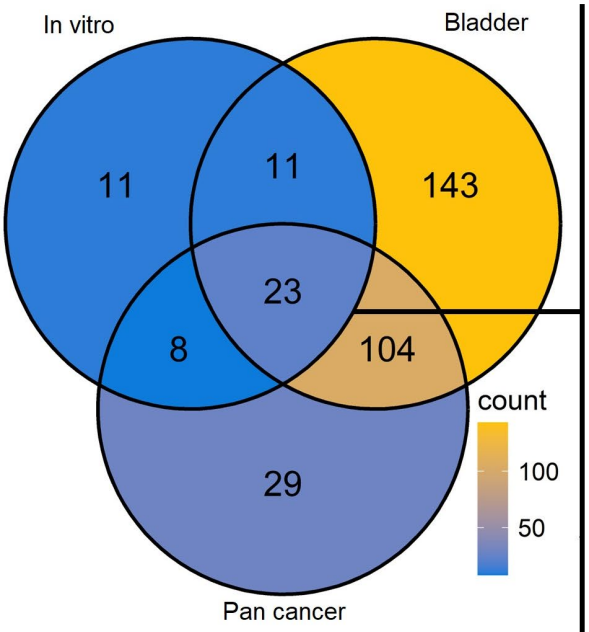
Identifying models (*in vitro*)

