Conquering Cancer Seminar:
Analysis of the initiation and progression of BRCA-mediated ovarian tumorigenesis at the single-cell level

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Introduction
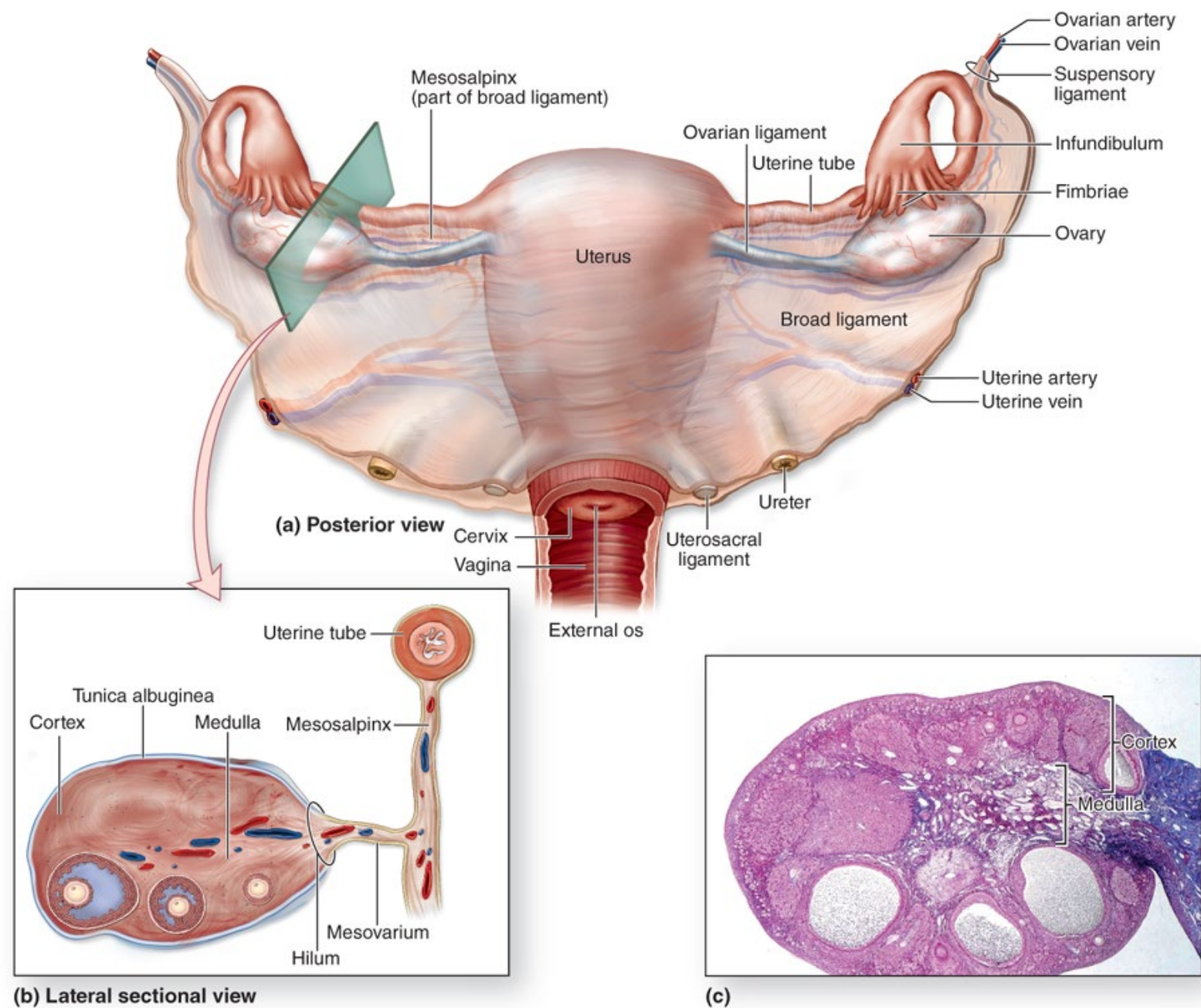
High grade serous ovarian cancer (HGSOC)

- HGSOC most common ovarian cancer, and most lethal Gyn cancer
  - **Presents at late stage and poor prognosis** – 5 yr survival ~ 35%; median survival ~3.5 years
  - No screening/early detection
  - **BRCA1/2**: 20-50% lifetime risk of epithelial ovarian cancer vs 1.8 % lifetime risk in the general population
    - 20% of HGSOC tumors have BRCA mutation
      - 13-15% = germline mut BRCA1/BRCA2, 7% somatic BRCA mut

- Knowledge gap
  - Lack knowledge about disease initiation/early stages
    - Current knowledge is based on whole tissue genomic data
    - Lack of information about cell-cell interaction/microenvironment
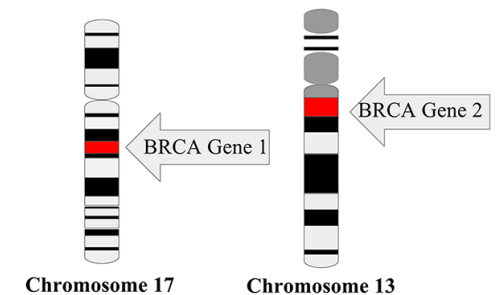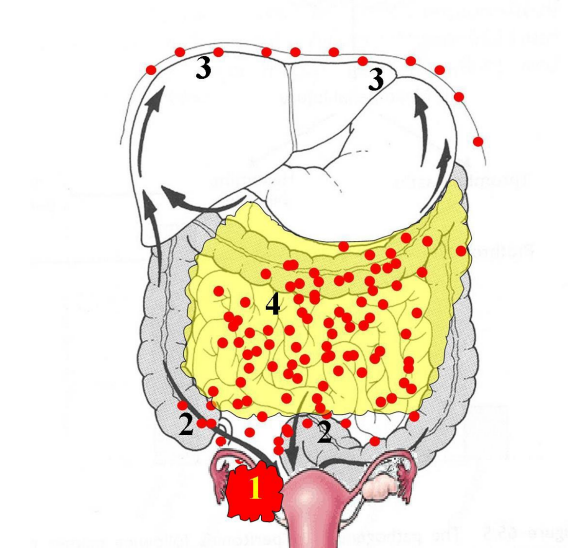
Background: Ovarian Histology and Physiology

