The single-cell pathology landscape of breast cancer

Hartland W. Jackson et al. 2020/01, Nature

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Complex single-cell phenotypes and spatial context are not at present reflected in the histological stratification.

- Histological and phenotypical differences between tumors guide cancer diagnosis, prognosis and the selection of treatment.

- Breast cancers are graded based on tumor structure and cellular morphology.

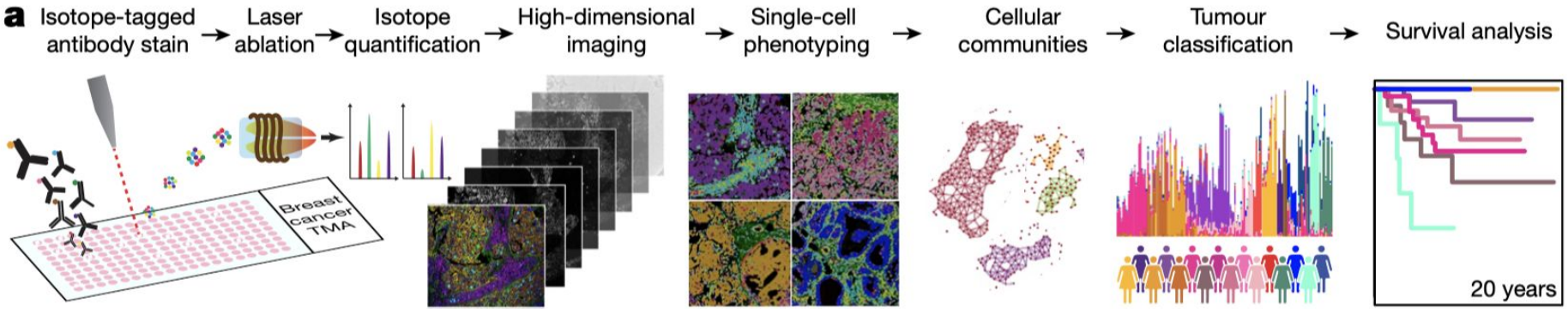
- Breast cancers are subcategorized when more than 1% of tumour cells contain hormone receptors or more than 10% express high levels of *HER2* protein or exhibit amplification of the *HER2* gene
    - This leaves a large portion of cells uncharacterized.

Spatially resolved single-cell phenotypes

To comprehensively quantify the cellular heterogeneity and spatial organization of breast cancer tissue

- Designed an imaging mass cytometry (IMC) panel specific to breast histology
- Used this to image samples from 281 tumours that represent all clinical subtypes and grades of pathology

