M2 Bio-Informatic Internship

10/06/2025 Presentation

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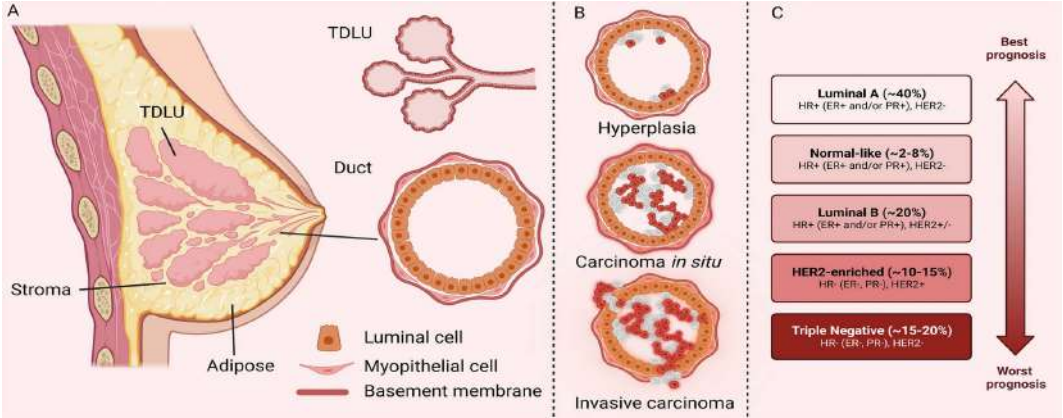
Context

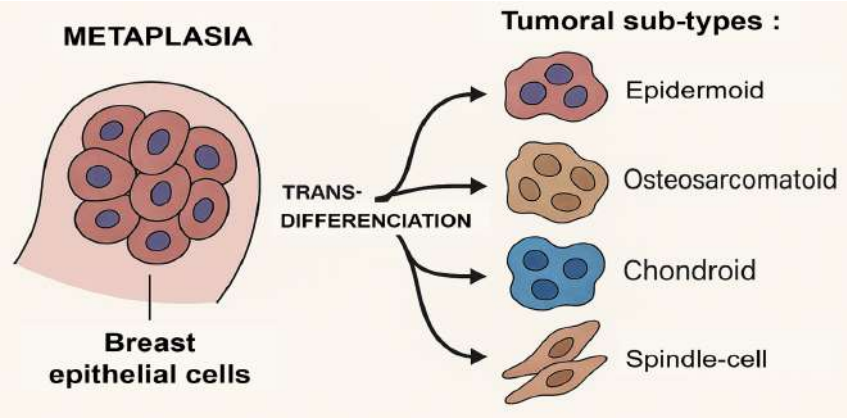


TNBC: Triple-Negative Breast Cancer
TDLU: Terminal Duct Lobular Units

Source: Chang-Yun Li
biomedcentral.com (CC BY 4.0)

MpBC (Metaplastic Breast Carcinomas) are rare forms of TNBC, lacking molecular diagnostic markers and specific therapies

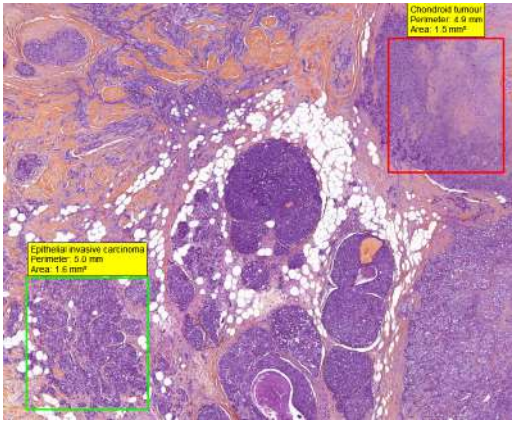
Trans-differenciation



MpBC can transdifferentiate into various aggressive tumor subtypes

Trans-differentiation

- **MpBC** exhibits a remarkable **plasticity**
- **Transdifferenciation** into multiple aggressive **tumor subtypes**
- **Mixed MpBC**
  - Each sample contains **at least 2 tumoral compartments**