- **Cortex**
  - Surface epithelium
  - Oocytes
  - Stromal cells
- **Medulla**
  - Stromal cells, fibroblasts, vasculature
- **Cell types**
  - Epithelial cells
  - Endothelial, fibroblasts
  - Immune cells
  - Oocytes
  - Granulosa cells
  - Theca cells
- Function:
  - Harbors/ produces oocytes and sex hormones

(a) Posterior view

Labels: Mesosalpinx (part of broad ligament), Ovarian artery, Ovarian vein, Suspensory ligament, Ovarian ligament, Infundibulum, Uterine tube, Fimbriae, Ovary, Uterus, Broad ligament, Uterine artery, Uterine vein, Ureter, Cervix, Uterosacral ligament, Vagina, External os

(b) Lateral sectional view

Labels: Uterine tube, Tunica albuginea, Cortex, Medulla, Mesosalpinx, Mesovarium, Hilum

(c)

Labels: Cortex, Medulla

OVARIAN EPITHELIAL CANCER
- High-Grade Serous Carcinomas
- Low-Grade Serous Carcinomas
- Clear cell carcinoma
- Endometrioid
- Mucinous

OVARIAN SEX CHORD-STROMAL TUMORS
Stromal tumors
1. Fibroma
2. Thecoma
3. Fibrosarcoma
4. Leydig cell tumor
5. Steroid cell tumor
6. Sclerosing stromal tumor

Sex chord tumors
1. Adult granulosa cell tumor
2. Juvenile granulosa tumor
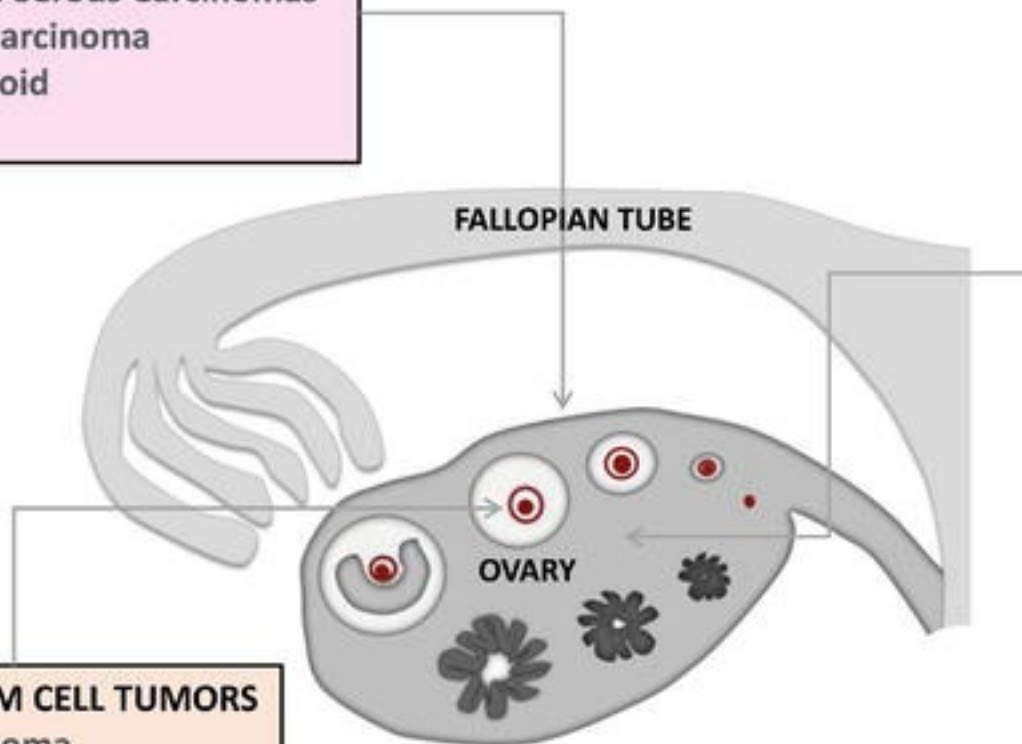3. Sertoli cell tumor
4. Sex chord tumor with annular tubules