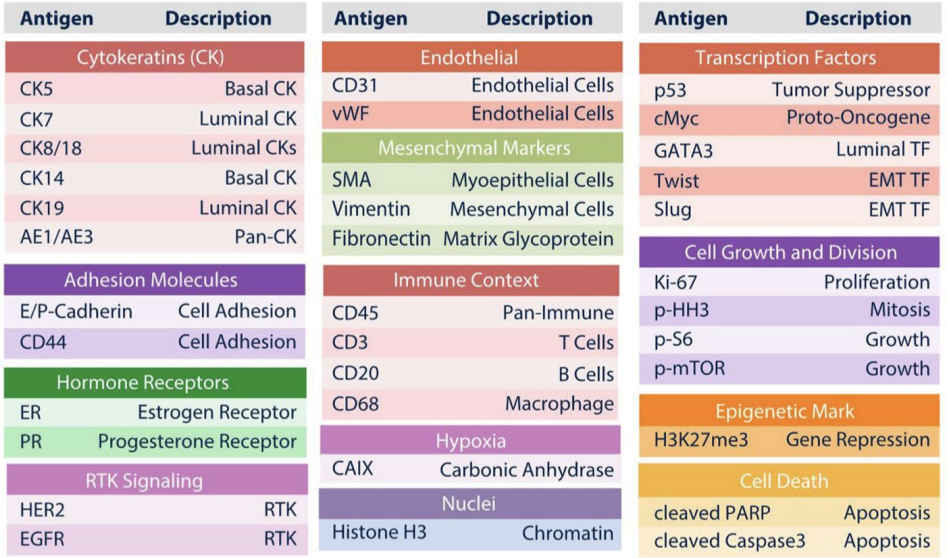
Fig.1



Spatially resolved single-cell phenotypes

Quantified 35 biomarkers, resulting in 720 high-dimensional pathology images of tumour tissue from 352 patients with breast cancer.

- Using a random forest pixel classifier (*Ilastik*) and *CellProfiler,* images were segmented into single cells and tumour and stromal regions.
- Identified 855,668 cells in 381 images
  - 289 tumour
  - 87 healthy breast
  - 5 liver controls
- Quantified the expression of both marker genes and the spatial features of each cell (Fig. 1a)



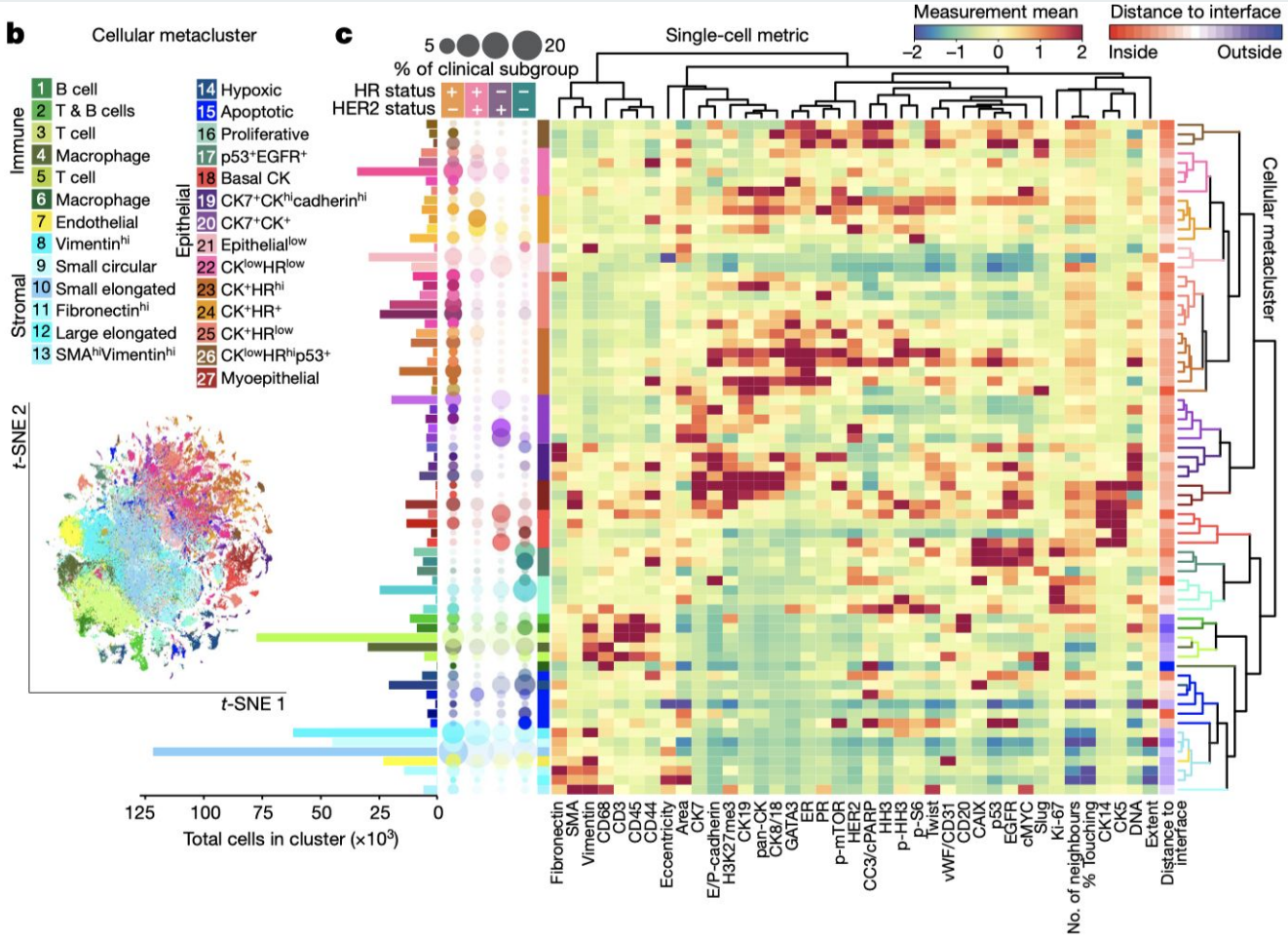
Antigen	Description
Cytokeratins (CK)	
CK5	Basal CK
CK7	Luminal CK
CK8/18	Luminal CKs
CK14	Basal CK
CK19	Luminal CK
AE1/AE3	Pan-CK
Adhesion Molecules	
E/P-Cadherin	Cell Adhesion
CD44	Cell Adhesion
Hormone Receptors	
ER	Estrogen Receptor
PR	Progesterone Receptor
RTK Signaling	
HER2	RTK
EGFR	RTK