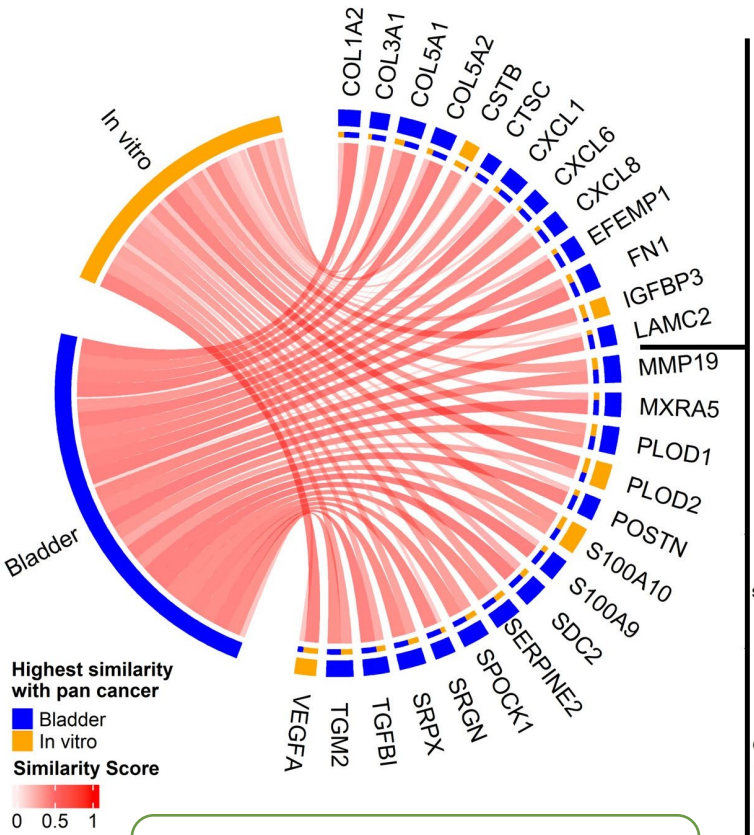


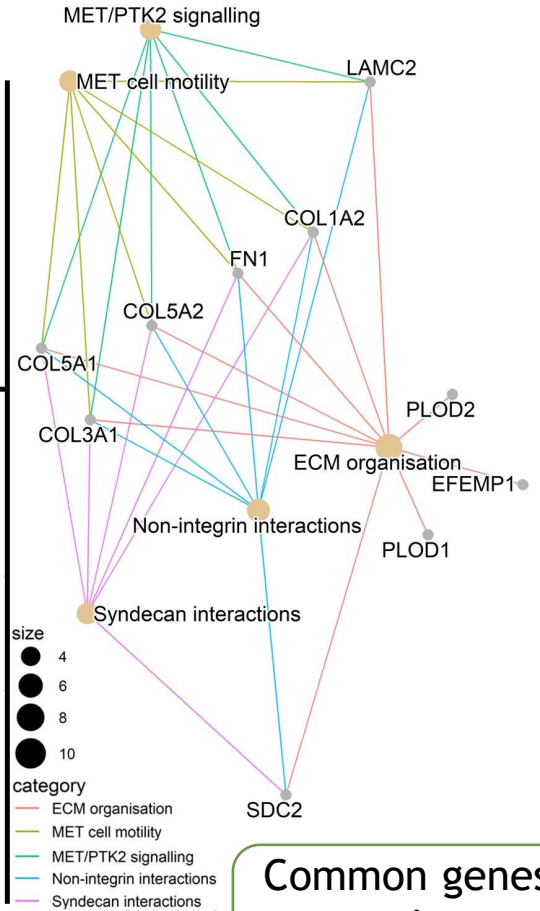


In vitro models recapitulate ECM hypoxia alterations found in the meta-analysis

Identifying models (*in vitro*)