Mixed sex chord-stromal tumors
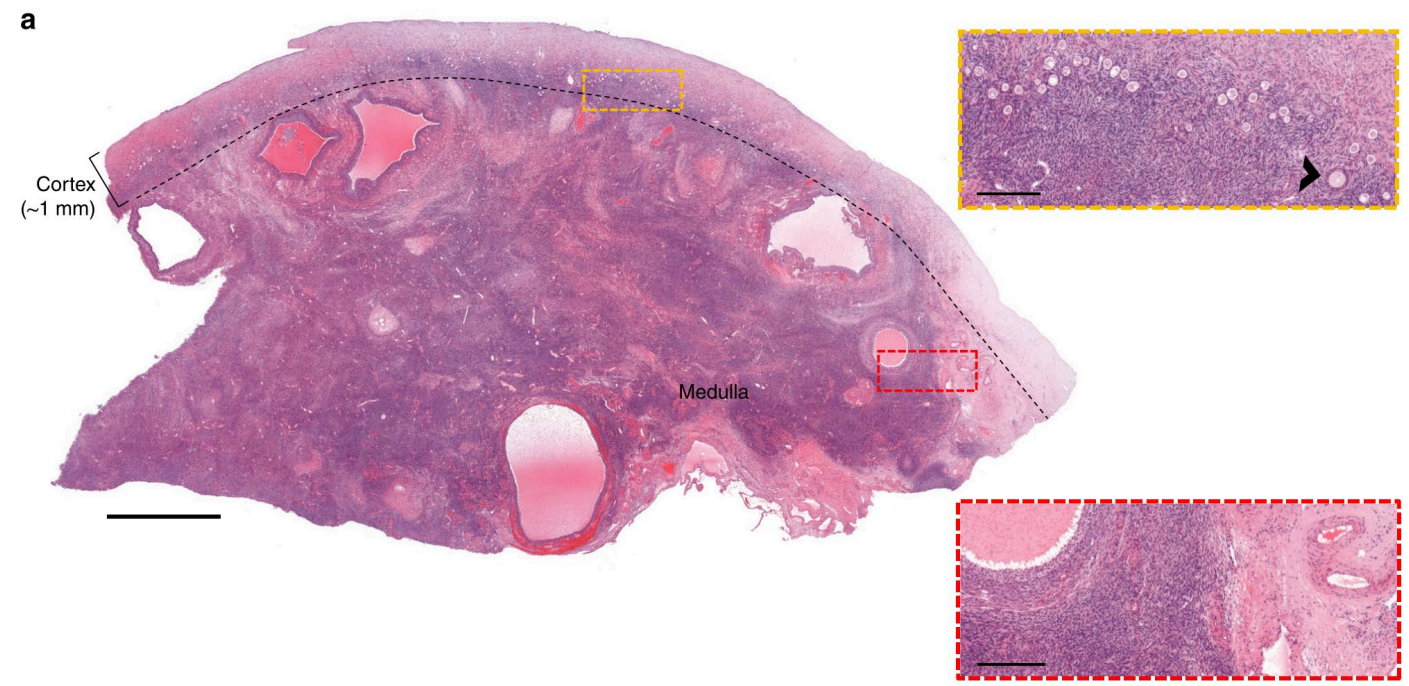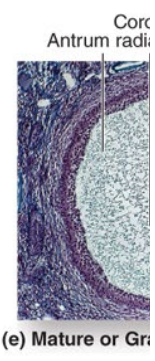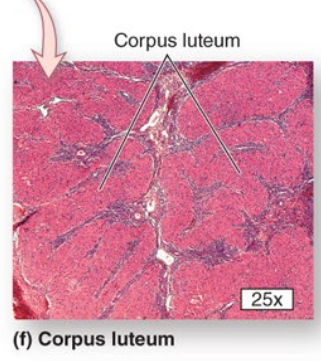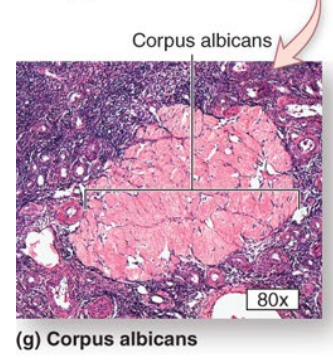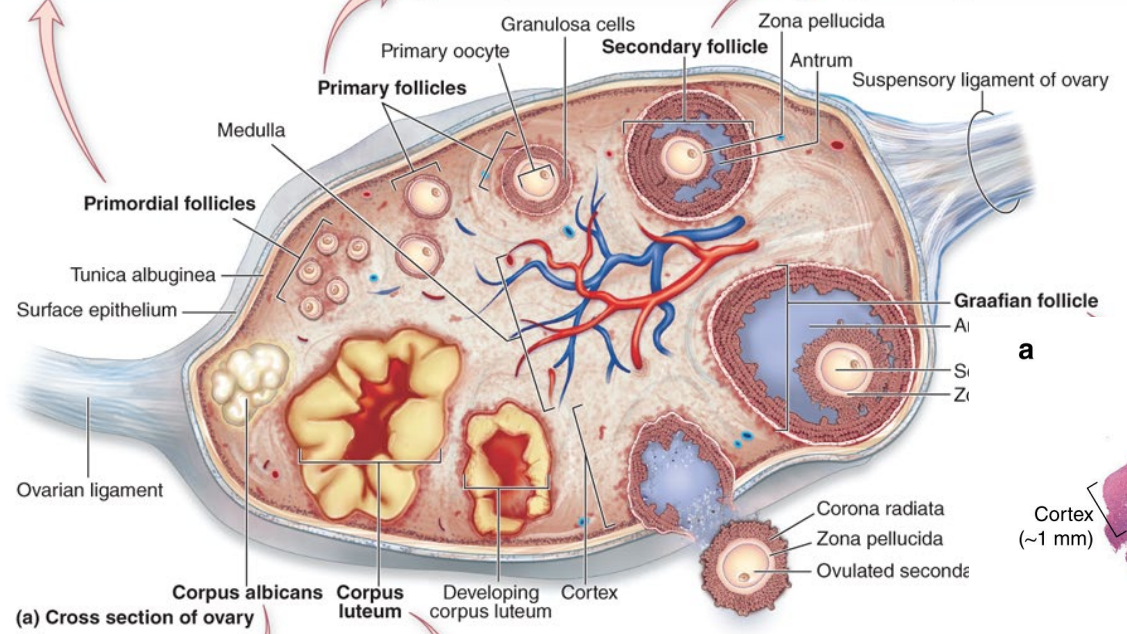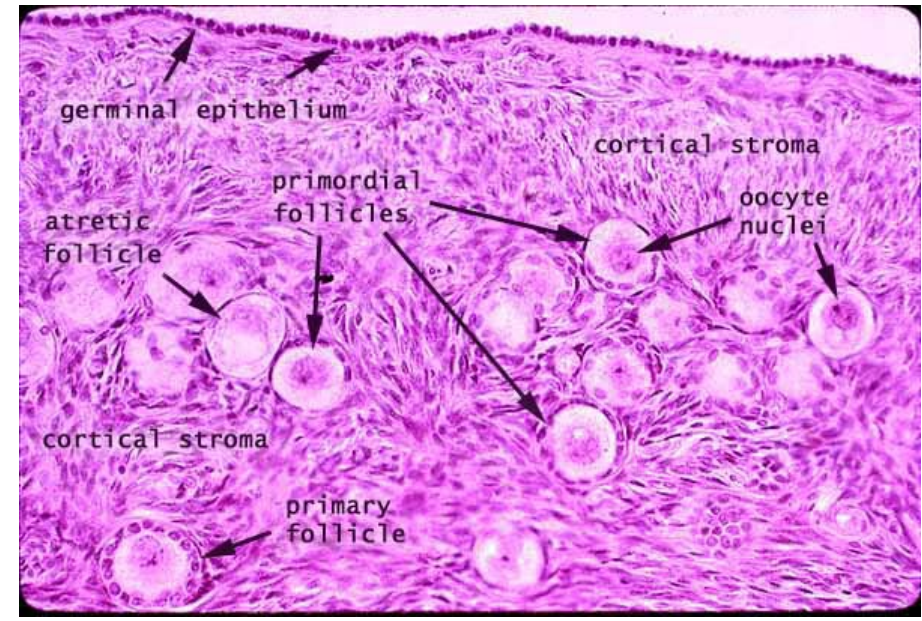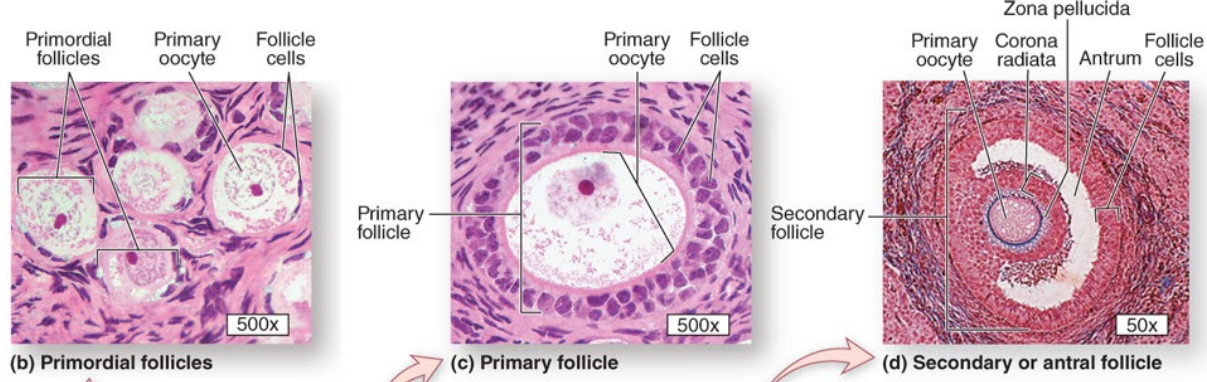1. Sertoli-Leydig cell tumor