Antigen	Description
Endothelial	
CD31	Endothelial Cells
vWF	Endothelial Cells
Mesenchymal Markers	
SMA	Myoepithelial Cells
Vimentin	Mesenchymal Cells
Fibronectin	Matrix Glycoprotein
Immune Context	
CD45	Pan-Immune
CD3	T Cells
CD20	B Cells
CD68	Macrophage
Hypoxia	
CAIX	Carbonic Anhydrase
Nuclei	
Histone H3	Chromatin

Antigen	Description
Transcription Factors	
p53	Tumor Suppressor
cMyc	Proto-Oncogene
GATA3	Luminal TF
Twist	EMT TF
Slug	EMT TF
Cell Growth and Division	
Ki-67	Proliferation
p-HH3	Mitosis
p-S6	Growth
p-mTOR	Growth
Epigenetic Mark	
H3K27me3	Gene Repression
Cell Death	
cleaved PARP	Apoptosis
cleaved Caspase3	Apoptosis

35 biomarkers

Fig. 1


b
Cellular metacluster
Immune
1 B cell
2 T & B cells
3 T cell
4 Macrophage
5 T cell
6 Macrophage
7 Endothelial
8 Vimentin^hi
Stromal
9 Small circular
10 Small elongated
11 Fibronectin^hi
12 Large elongated
13 SMA^hiVimentin^hi
Epithelial
14 Hypoxic
15 Apoptotic
16 Proliferative
17 p53+EGFR+
18 Basal CK
19 CK7+CK^hicadherin^hi
20 CK7+CK+
21 Epithelial^low
22 CK^lowHR^low
23 CK+HR^hi
24 CK+HR+
25 CK+HR^low
26 CK^lowHR^hip53+
27 Myoepithelial
t-SNE 2
t-SNE 1
125 100 75 50 25 0
Total cells in cluster (×10^3)
c
5 20
% of clinical subgroup
HR status
HER2 status
Single-cell metric
Measurement mean
−2 −1 0 1 2
Distance to interface
Inside Outside
Cellular metacluster
Fibronectin
SMA
Vimentin
CD68
CD3
CD45
CD44
Eccentricity
Area
CK7
E/P-cadherin
H3K27me3
CK19
pan-CK
CK8/18
GATA3
ER
PR
p-mTOR
HER2
CC3/cPARP
HH3
p-HH3
p-S6
Twist
vWF/CD31
CD20
CAIX
p53
EGFR
cMYC
Slug
Ki-67
No. of neighbours
% Touching
CK14
CK5
DNA
Extent
Distance to interface

Spatially resolved single-cell phenotypes

Overview of patient clinical metadata for cohort from Basel Univ. Hospital

Supplementary Table 1.