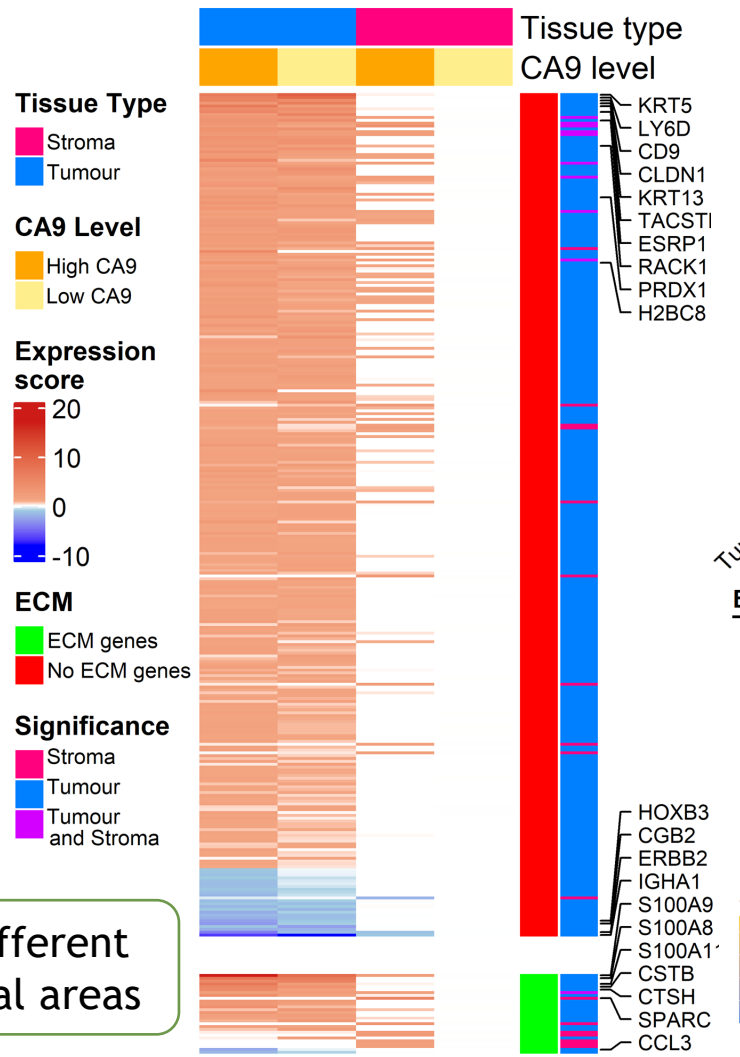


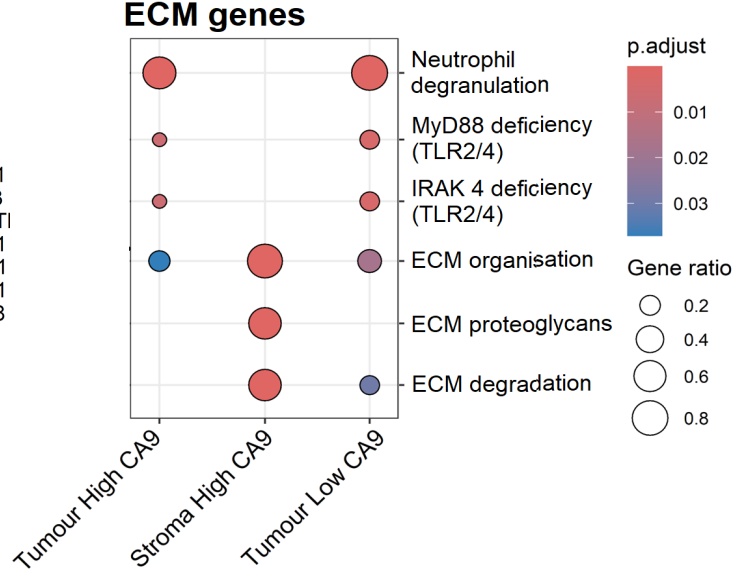
In vitro atlas:
53 ECM genes Significant at protein and RNA levels

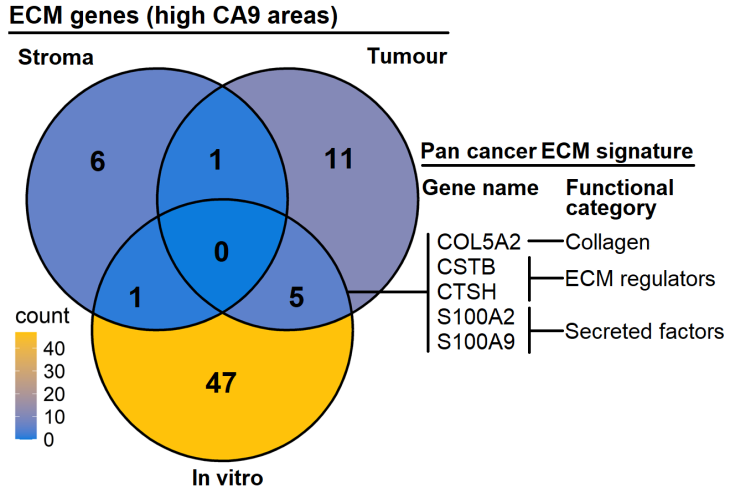
Common genes had similar expression patters

Common genes regulate MET motility

Identifying models (FFPE)





