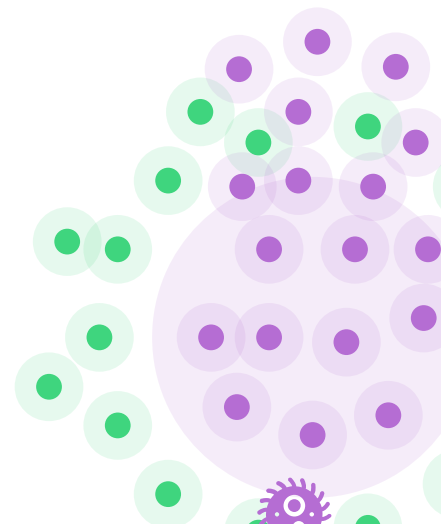# Single-cell RNA-sequencing in cancer research

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Summary

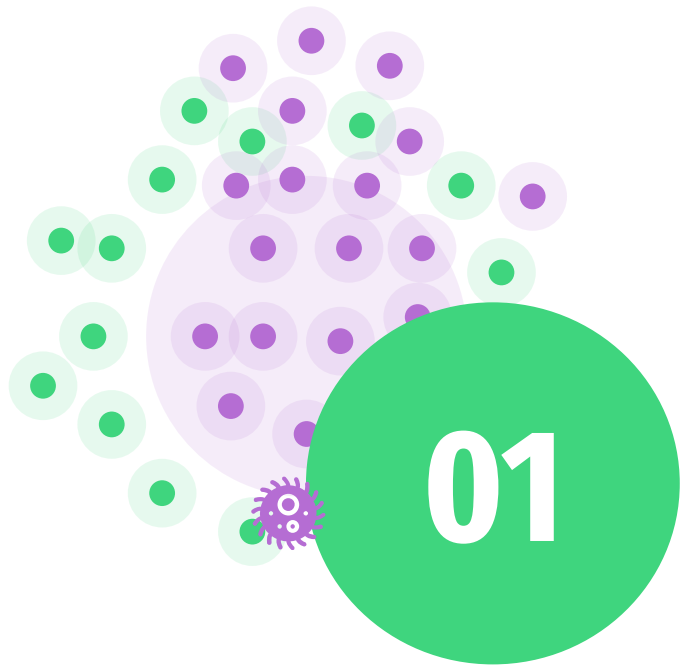|		
01	**Background**	Malignant tumors Overview of scRNA-seq scRNA-seq and tumor research
02	**Tumor heterogeneity**	Tumorigenesis and application scRNA-seq limitations
03	**Immune Escape**	scRNA-seq and immune escape The lack of tumor cell immunogenicity Tumor intercellular comunnication

