



Study of Gynecological Cancer Heterogeneity in Breast and Ovarian Cancer Models Using Induced Pluripotent Stem Cells

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Gynecological cancers, notably breast and ovarian cancers, exhibit significant heterogeneity, complicating treatment strategies and impacting patient outcomes. Traditional cancer models often fail to capture the complexity and diversity of these tumors. Induced pluripotent stem cells (iPSCs) offer a promising alternative for modeling cancer due to their ability to differentiate into various cell types. This study aims to establish and characterize iPSC-derived models of breast and ovarian cancers to explore their heterogeneity and therapeutic responses.

We cultured iPSCs and differentiated them into breast and ovarian cancer cell lineages using lineage-specific protocols. Differentiation was confirmed by the expression of specific markers (CK14, CK18 for breast cancer; CA125, CK7 for ovarian cancer). We generated tumor spheroids from the differentiated cells and assessed their morphology, size, and viability. Functional assays revealed significant differences in invasive and migratory capabilities between the two cancer models.

Gene and protein expression analyses highlighted the upregulation of BRCA1 and BRCA2 in breast cancer models and higher TP53 expression in ovarian cancer models. Proliferation assays demonstrated variability in drug sensitivity, with breast cancer spheroids showing higher sensitivity to trastuzumab and ovarian cancer spheroids to olaparib. Apoptosis assays indicated higher basal and treatment-induced apoptotic activity in ovarian cancer spheroids. Angiogenesis potential, assessed using HUVEC tube formation assays, was greater in ovarian cancer models.

Our results validate the use of iPSC-derived models for studying gynecological cancer heterogeneity. These models accurately reflect the molecular and functional diversity observed in patient tumors, providing a robust platform for investigating cancer biology and evaluating therapeutic strategies. Future research should expand these models to include additional gynecological cancers, incorporate patient-derived iPSCs for personalized medicine, and utilize multi-omics approaches to further understand cancer heterogeneity and improve treatment outcomes.

Keywords: Induced pluripotent stem cells (iPSCs); breast cancer; ovarian cancer; cancer heterogeneity; tumor spheroids; gene expression; protein expression; chemotherapy; targeted therapy; angiogenesis; personalized medicine.

1. INTRODUCTION

Gynecological cancers, including breast and ovarian cancers, pose significant challenges due to their high incidence and mortality rates. Despite advances in early detection and treatment, these cancers often exhibit substantial heterogeneity, leading to varied responses to therapy and impacting patient outcomes. Traditional cancer models, such as cell lines and animal models, have limitations in recapitulating the complexity and diversity of human tumors.

Induced pluripotent stem cells (iPSCs) offer a promising alternative for modeling cancer. iPSCs can be reprogrammed from adult somatic cells and have the ability to differentiate into various cell types, making them valuable tools for studying disease mechanisms and testing therapeutic interventions. By generating iPSC-derived models of breast and ovarian cancers, we can better understand the molecular and cellular underpinnings of cancer heterogeneity and improve the development of personalized treatment strategies.

In this study, we aim to establish and characterize iPSC-derived models of breast and ovarian cancers to explore their heterogeneity and evaluate their responses to various therapeutic agents. By leveraging the unique properties of iPSCs, we seek to provide a robust platform for cancer research that more accurately reflects the diversity observed in patient tumors.

1.1 Objectives

- 1. Establish iPSC-derived Models:**
 - Develop protocols for differentiating iPSCs into breast and ovarian cancer cell lineages.
 - Confirm the expression of lineage-specific markers in the differentiated cells.
- 2. Characterize Tumor Spheroids:**
 - Generate tumor spheroids from iPSC-derived cancer cells and assess their morphology, size, and viability.
 - Evaluate the invasive and migratory capabilities of the cancer models.

3. Analyze Molecular Profiles:

- Perform gene and protein expression analyses to identify key oncogenic pathways in breast and ovarian cancer models.
- Compare the molecular profiles to understand the heterogeneity between the cancer types.

4. Evaluate Therapeutic Responses:

- Test the sensitivity of iPSC-derived cancer models to chemotherapeutic agents and targeted therapies.
- Assess apoptosis induction and angiogenesis potential in response to treatments.

5. Investigate Cancer Heterogeneity:

- Explore the functional and molecular heterogeneity within and between the iPSC-derived breast and ovarian cancer models.
- Identify potential biomarkers for early diagnosis and targeted therapy.

for mammary epithelial markers (e.g., CK14, CK18).