FALLOPIAN TUBE

OVARY

OVARIAN GERM CELL TUMORS
1. Dysgerminoma
2. Immature teratoma
3. Yolk sac tumors
4. Mixed germ cell tumors

SMALL CELL CARCINOMA OF THE OVARY
1. SCCO - hypercalcemic type
2. SCCO - pulmonary type

(b) Primordial follicles
Primordial follicles — Primary oocyte — Follicle cells — 500x

(c) Primary follicle
Primary oocyte — Follicle cells — Primary follicle — 500x

(d) Secondary or antral follicle
Zona pellucida — Primary oocyte — Corona radiata — Antrum — Follicle cells — Secondary follicle — 50x

(a) Cross section of ovary
Primordial follicles — Medulla — Primary oocyte — Primary follicles — Granulosa cells — Secondary follicle — Zona pellucida — Antrum — Suspensory ligament of ovary — Tunica albuginea — Surface epithelium — Primordial follicles — Graafian follicle — Ovarian ligament — Corpus albicans — Corpus luteum — Developing corpus luteum — Cortex — Corona radiata — Zona pellucida — Ovulated seconda

(g) Corpus albicans
Corpus albicans — 80x

(f) Corpus luteum
Corpus luteum — 25x

(e) Mature or Gr
Coro — Antrum radia

Source: Anthony L. Mescher:
Junqueira's Basic Histology Text and Atlas, 16e
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germinal epithelium — cortical stroma — atretic follicle — primordial follicles — oocyte nuclei — cortical stroma — primary follicle