Letícia Okada

01

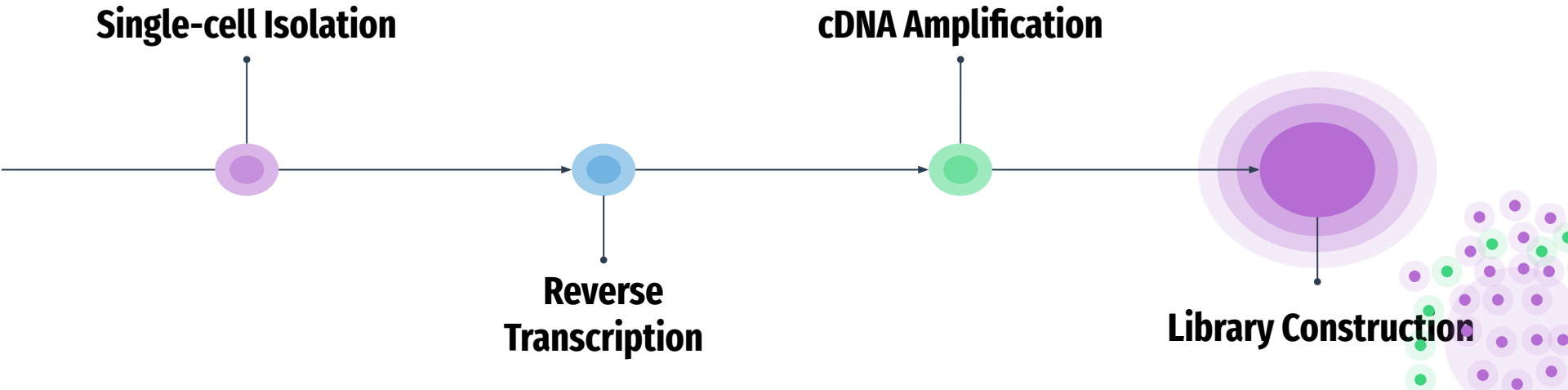# Background

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Malignant tumors

Caused by **genetic mutations**, due to endogenous or environmental factors, leading to **tumor development**.

It is a complex, multi-stage process in which various genetic mutations lead to **changes in DNA**, resulting in **transformation into tumor cells**.

Tumor heterogeneity plays a role in **cancer progression**, resulting in **different phenotypes and behaviors**, which complicates the understanding of cells and the effectiveness of treatment

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scRNA-seq

➔ Single-cell RNA-sequencing is a sequencing technology that allows the **analysis** of the entire **transcriptome** at the **cellular level**, differentiating different cell types

➔ Can identify the **expression of different genes** and **epigenetic factors** caused by mutations

Single-cell Isolation

cDNA Amplification

Reverse Transcription
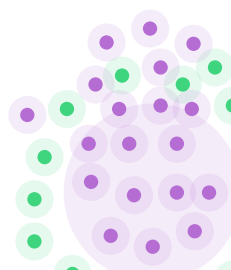
Library Construction

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scRNA-seq and tumor research

scRNA-seq is essential for understanding tumor heterogeneity, identifying **key mutations** and monitoring **tumor progress,** as well as signaling pathways and biological processes involved

Transcriptome analysis of immune cells in tumor tissues assists in classifying immune cells, mechanisms of escape and drug resistance, and developing **specific therapeutic targets**

02
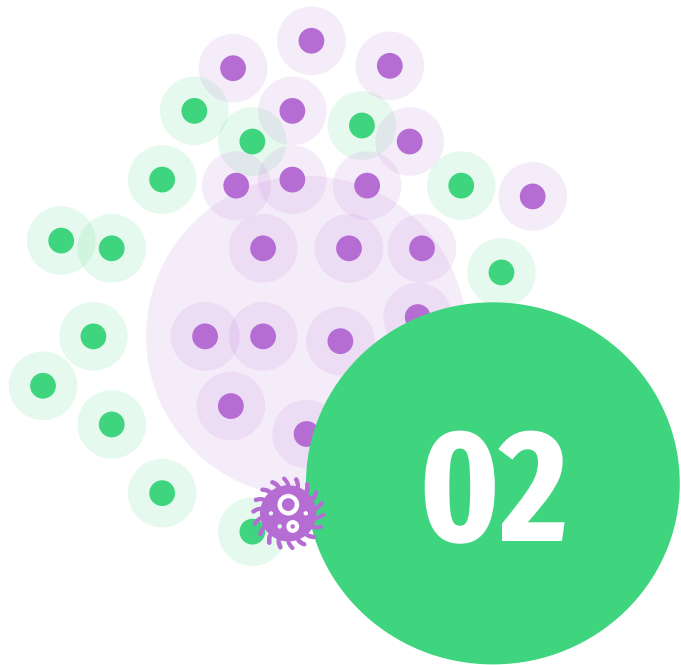
Tumor heterogeneity and development by scRNA-seq
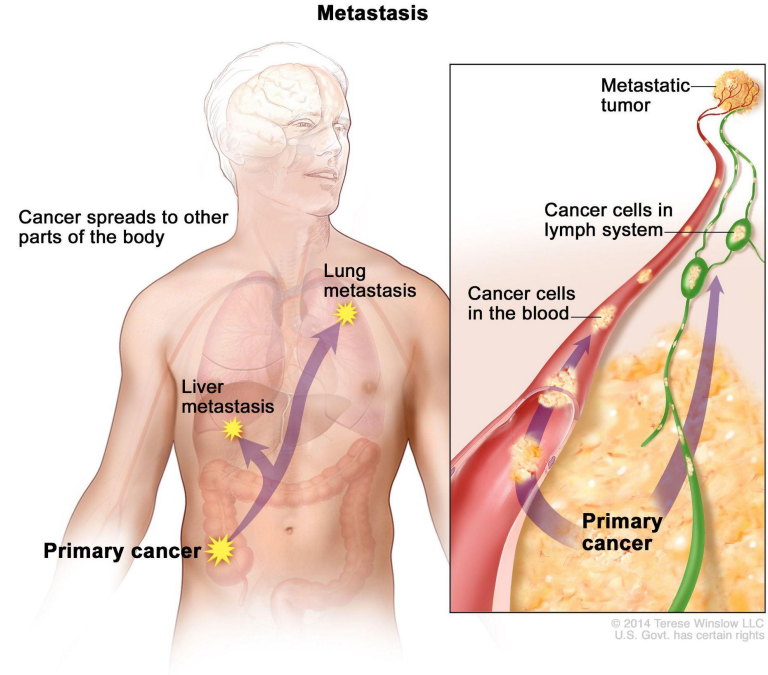
Gustavo Fortunato