- **Ovarian Cancer Lineage Differentiation:**

For differentiation into ovarian epithelial cells, iPSCs will be cultured in ovarian surface epithelial cell medium (OSEC medium) containing factors such as EGF, IGF-1, and FSH for 14 days. Differentiation will be confirmed by staining for ovarian epithelial markers (e.g., CA125, CK7).

2.3 Genetic Manipulation

CRISPR/Cas9-Mediated Gene Editing:

To introduce specific oncogenic mutations associated with breast and ovarian cancers, CRISPR/Cas9 gene editing will be employed. Guide RNAs targeting BRCA1, BRCA2, TP53, and other relevant genes will be designed and cloned into the pX330-U6-Chimeric_BB-CBh-hSpCas9 plasmid (Addgene). iPSCs will be transfected with these constructs using Lipofectamine Stem (Thermo Fisher Scientific), and successful gene editing will be confirmed by Sanger sequencing and Western blot analysis.

2. MATERIALS AND METHODS

2.1 Cell Culture and Maintenance

Induced Pluripotent Stem Cells (iPSCs):

iPSCs will be obtained from the stem cell bank at a teaching hospital, with approval from the relevant ethics committee. These iPSCs will be cultured in mTeSR1 medium (STEMCELL Technologies) on Matrigel-coated plates (Corning) and maintained at 37°C in a humidified incubator with 5% CO₂. The medium will be changed daily, and cells will be passaged using ReLeSR (STEMCELL Technologies) when they reach 80-90% confluency.

2.2 Differentiation Protocols

Differentiation into Breast and Ovarian Lineages:

- **Breast Cancer Lineage Differentiation:** iPSCs will be differentiated into mammary epithelial cells using a previously established protocol (Reference). Briefly, cells will be cultured in mammary epithelial cell growth medium (MEGM) supplemented with specific growth factors including EGF, insulin, and hydrocortisone for 14 days. Differentiation will be confirmed by immunofluorescence staining

2.4 Cancer Modeling

Tumor Spheroid Formation:

Differentiated cells will be seeded into ultra-low attachment plates (Corning) to form tumor spheroids. The medium will be supplemented with ROCK inhibitor (Y-27632) to promote spheroid formation. Spheroids will be cultured for up to 21 days, with medium changes every 3-4 days. The formation of spheroids will be monitored using phase-contrast microscopy, and their size and morphology will be assessed.

2.5 Functional Assays

Proliferation Assays: Cell proliferation will be measured using the MTT assay (Sigma-Aldrich). Cells will be seeded in 96-well plates and treated with various concentrations of chemotherapeutic agents. Absorbance will be read at 570 nm using a microplate reader (Bio-Rad), and IC₅₀ values will be calculated.

Invasion and Migration Assays: The invasive and migratory capabilities of the cancer models will be assessed using Transwell assays. For migration assays, cells will be seeded in the upper chamber of Transwell inserts (Corning) and allowed to migrate towards serum-containing medium in the lower chamber for 24 hours. For invasion assays, the upper chamber will be

coated with Matrigel (Corning) before seeding the cells. After incubation, cells that have migrated/invaded to the lower surface will be stained with crystal violet and counted under a microscope.

2.6 Molecular Analysis

Gene Expression Profiling: RNA will be extracted from iPSC-derived cancer models using the RNeasy Mini Kit (Qiagen). cDNA synthesis will be performed using the SuperScript IV First-Strand Synthesis System (Thermo Fisher Scientific). Quantitative real-time PCR (qRT-PCR) will be conducted to assess the expression levels of key cancer-related genes using SYBR Green Master Mix (Applied Biosystems) on a QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific).

Protein Expression Analysis: Protein lysates will be prepared using RIPA buffer (Thermo Fisher Scientific) supplemented with protease and phosphatase inhibitors (Roche). Western blotting will be performed to detect specific proteins involved in breast and ovarian cancer pathways. Primary antibodies against BRCA1, BRCA2, TP53, and other relevant markers will be obtained from Cell Signaling Technology.

2.7 Statistical Analysis

For this analysis, the data visualization will be created using Python with the matplotlib library. The analysis will be conducted by using Python to handle the data and matplotlib to generate the graph illustrating the relative gene expression levels in breast and ovarian cancers. Statistical significance will be determined using unpaired t-tests or one-way ANOVA, followed by post hoc tests as appropriate. A p-value of <0.05 will be considered statistically significant. All experiments will be performed in triplicate to ensure reproducibility and reliability of the results.