Research questions

Unresolved questions

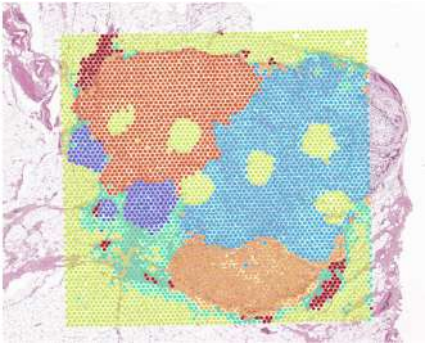
1. Understanding the **evolutionary trajectories** in MpBC.
   - Internal determinants (genetic and epigenetic)
   - External determinants (tumor microenvironment)
2. Identify molecular biomarkers to **improve diagnostic** precision.
3. Discover genetic and epigenetic features that may serve as potential **therapeutic targets**.

Internship Aims

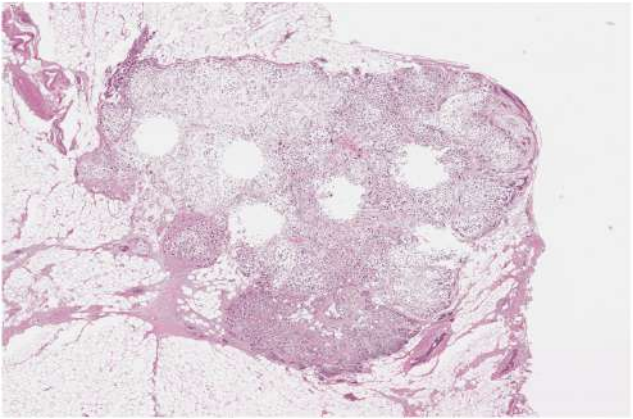
- Determine **expression markers** specific to different tumor sub-types.
- Analyze **genomic divergence** between tumoral compartments.

Data

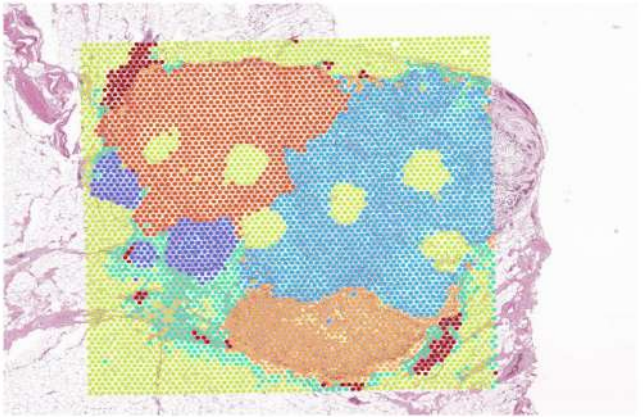
- **Spatial transcriptomic counts** (*Visium*, 10X Genomics)
- **16 mixed MpBC** samples
- Different tumor **transdifferentiation** states captured