Tumorigenesis

➜ Tumorigenesis results from somatic mutation accumulation;

➜ Somatic mutations mainly include gene mutation heterogeneity (base pair replacement, insertion, and deletion);

➜ And genomic instability (chromosomal instability, chromosome rearrangement, copy number variations and microsatellite instability);

➜ scRNA-seq, is used to study the genetic and molecular characteristics of various stages of tumor development.
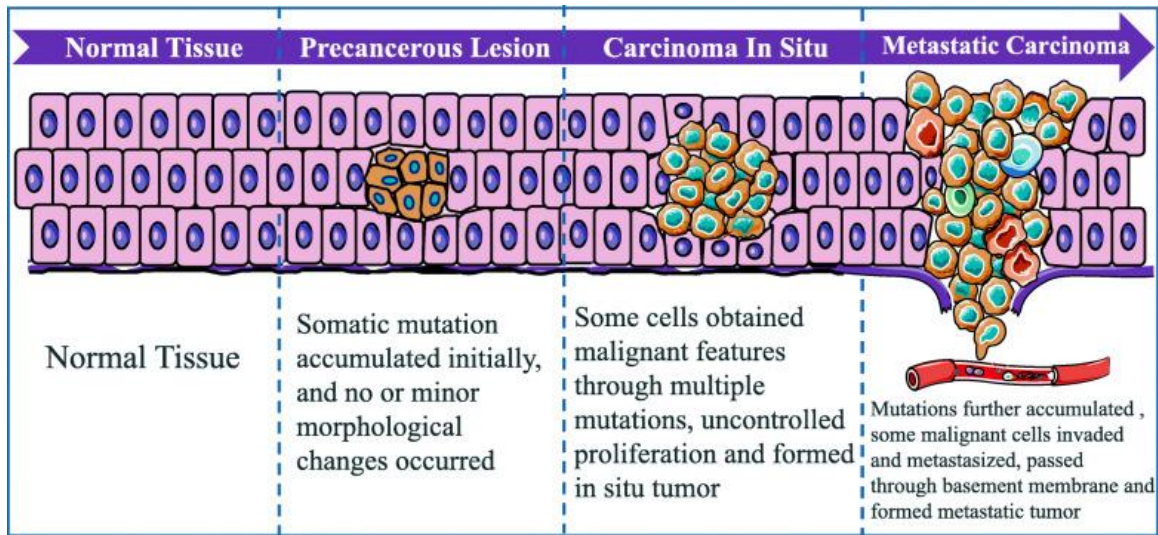

Cancer cells dividing — Normal

Gustavo Fortunato
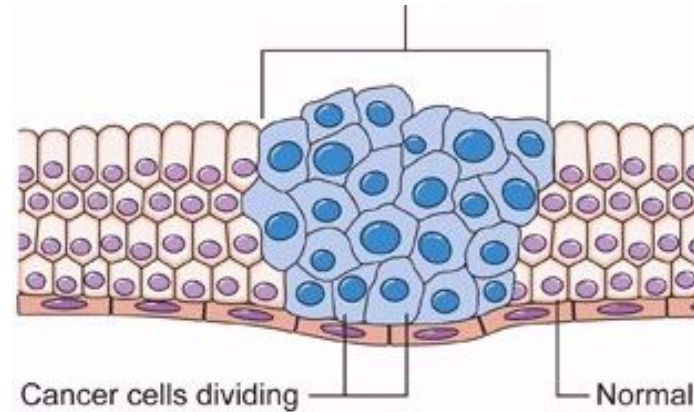
Tumor metastasis

➔ Tumor metastasis is one of the leading causes of death;