a

Cortex (~1 mm) — Medulla

Background: **Pathophysiology**

- Development of ovarian cancer
    - **Genomic instability**, TP53
    - **Incessant ovulation**:
        - Recurrent ovulation as a transforming event → damage to epithelium
    - **Primary site of origin? + inclusion cyst**:
        - extra-ovarian epithelia (endometrium or fallopian tube epithelium)
            - endometriosis or fallopian tube epithelium seeded on the ovary as inclusion cysts
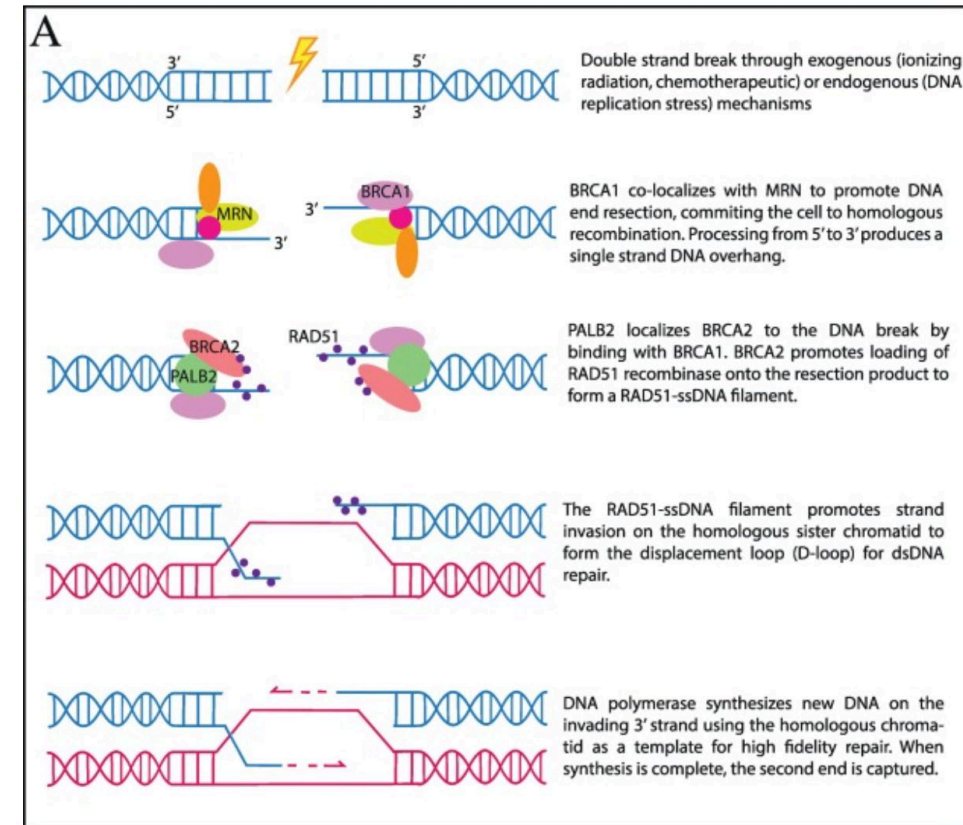   - transformed epithelial cells seed the ovary and its microenvironment → neoplasia
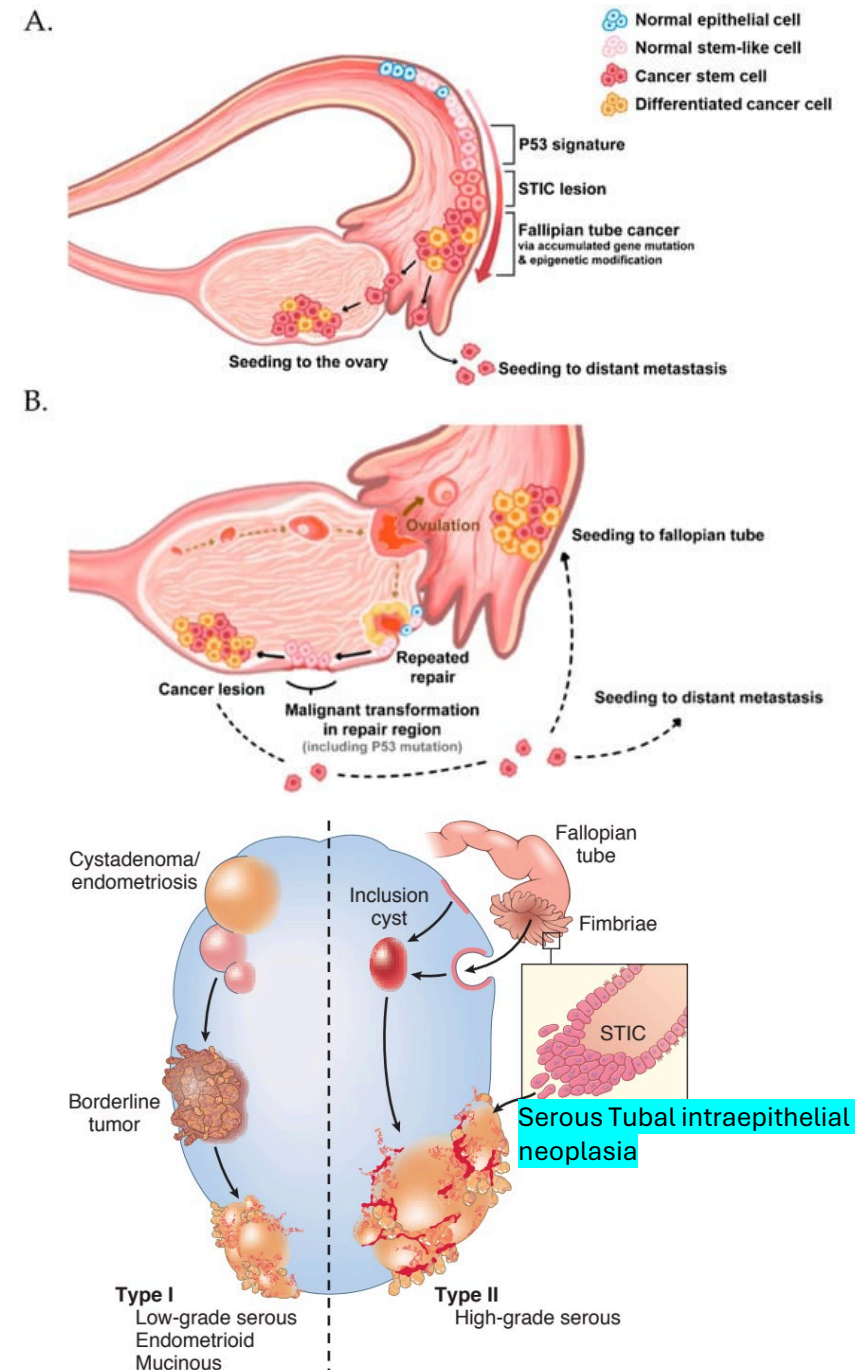
Genetic susceptibility: BRCA mutation / HRD

= ovarian cancer susceptibility genes

BRCA1 = 50% OC risk

BRCA2 = 20% OC risk

- BRCA1 and BRCA2 = proteins involved in homologous recombination DNA repair

  o Homologous recombination (HR) is one method to repair double-strand DNA breaks (DSB)

- 50% of tumors possess mutations in HR genes = homologous recombination deficiency or HRD, which includes the BRCA genes

A

Double strand break through exogenous (ionizing radiation, chemotherapeutic) or endogenous (DNA replication stress) mechanisms

BRCA1 co-localizes with MRN to promote DNA end resection, commiting the cell to homologous recombination. Processing from 5' to 3' produces a single strand DNA overhang.

PALB2 localizes BRCA2 to the DNA break by binding with BRCA1. BRCA2 promotes loading of RAD51 recombinase onto the resection product to form a RAD51-ssDNA filament.