MpBC sample

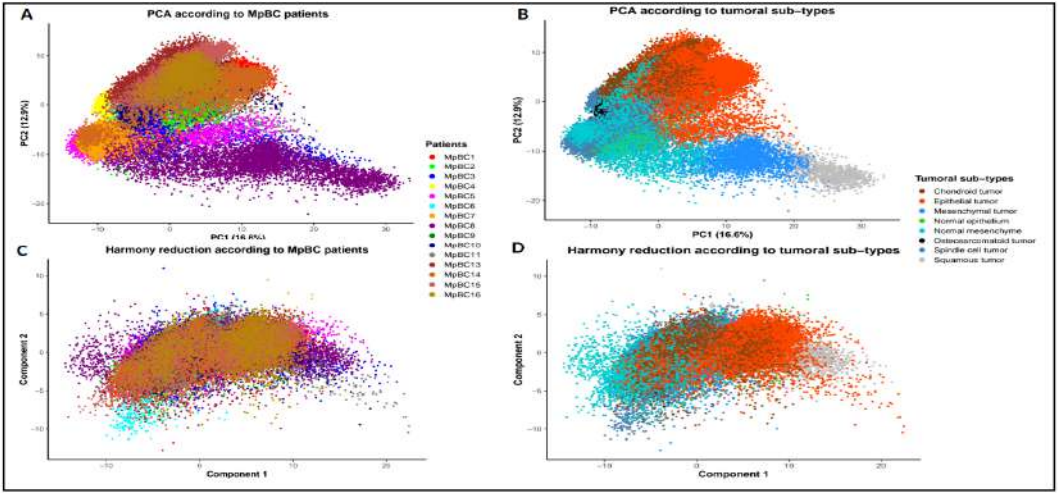


Expert annotations



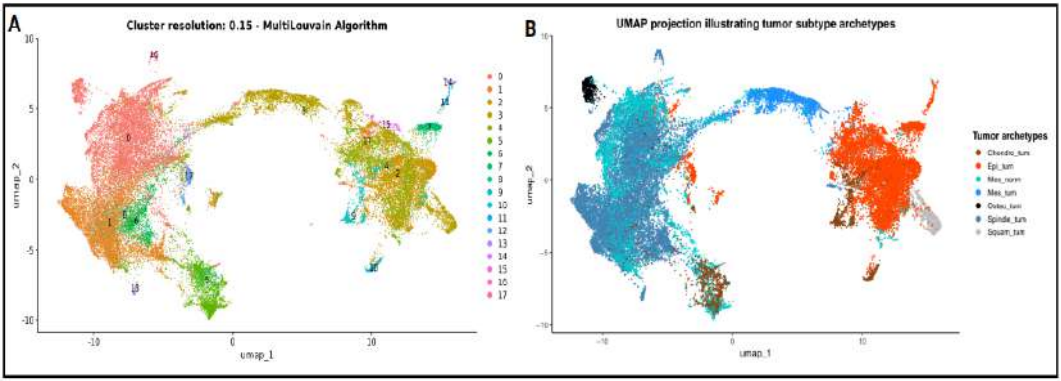
Mixed MpBC with several tumor cell types covered by **Visium spots**, **grouped by k-means clustering** and **annotated by a pathologist**.

Batch effect correction : Harmony



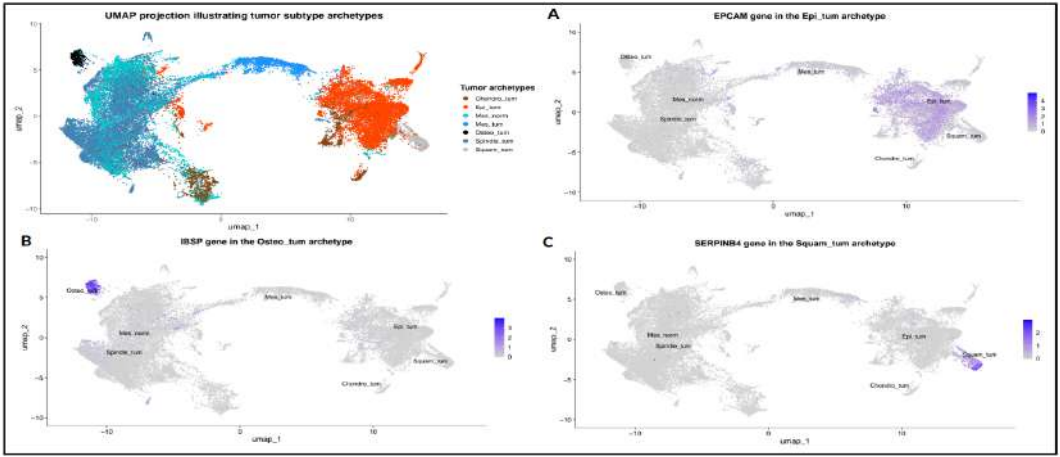
Improve integration with fewer isolated patients