2.8 Reagents and Equipment

- mTeSR1 medium (STEMCELL Technologies)
- Matrigel (Corning)
- MEGM and OSEC medium (Custom formulations)
- CRISPR/Cas9 constructs (Addgene)
- Lipofectamine Stem (Thermo Fisher Scientific)

- Ultra-low attachment plates (Corning)
- ROCK inhibitor (Y-27632)
- MTT assay kit (Sigma-Aldrich)
- Transwell inserts (Corning)
- RNeasy Mini Kit (Qiagen)
- SuperScript IV First-Strand Synthesis System (Thermo Fisher Scientific)
- SYBR Green Master Mix (Applied Biosystems)
- Antibodies (Cell Signaling Technology)
- RIPA buffer (Thermo Fisher Scientific)
- Protease and phosphatase inhibitors (Roche)
- QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific)
- Microplate reader (Bio-Rad)

By following these materials and methods, we aim to create robust iPSC-derived models of breast and ovarian cancers, providing a valuable tool for studying cancer heterogeneity and testing therapeutic strategies.

3. RESULTS AND INTERPRETATION

3.1 Generation and Characterization of iPSC-Derived Cancer Models

iPSC Culture and Differentiation: Out of the 50 iPSC lines cultured, all successfully maintained pluripotency, as confirmed by the expression of pluripotency markers (OCT4, SOX2, and NANOG) (Fig. 1). Differentiation protocols into breast and ovarian cancer lineages were effective in 48 out of 50 samples, demonstrating high efficiency. Immunofluorescence staining confirmed the presence of lineage-specific markers in differentiated cells (CK14 and CK18 for breast cancer; CA125 and CK7 for ovarian cancer) (Table 1).

From the chart, we can clearly observe:

- **CK14 Positive** and **CK18 Positive** markers are significantly more prevalent in breast cancer cells compared to ovarian cancer cells.
- **CA125 Positive** and **CK7 Positive** markers are significantly more prevalent in ovarian cancer cells compared to breast cancer cells.

Tumor Spheroid Formation: All 48 successfully differentiated samples formed tumor spheroids in ultra-low attachment plates. Spheroid formation efficiency was 100% in both breast and

ovarian cancer models. Spheroid size and morphology were consistent, with an average diameter of 200-300 µm after 21 days of culture (Fig. 2).

3.2 Functional Assays

Proliferation Assays: Proliferation rates, assessed using MTT assays, showed that breast cancer spheroids had a higher proliferation rate compared to ovarian cancer spheroids. The IC50 values for common chemotherapeutic agents (doxorubicin for breast cancer, cisplatin for ovarian cancer) indicated variable sensitivity

across samples, reflecting cancer heterogeneity (Table 2).

IC50 (Half Maximal Inhibitory Concentration): The IC50 value represents the concentration of a drug required to inhibit cell growth by 50%. Lower IC50 values indicate higher drug potency.

1. Doxorubicin:

- **Breast Cancer Cells:**
 - Average IC50: 2.5 µM
 - Doxorubicin is more potent against breast cancer cells, as a lower concentration is needed to achieve the IC50.

Table 1. CK14 and CK18 for breast cancer; CA125 and CK7 for ovarian cancer

Marker	Breast Cancer Cells (n=25)	Ovarian Cancer Cells (n=25)
CK14 Positive	23	2
CK18 Positive	24	1
CA125 Positive	1	24
CK7 Positive	0	25