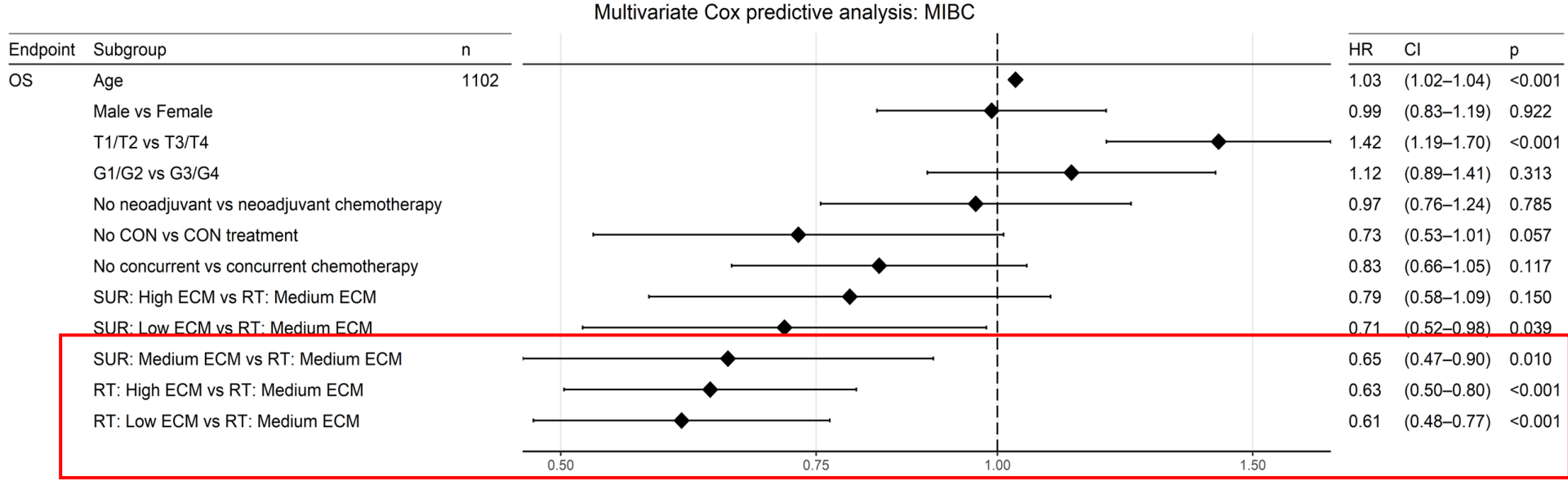


Hypoxic ECMs are different in tumour and stromal areas

Signature validation: meta-analysis

- Using available clinical and transcriptomic data from the meta-analysis cohorts
- Patients were tertile-stratified based on signature expression levels
- Signature evaluated with Cox multivariate analysis

Prognostic (medium or high scores do worse):
Glioblastoma (n=1,011; p=0.0000054),
Bladder (n=1,102; p=0.0027)
Lung (n=764; p=0.0029)
Pancreatic (n=271; p=0.00035)
Prostate: (n=689; p=0.035)



Mechanistic evaluation

Immunofluorescence

ECM fibres: FN, COL5, COL1
Co-localisation: E/N-cadherin; Vinculin/Paxillin

Co-localisation
24h inc.

Culture cells 7 days:
- Hypoxia (0.2% O_2)
- Normoxia (20.9% O_2)

decellularized

Plate with ECM coating

Irradiation (0-8Gy) and seeding of new cells (20.9% O_2)

Four bladder cancer cell lines:
- T24, UMUC3, J82
- n= 3 biological repeats with n = 20 technical repeats / cell line