UMAP Projection



Clear epithelial-mesenchymal axis (umap1), with few ambiguous spots

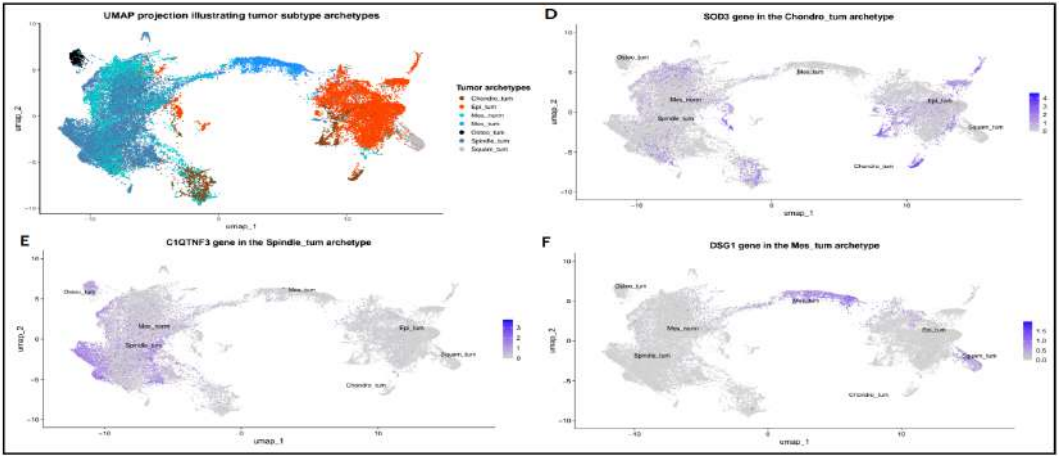
Expression markers



Analysis with **MAST** (Model-based Analysis of Single-cell Transcriptomics)

Some phenotypic markers identified are specific to clusters...

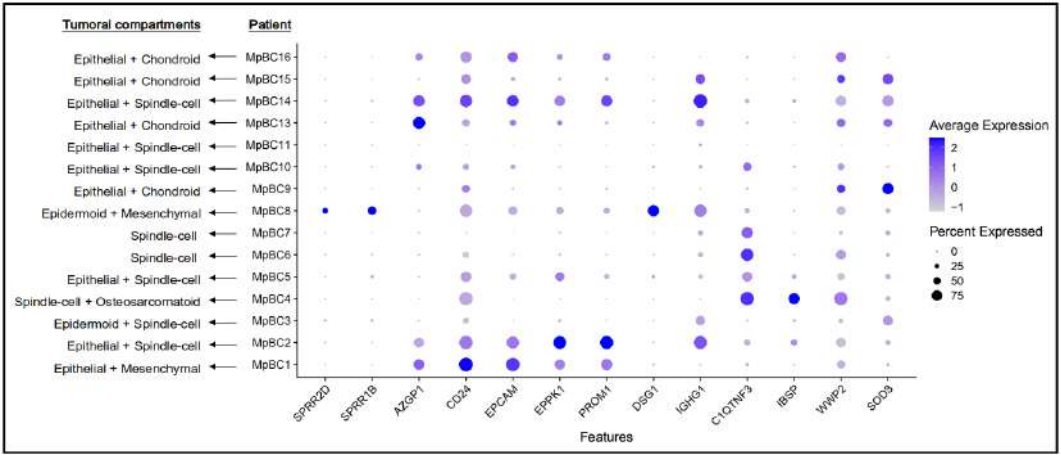
Expression markers



Few phenotypic markers aren't specific enough (Visium limitations)