Metadata	Values	Patient Counts/ Means
Grade	1	19
	2	26
	3	27
Tumor Size [mm]	8 - 80	26.93
Age [years]	42 - 60	53.77
Gender	Female	72
	Male	0
Menpausal	Pre	0
	Post	72

[Age]
US: 43.3
Taiwan2:53.4

[Menopausal]
US
Pre: 76
Post: 53
Taiwan2
Pre: 47
Post: 69

Spatially resolved single-cell phenotypes

Supplementary Table 1. (cont'd)

Metadata	Values	Patient Counts/ Means
ER Status	Positive	51
	Negative	21
PR Status	Positive	43
	Negative	29
HER2 Status	Positive	14
	Negative	58
Clinical Subtype	HR+HER2+	12
	HR+HER2-	39
	HR-HER2+	2
	TripleNeg	9

[ER]
US
Pos: 48
Neg: 29
Missing: 53

Taiwan2
Pos: 96
Neg: 18
Missing: 2

[PR]
US
Pos: 35
Neg: 38
Missing: 57

Taiwan2
Pos: 79
Neg: 31
Missing: 6

Spatially resolved single-cell phenotypes

Supplementary Table 1. (cont'd)

Metadata	Values	Patient Counts/ Means
ER Status	Positive	51
	Negative	21
PR Status	Positive	43
	Negative	29
HER2 Status	Positive	14
	Negative	58
Clinical Subtype	HR+HER2+	12
	HR+HER2-	39
	HR-HER2+	2
	TripleNeg	9

[HER2]
US
Pos: 3
Neg: 68
Missing: 59

Taiwan2
Pos: 19
Neg: 90
Missing: 7

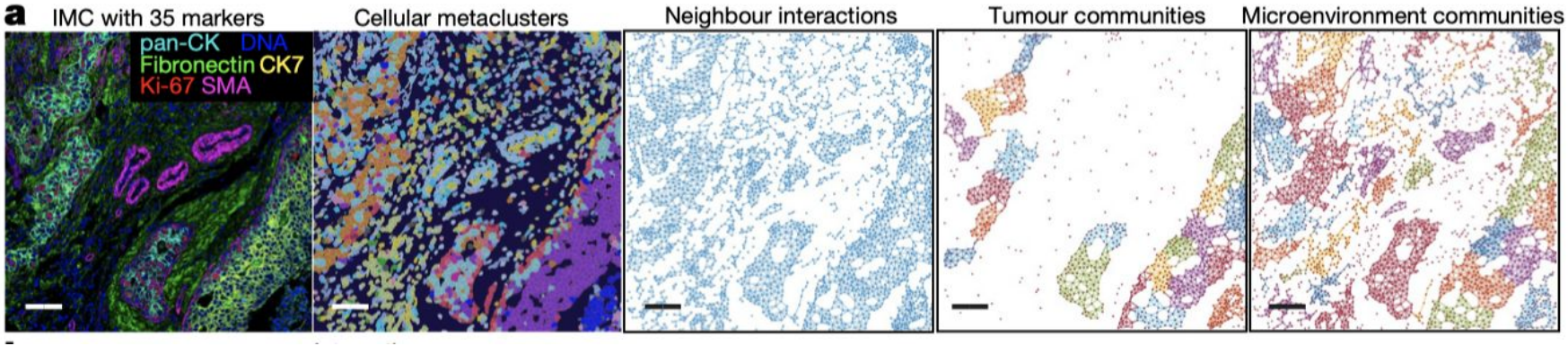
[Subtype]
US
HER2+: 3
HR+HER2-: 42
TripleNeg: 26
Noninvasive or benign: 59