The RAD51-ssDNA filament promotes strand invasion on the homologous sister chromatid to form the displacement loop (D-loop) for dsDNA repair.

DNA polymerase synthesizes new DNA on the invading 3' strand using the homologous chromatid as a template for high fidelity repair. When synthesis is complete, the second end is captured.

Background:
Treatment and clinical course/prognosis

- **Treatment**
  - Cytoreductive surgery
  - Carboplatin/Paclitaxel +/- Bevacizumab – 6 cycles, ? Maintenance therapy
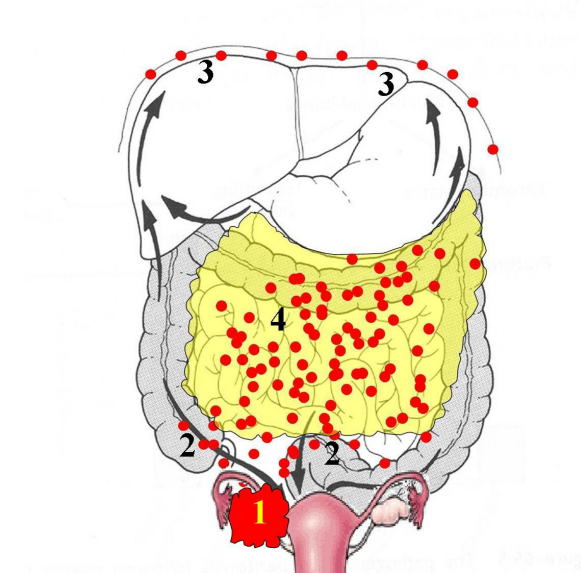
- **Clinical course/Prognosis**
  - 75% present at stage 3+
  - 65% recurrence within 2 years, 75% at 3 years
  - 5-year cause-specific survival: stage 3 (42%) stage 4 (26%)
  - Fast growth of tumor nodules encasing organs → ascites, carcinomatosis, bowel obstruction

- **Improvements**
  - 5-10% improvement in survival rate over 30 years
  - Maintenance therapies

- **Still NO screening or early detection**

Aim / hypothesis

- Identify cell populations, RNA expression patterns and cell/cell interactions within the ovary that lead to changes in epithelial cells that can cause ovarian cancer

- We hypothesize that BRCA+/mut carriers exhibit alterations in ovarian epithelial and stromal cell populations including changes in genetic expression, concentrations, and cellular interactions, that promote epithelial cell changes leading to cancer initiation.
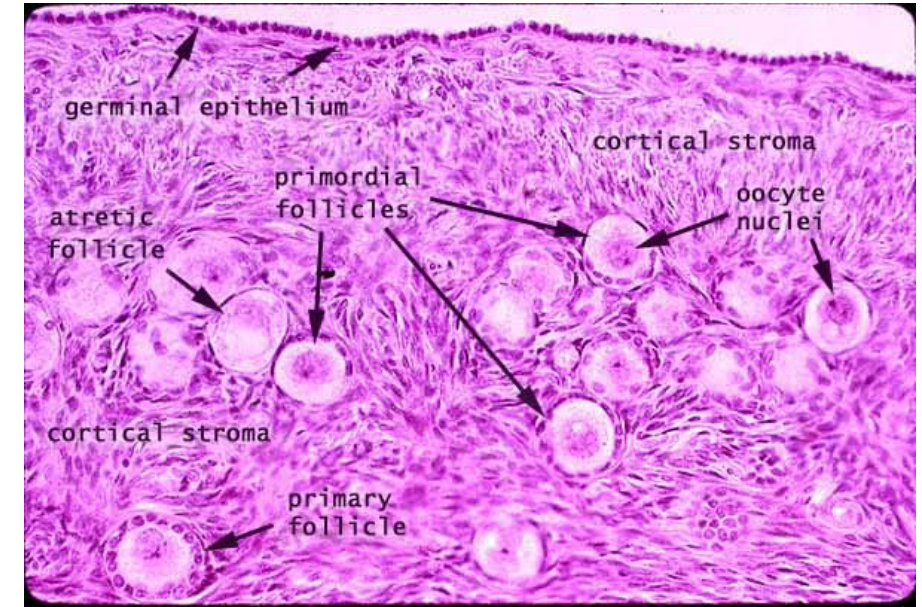
- Translational discovery:
  - These cell populations and RNA expression pattern changes could identify potential:
    - Precursor lesions/cell types
    - Biomarkers for pre-cancerous lesions and prevention techniques
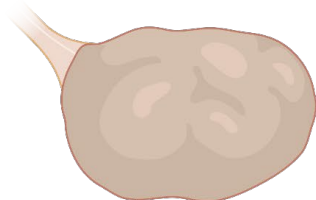   - Therapeutic targets
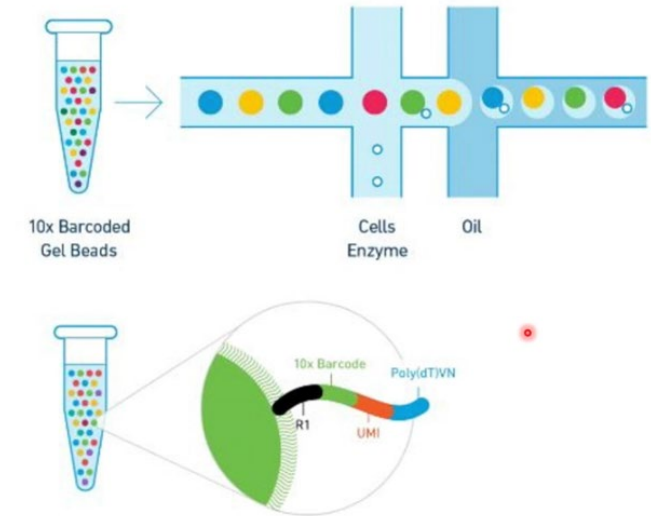
Methods