Different **spot purity** depending on the **subtypes**

Markers specificities



Some cluster-specific markers are also patient-specific markers

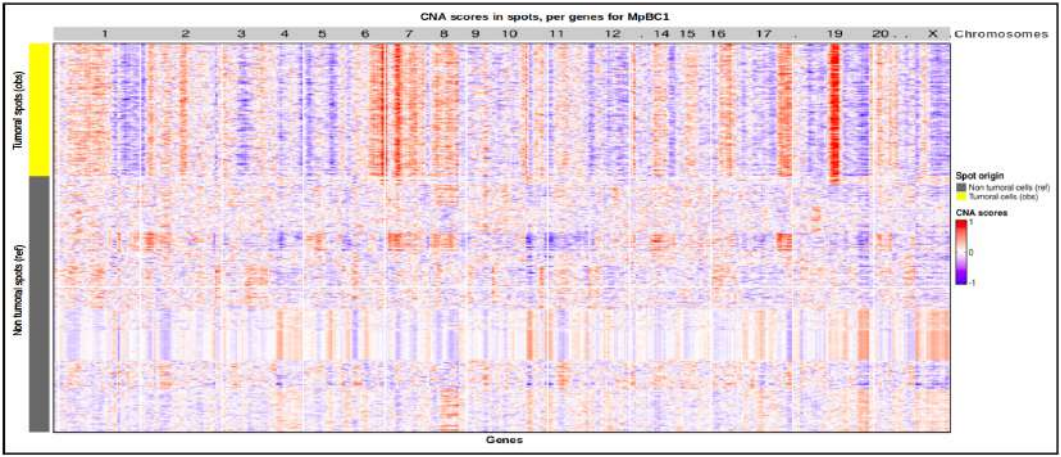
Analysis conclusions

- Analysis reveals **archetype-specific markers**
  - **Epidermoid**
  - **Epithelial**
  - **Osteosarcomatoid**
- Some markers remain **non-specific or not representative of the archetypes**
  - **Spindle-cell**
  - **Chondroid**
  - **Mesenchymal**
- **Spatial transcriptomics** combined with **pathologist annotations** is **promising**, but **still limited** by the **cellular purity of Visium spots**.

CNA in cancer

- **Frequent in cancer:** CNA are a hallmark of cancer cells.
- **Types of alterations:**
  - Can be **focal** (targeting specific genes or regions)
  - Or **broad**, affecting entire cytobands or chromosome arms
- **Functional impact:**
  - CNA can lead to oncogene amplification or tumor suppressor loss
- **Clonal evolution insight:**
  - CNA profiles help reconstruct **evolutionary trajectories**
  - Reveal selection of clones and subclones in tumor

Raw InferCNVPlus results

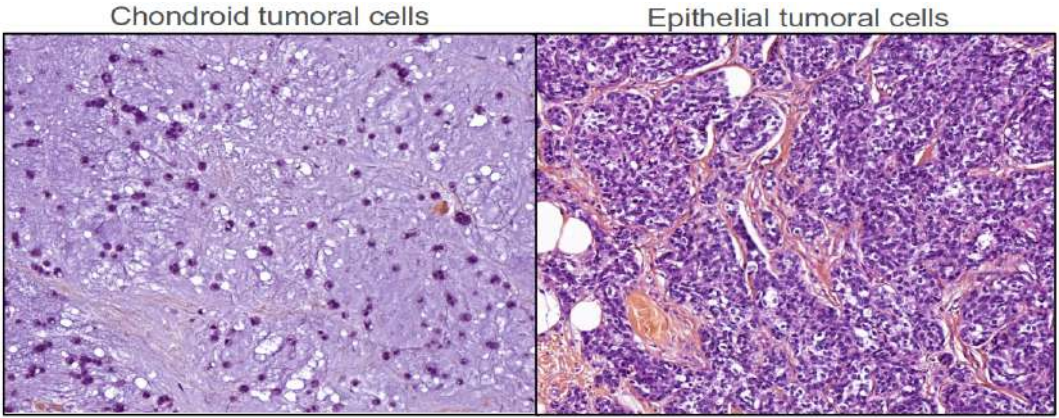


Raw CNA scores in tumoral (obs) or non tumoral (ref) spots for each gene expressed

Heatmap

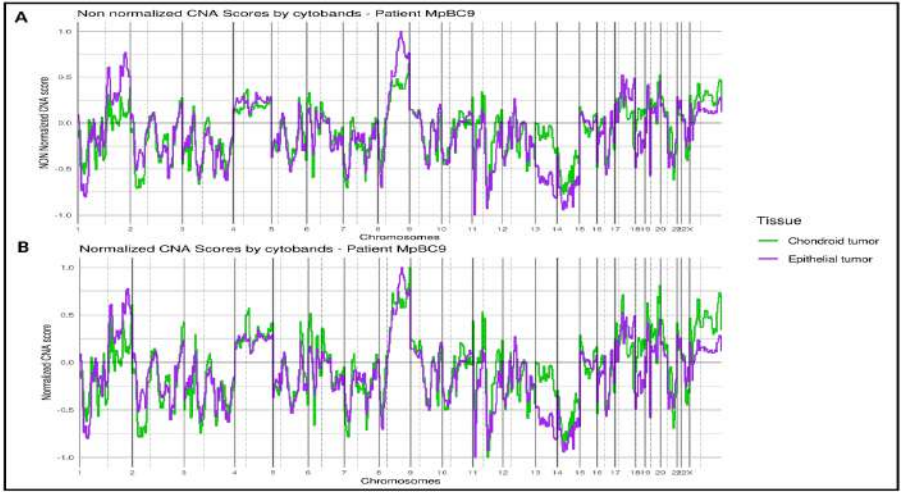
CNA scores in each tumoral compartments for each cytoband