➔ Compared the gene expression profiles of metastatic and primary tumors, the second has differential genes expression;

➔ scRNA-seq can provide comprehensive information about process of primary tumor metastasis.

Credit: National Cancer Institute.

Application of scRNA-seq in tumorigenesis

Credit Zhang, Yijie et al. "Single-cell RNA sequencing in cancer research."。

Gustavo Fortunato

Cancer stem cell (CSC)

➔ CSCs can divide and expand the pool of CSCs and differentiate into heterogeneous non-tumorigenic cancer cell types;

➔ CSCs are thought to have the potential to drive tumor growth and promote disease progression, and are associated with distant metastasis and resistance to treatment;

➔ It is necessary to focus sequencing analyses on these cells to prevent tumor growth.

Credit: PromoCell.
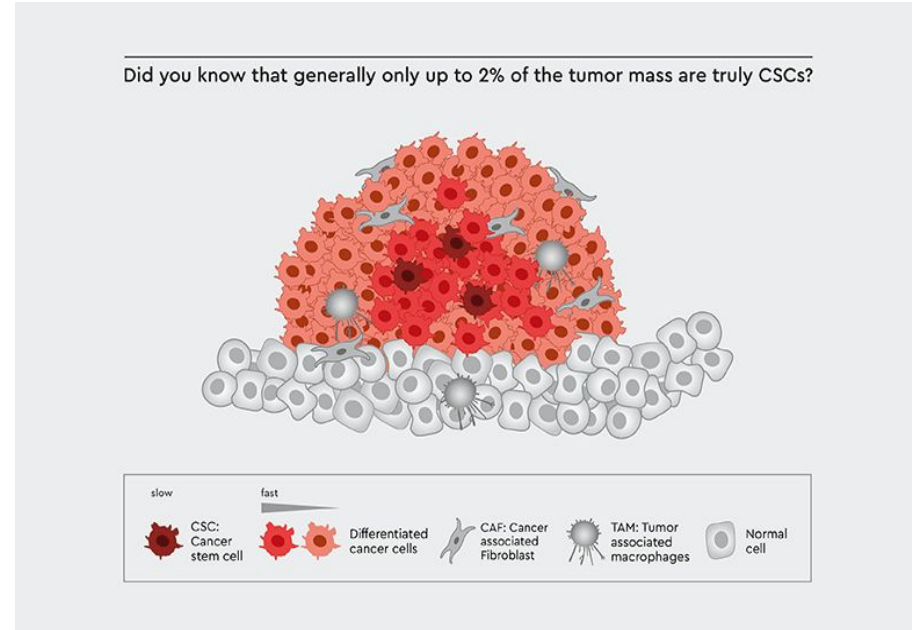
scRNA-seq limitations

➔ Single cell RNA sequencing has limitations in detecting somatic mutations and tumor heterogeneity.