- **Tissue procurement**
  - FFPE archived oophorectomy tissue
    - **Benign oophorectomy** **BRCA** **wildtype**
    - **Pre-neoplastic/prophylactic** **BRCA** **mut**
    - **Ovarian Tumor** **BRCA** **mut**
    - **Met Tumor** **BRCA** **mut**

- **Single-cell RNA sequencing**

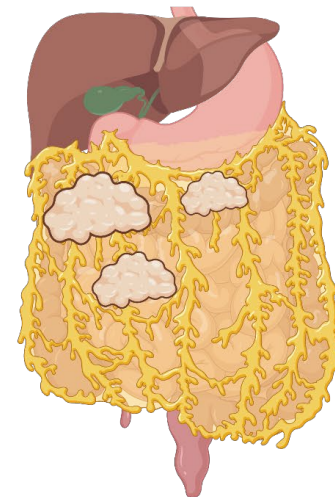- **Spatial omics and multiplexed imaging**

Normal Ovary (control)
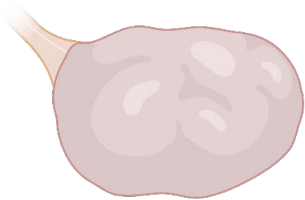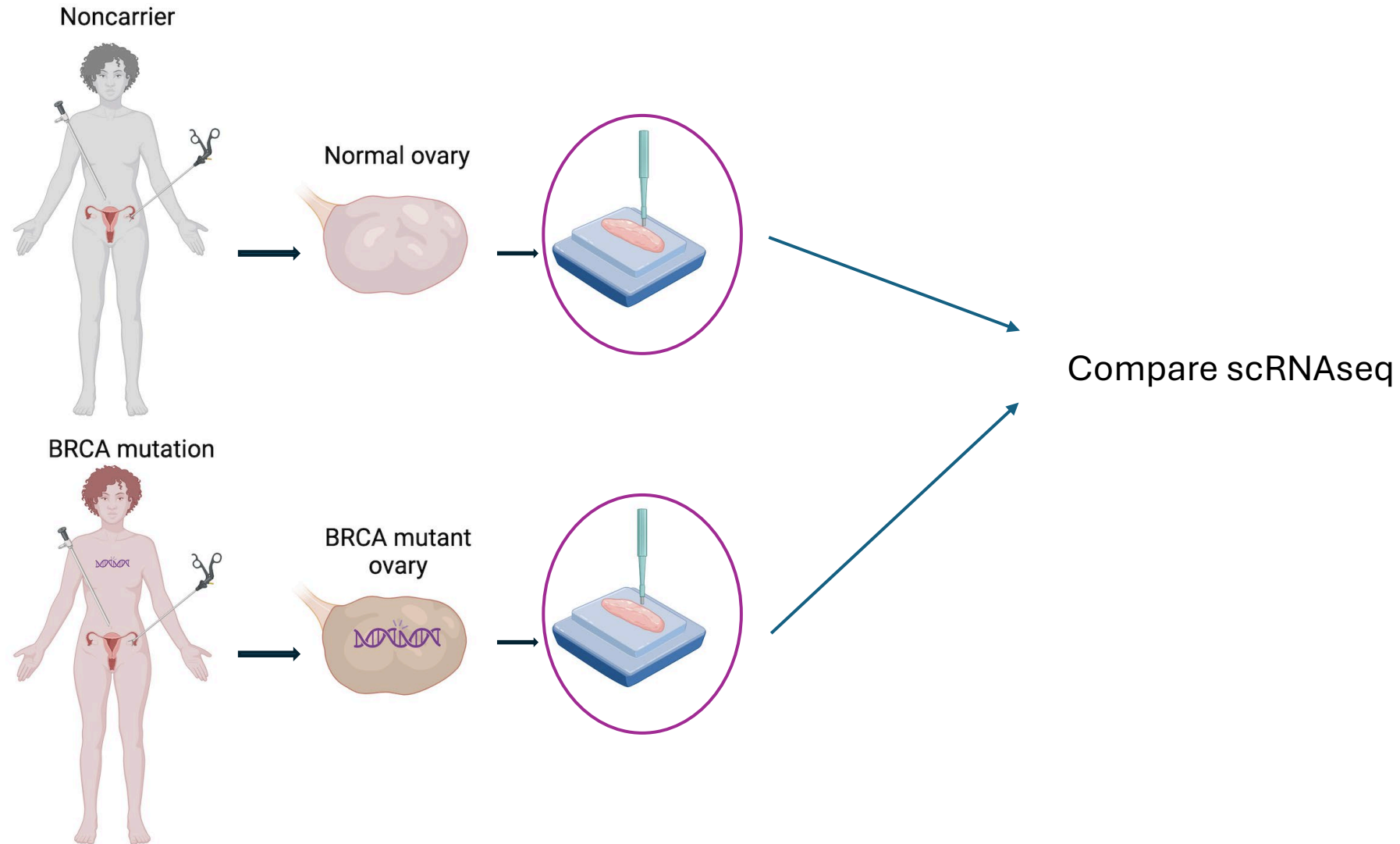
Pre-neoplastic BRCA+/mut ovary