RNA sequencing depth per spot



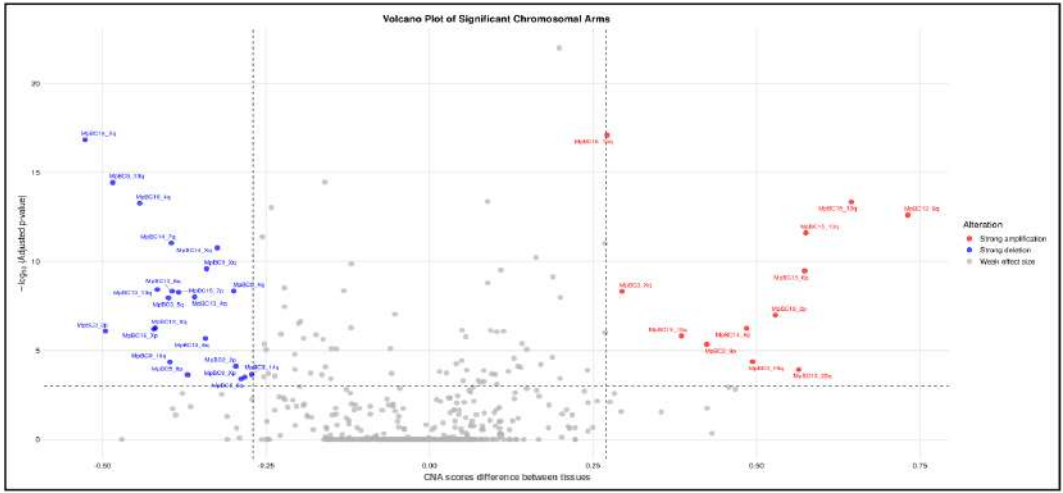
Normalization to correct for differences in RNA sequencing depth across spots

Genomic CNA profil



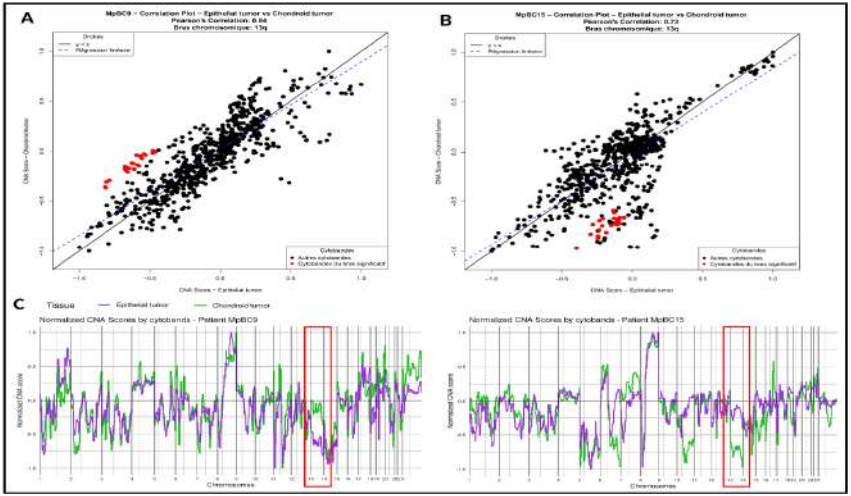
Some genomic regions are higly altered even after the normalisation

VolcanoPlot



Some chromosomic arms significatively altered for few patients

Correlation Plot



Significant cytobands deviate from other cytoband distributions

Analysis Conclusions

- **Limited divergence** in CNA profiles between paired tumor compartments
  - Suggests a **shared clonal origin** for tumor subtypes
- **Rare cases** of chromosomal arm-level divergence between compartments
  - May reflect **subclonal evolution**, where one compartment originates from the other

Conclusion

Main results

- Identified **phenotypic markers** specific to subtypes, but there is still limits (Visium resolutions).
  - Emphasizes the need for single-cell resolution.
- **Limited genomic divergence** suggests a common clonal origin and transdifferentiation in MpBC.