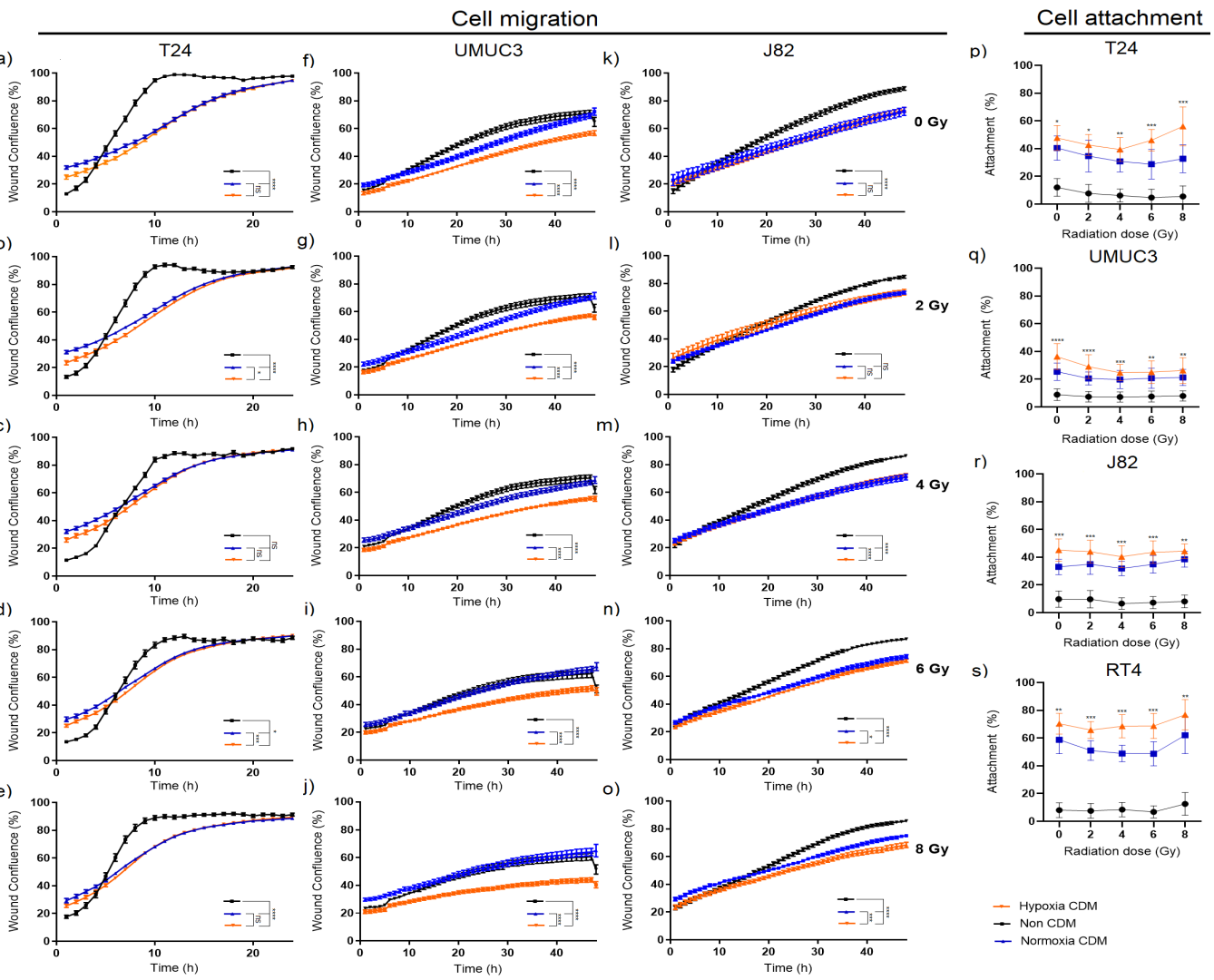
24h inc.

30min inc.