Taiwan2
HER2+: 19
HR+HER2-: 79
TripleNeg: 11
Missing: 7

Multicellular breast cancer architecture

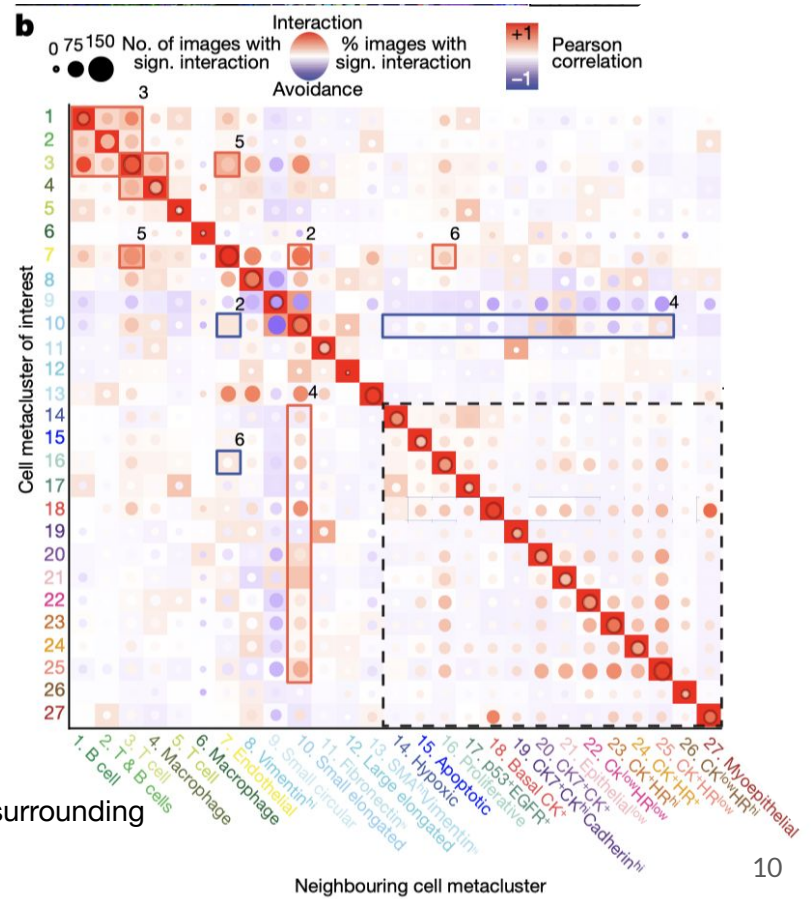
Based on the previous single-cell phenotypes, they defined patterns of multicellular architecture in breast tumour tissue

- Evaluated regional correlations between cellular metaclusters to determine whether cells co-occurred across all images



Multicellular breast cancer architecture

- Used neighbourhood analysis based on permutation tests
  - To quantify cell colocalization
  - To identify statistically significant interaction or avoidance between pairs of cell phenotypes