Future Work

1. Perform snRNA-seq on MpBC

- Characterize MpBC subtypes using **more specific molecular markers**.
- Build a **MpBC-specific transcriptomic atlas** for each tumor subtype.

2. Visium Spot Deconvolution

- Apply **spot deconvolution algorithms** (e.g., RCTD) to estimate cell type composition per spot.
- Improve the assignment of transcriptomic profiles to specific tumor subtypes.

Future Work

3. Microdissection of Tumoral Compartments

- Perform exome sequencing to explore **intrinsic molecular determinants**.
- Detect point mutations, driver alterations, and validate CNA findings.
- Conduct epigenetic profiling, including methylome analysis.

4. Long-term Objectives: Clinical Applications

- Discover **subtype-specific molecular markers**.
- Identify **targetable pathways** relevant to MpBC.

Thank you for listening !

Tutor : Dr Pierre Martinez

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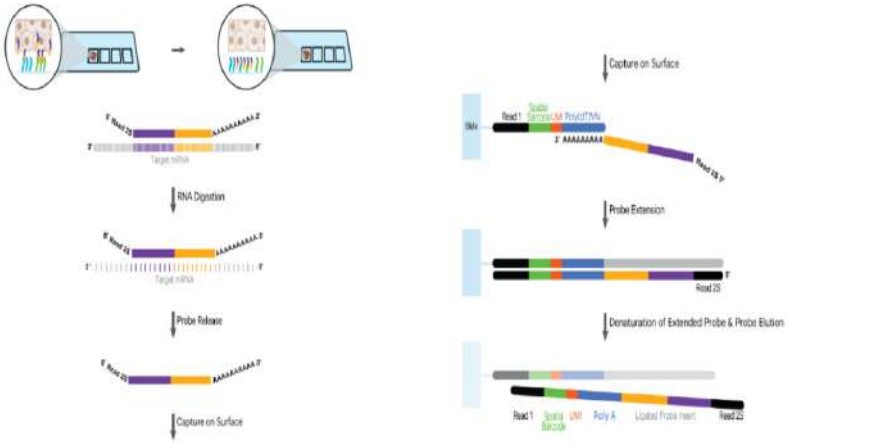
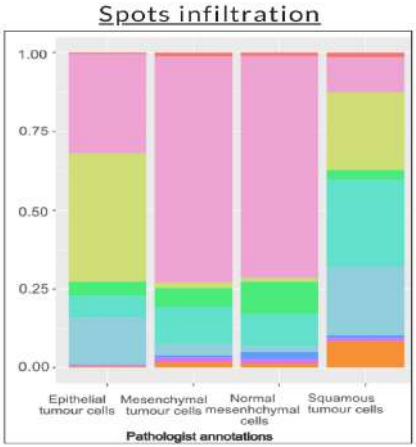
FFPE-Visium workflow



Source: 10x Genomics

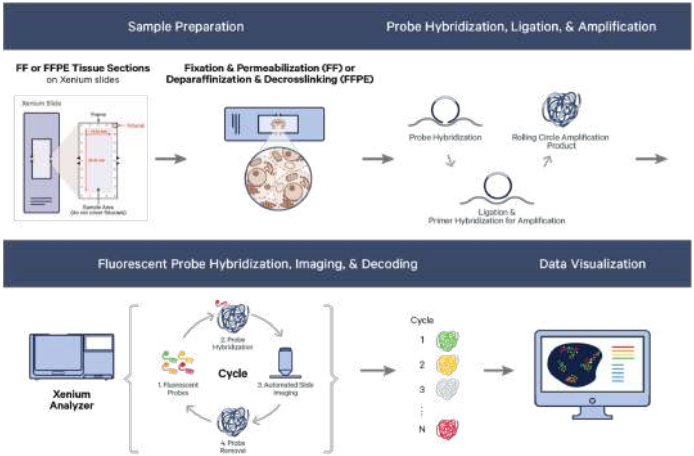
Probe hybridization and ligation to capture RNA in each spots

RCTD deconvolution



Source: Ines Kardous M2 Internship 2024

Xenium



Source: 10x Genomics