01 **Low sensitivity of low-abundance transcripts**

02 **High level of noise in scRNA-seq data**

03 **Insufficient sequencing depth at single-cell level**

03

scRNA-seq and immune escape

Beatriz Oliveira

Beatriz Oliveira
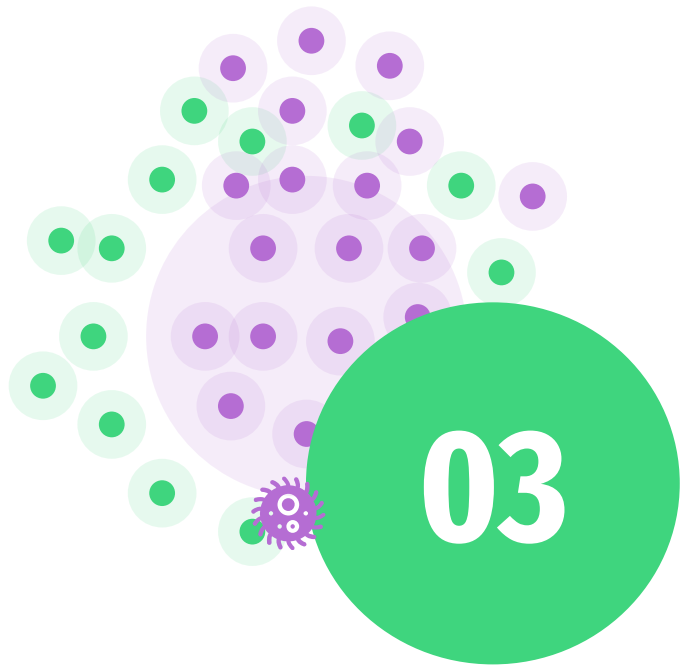
scRNA-seq and immune escape

➔ CTLA-4 and PD-1: receptors associated with T cell regulation

01 **Prevention of autoimmunity**

02 **Regulation of immune responses to microorganisms**

Tumors: ⬆ CTLA-4 and PD-1

⬇ T response

Célula cancerosa

CTLA-4

Treg

PD-L1

PD-1

Linfócitos T citotóxicos

CTLA-4

B7

Células dendríticas

Beatriz Oliveira
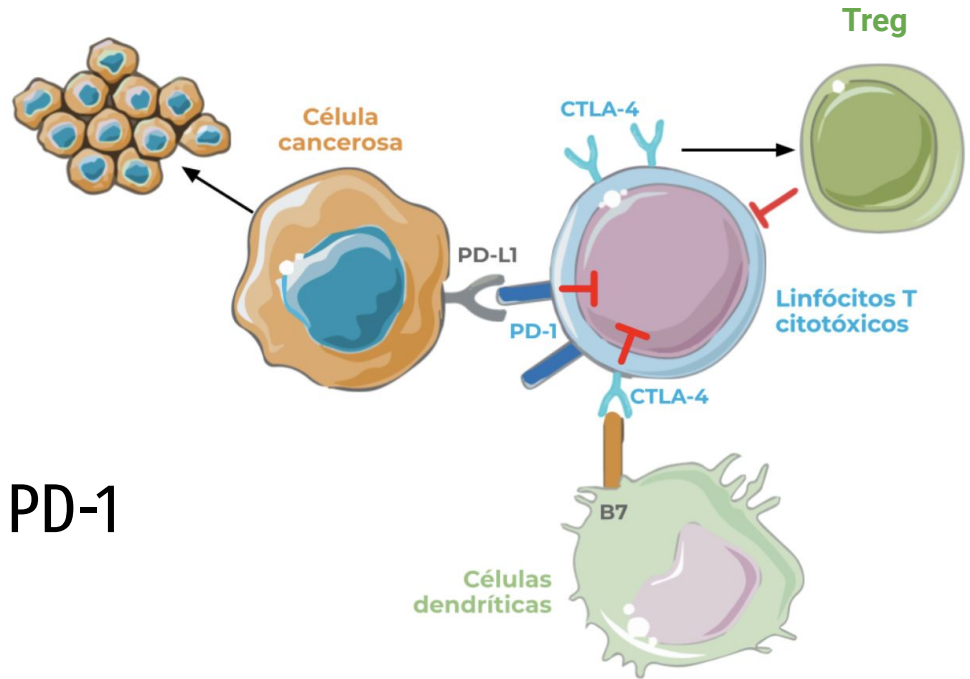
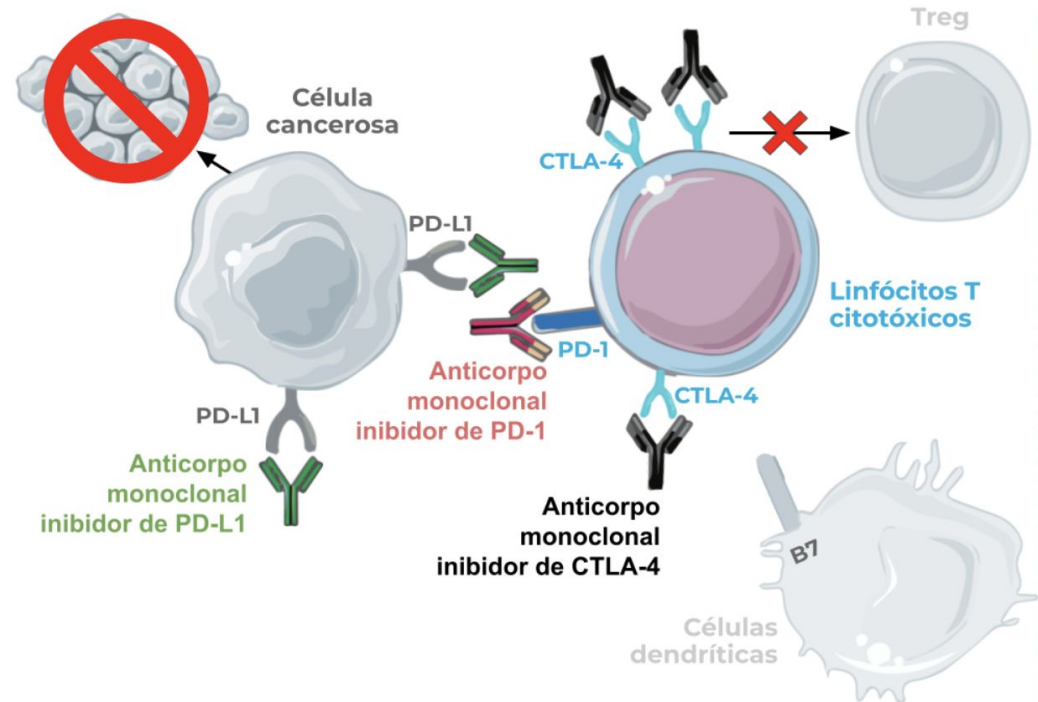# Immune Checkpoint Inhibitor Therapy