Ovarian BRCA+/ mut tumor
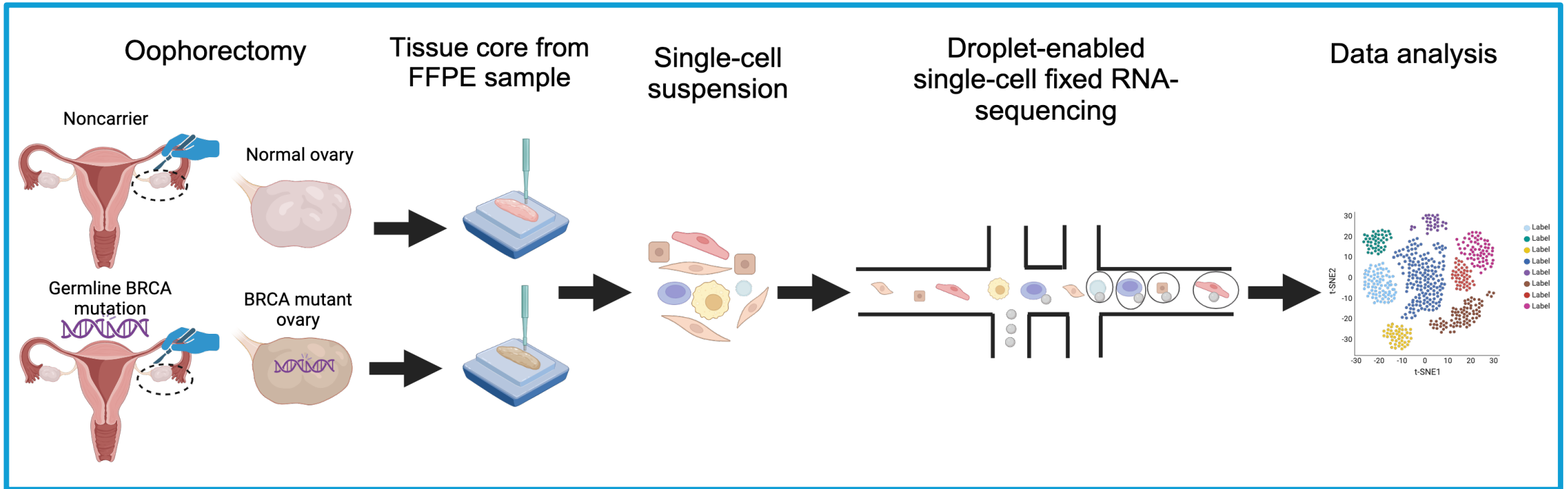
Metastatic BRCA +/mut tumor

Normal ovary vs BRCA carrier ovary
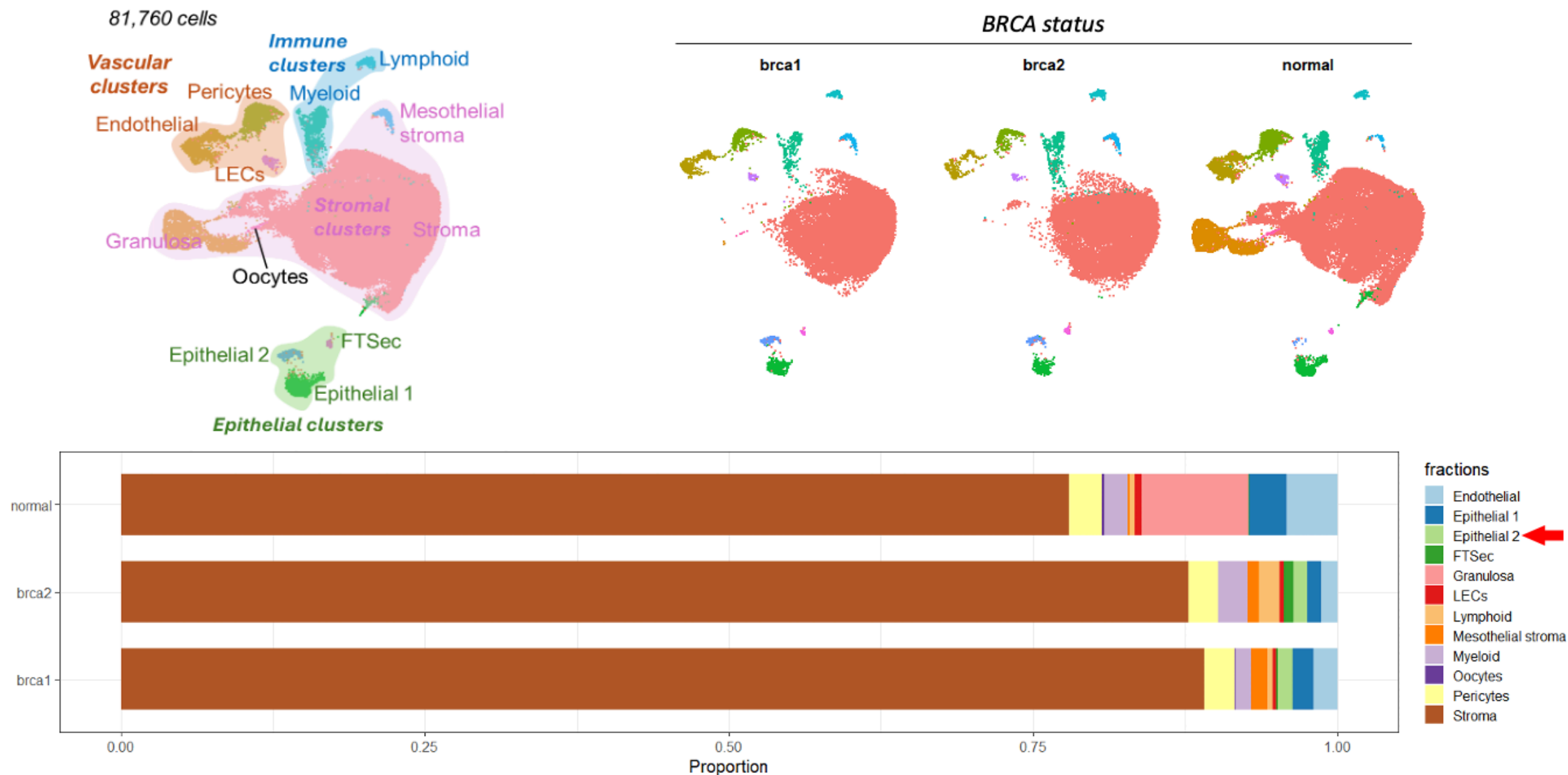
Single cell RNA sequencing

Single cell RNA seq workflow

Genotype	No. of samples
Noncarrier	8
BRCA1 mut	5
BRCA2 mut	5

Data analysis

Figure 2. Cell cluster types and frequencies in Normal vs BRCA+/mut ovarian

Marker genes for epithelial 2