Scratch assay

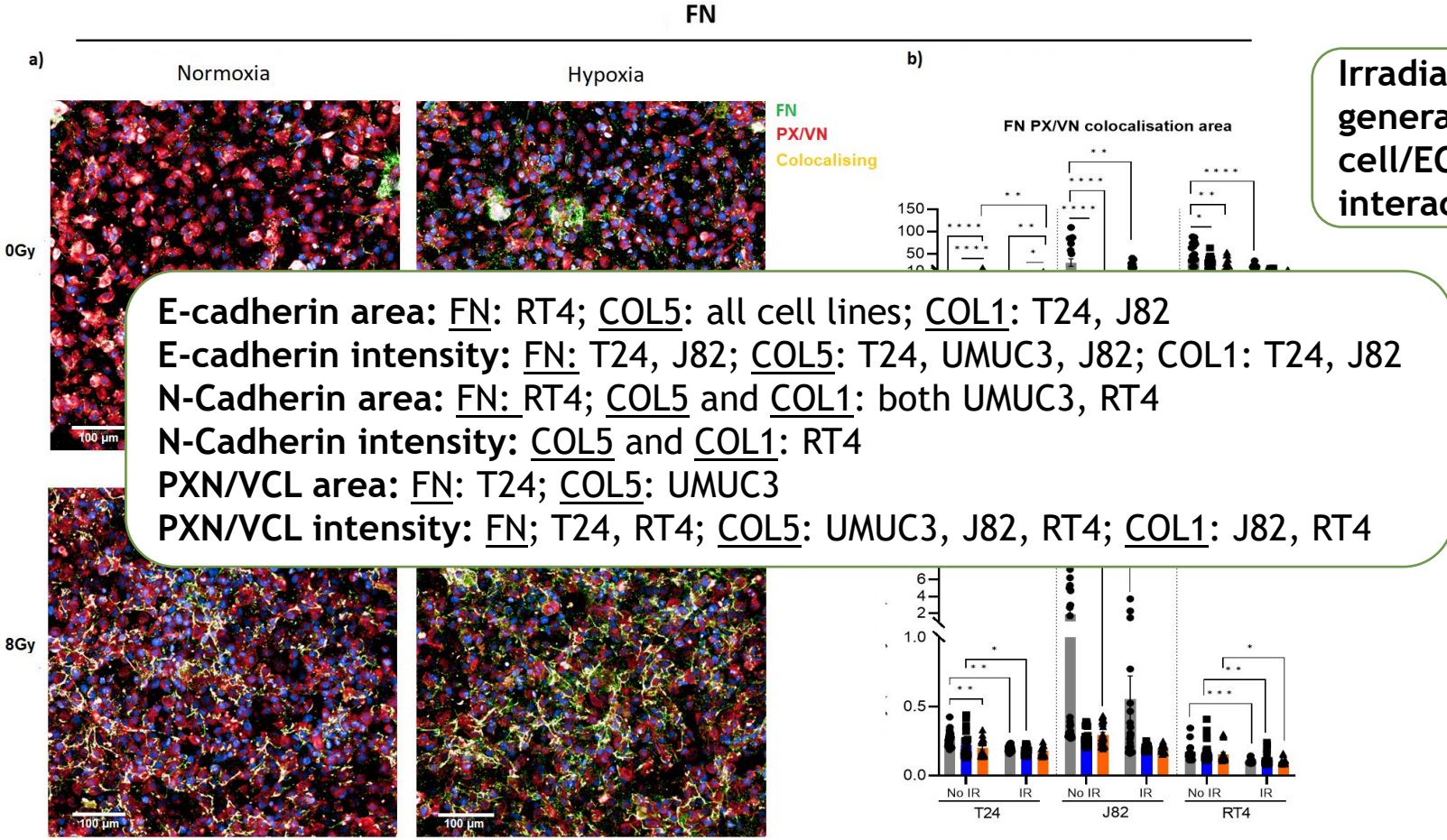
Attachment assay

Mechanistic evaluation



Hypoxic ECMs promote adhesion and impair migration, effect enhanced by irradiation

Mechanistic validation: ECM fibres colocalisation



Irradiation generally impairs cell/ECM interactions

E-cadherin area: FN: RT4; COL5: all cell lines; COL1: T24, J82
E-cadherin intensity: FN: T24, J82; COL5: T24, UMUC3, J82; COL1: T24, J82
N-Cadherin area: FN: RT4; COL5 and COL1: both UMUC3, RT4
N-Cadherin intensity: COL5 and COL1: RT4
PXN/VCL area: FN: T24; COL5: UMUC3
PXN/VCL intensity: FN; T24, RT4; COL5: UMUC3, J82, RT4; COL1: J82, RT4

Conclusions

- Hypoxia induces prevalent pan-cancer alterations in the ECM
- Those alterations linked to MET-mediated cell migration and immune signalling
- A hypoxic-ECM signature is both prognostic and predictive of radiotherapy benefit
- High-score patients benefit from radiotherapy and had reduced metastatic events
- Highly hypoxic ECMs (0.2% O2) have fewer collagen fibres and reduce cell migration (effect enhanced by radiotherapy)
- Radiation impairs cell/ECM interactions, providing context for the signature’s predictive capacity

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