➔ CTLA-4 and PD-1: receptors associated with T cell regulation

ICI: **Inhibition of the expression of these receptors**

➔ Intensification of tumor killing by T cells
➔ Containment of the progression of advanced cancers

Role of scRNA-seq
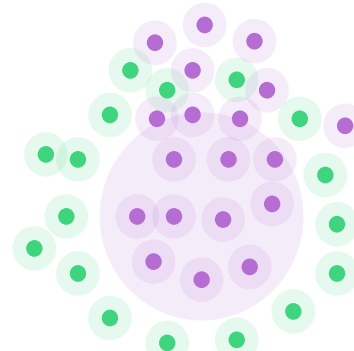
➜ In this context, scRNA-seq can be used:

A

To analyze the **heterogeneity of** immune-related genes in **tumor tissues** and to design a **combination therapy** targeted at a variety of tumor antigens

B

For tumor **patients who are not sensitive to** anti-CTLA-4 and anti-PD-1/PD-L1 **treatment**, to analyze specific tumor cells and **discover the mechanism of drug resistance**

The lack of tumor cell immunogenicity

➜ High multiplication levels
➜ Genetic and epigenetic changes
➜ Immunogenic proteins that were expressed are no longer expressed
➜ New proteins may or may not generate an immune response

Failure to produce tumor antigen

Antigen-loss variant of tumor cell

Lack of T cell recognition of tumor
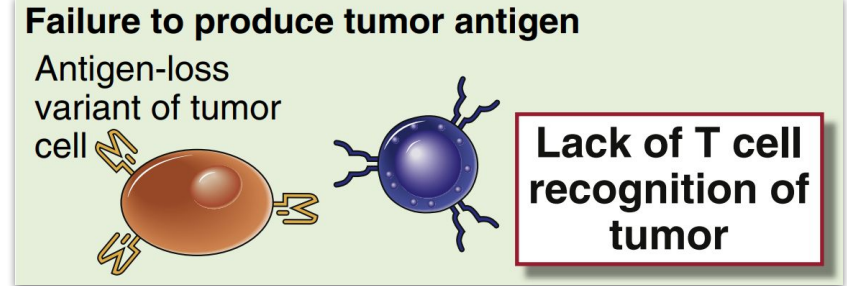
ABBAS, Abul K.; PILLAI, Shiv; LICHTMAN, Andrew H. **Imunologia** celular e molecular.