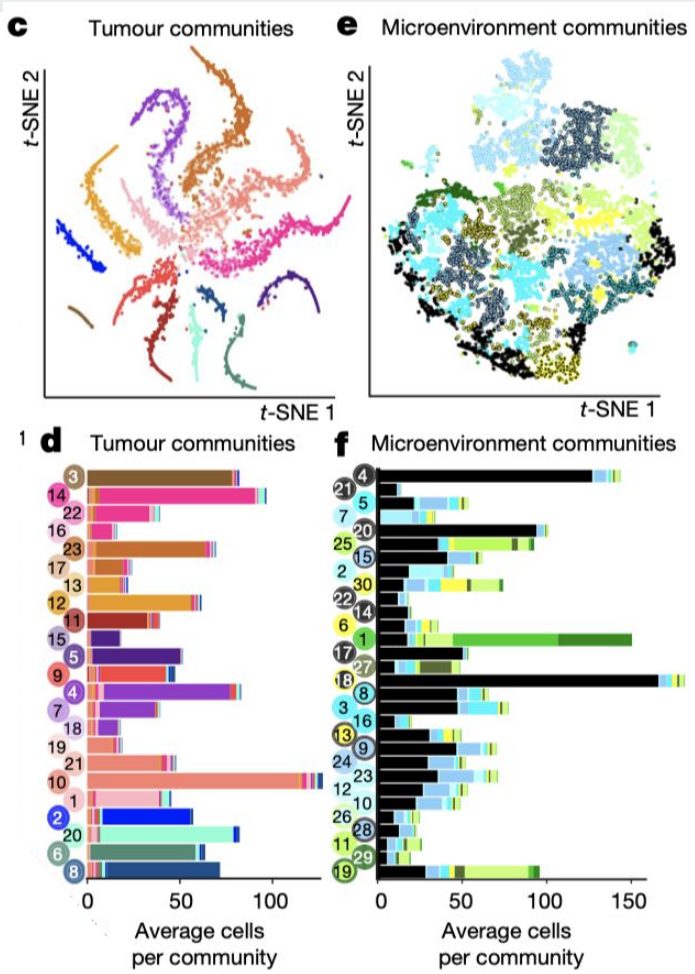
(1) tumour epithelium
(2) endothelium
(3) immune cells
(4) surrounding stroma
(5) endothelium and T cells
(6) proliferating epithelium surrounding endothelial cells.

Multicellular breast cancer architecture

- clustered by PhenoGraph on the basis of the min to max normalized absolute number of cells from each cellular metacluster
  - **(c, d)** Individually coloured epithelial communities (n = 8,495)
  - **(e, f)** microenvironment communities (n = 12,854)

- Barplots indicate the average number of cells from each cellular metacluster

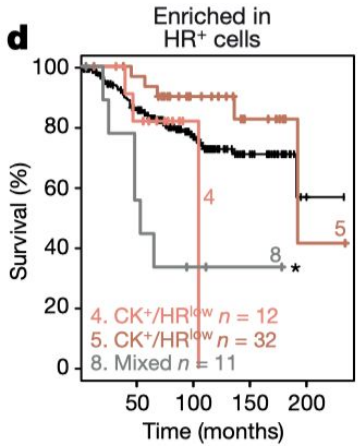
- **(f)** Black represents tumour cell phenotypes.



Single-cell pathology and risk

Investigated how the organization of single cells into communities contributes to the tissue architecture of breast cancer and its subtypes

- **(Fig3a, b)** Observed variable structures and cellular densities, and relationships between cellular phenotype and tissue organization
- **(Fig. 3b)**
  - Heterogeneous tumours consist of multiple phenotypically pure communities
  - Homogeneous tumours have only a few clustered bands
- **(Fig. 4d)** Patients with these heterogeneous tumours of subgroup SCP 8 had very poor outcomes.



Quantification of intratumour heterogeneity

Investigated the reproducibility and spatial variability of SCP classifications in two central and two peripheral tumour regions from 72 patients in an independent cohort.

- Used the same approach
  1) to independently define single-cell phenotypes
  2) match them to cellular metaclusters
  3) classify each imaged region into SCP subgroups and stromal architectures
- All cellular metaclusters and SCP subgroups that were identified in the first cohort were present in the second cohort.

Discussion

- Single-cell pathology can better segregate patients with distinct clinical outcomes than can the current strategy of clinical subtyping.
- When it comes to the patient outcome, the information yielded by the multicellular structures was superior to that yielded by single-cell data alone.
- Observed that phenotypic and spatial heterogeneity varied between clinically established subtypes and identified breast cancer phenotypes that co-occur
- Multicellular spatial information provides a basis for future study of how spatial and phenotypic tissue features influence disease outcome.