. . . . .

NGS scans regions and combines them with MHC features to predict antigens that can be used for treatment.

. . . . .

Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing

Genes with high expression that could be used as targets in combined immunotherapy for tumor adenocarcinoma
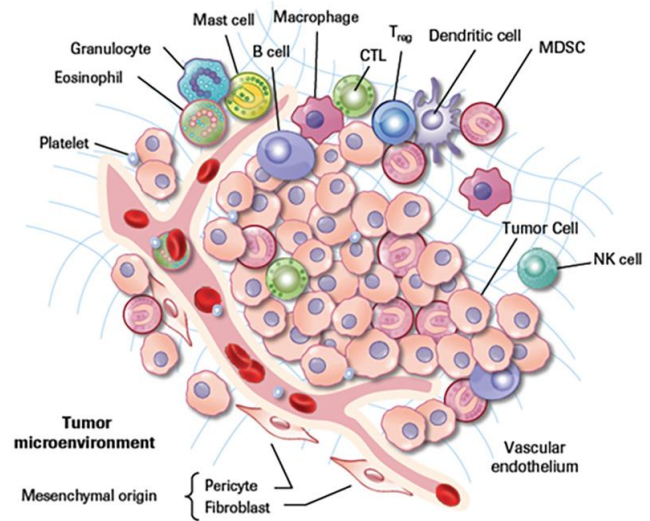
Beatriz Oliveira

Beatriz Oliveira

Tumor intercellular communication and scRNA-seq

➔ Interaction of different cell types: malignant, immune and stromal

> The cellular composition and the interaction between these components play a fundamental role in the development of cancer

scRNA-seq can be used to:

- to characterize the abundance and functional status of tumor cell types;
- to detect cell-cell communication;
- to analyze the relationship between cells in different individuals;
- to study the pathophysiological characteristics of tumor microenvironment, so as to predict the development of tumor and prognosis

Conclusions

scRNA-seq

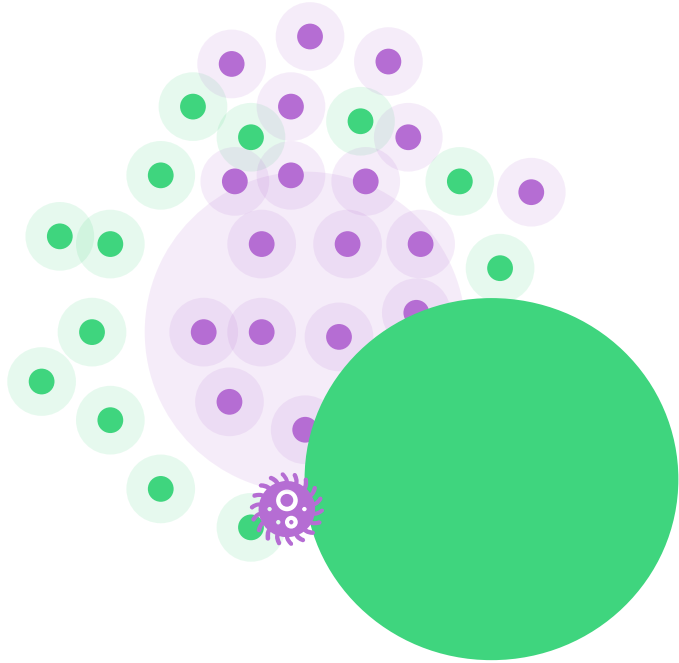**01** Contributes to the **construction of transcriptomic databases**, to understand tumor biology and cellular heterogeneity.

02 It allows the verification of the **molecular mechanisms of oncogenesis,** identification of genetic mutations in somatic cells and assessment of the risk of tumor susceptibility.

04 It offers valuable insights for the development personalized immunotherapies, improving the effectiveness of **clinical treatment and disease prognosis.**

03 The combination of scRNA-seq with other omics technologies expands its applications in **epigenetics and proteomics, boosting cancer research.**

Beatriz Oliveira

Obrigado!