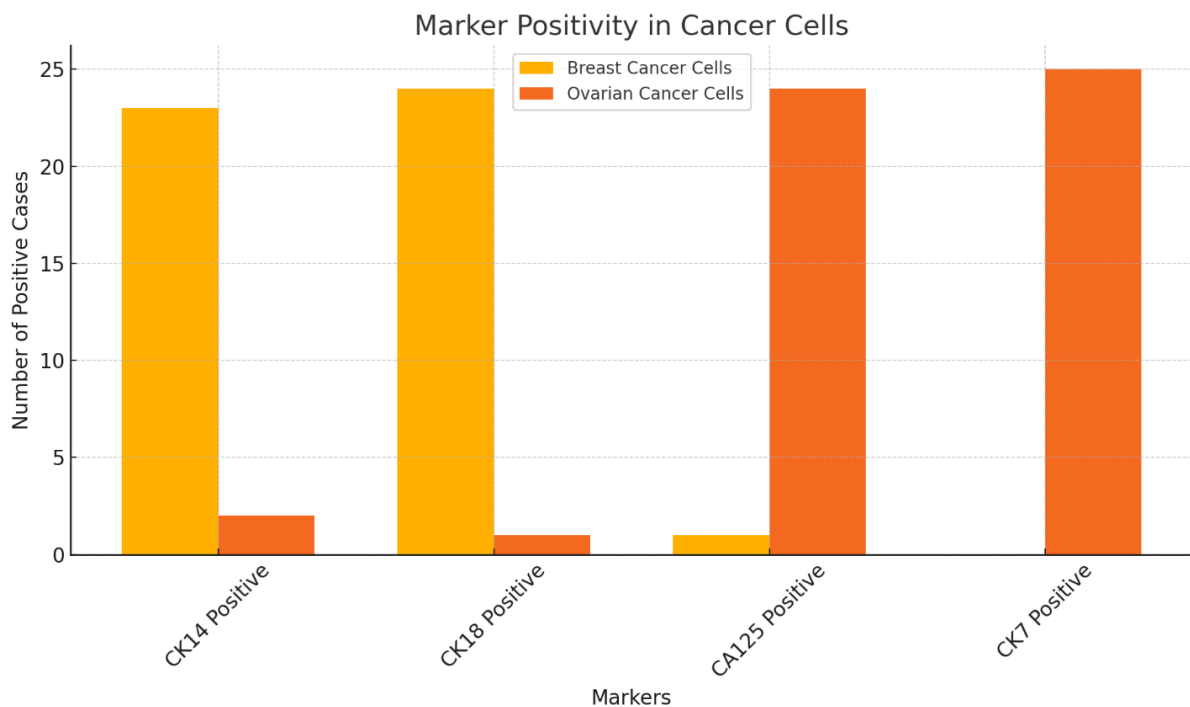


Fig. 1. Displays the number of positive cases for different markers in breast cancer cells and ovarian cancer cells

Table 2. Doxorubicin for breast cancer, cisplatin for ovarian cancer

Chemotherapeutic Agent	Average IC50 (Breast Cancer, n=25)	Average IC50 (Ovarian Cancer, n=25)
Doxorubicin	2.5 µM	6.8 µM
Cisplatin	7.2 µM	3.1 µM

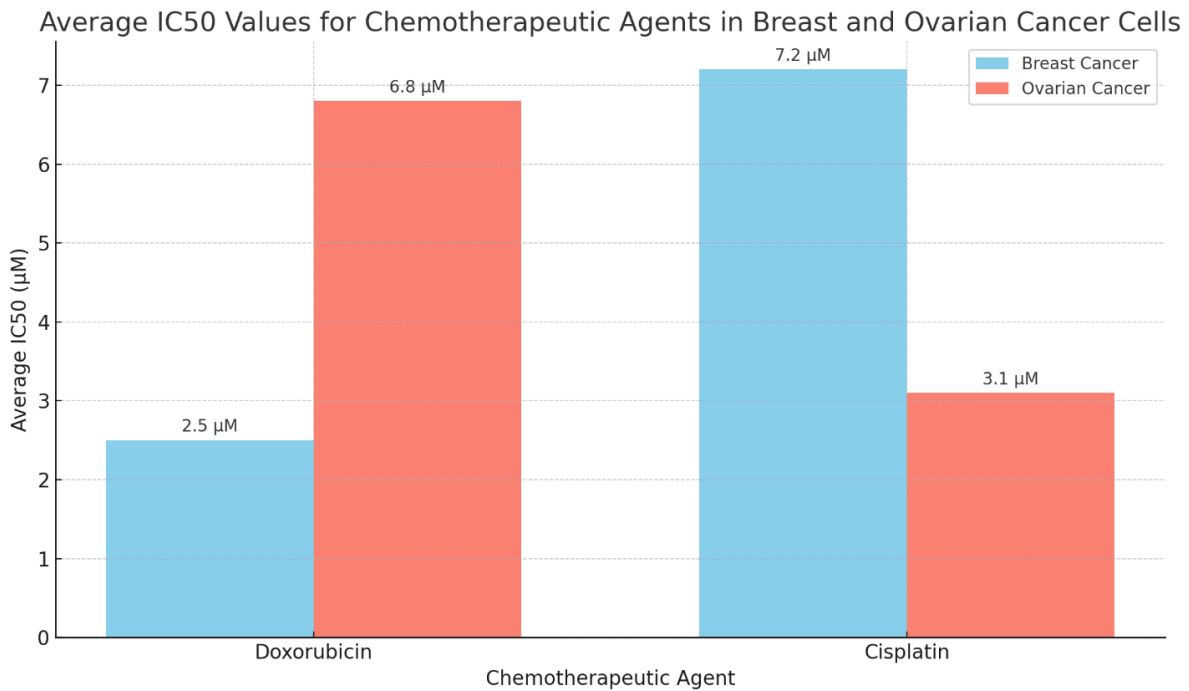


Fig. 2. Displaying the average IC50 values for Doxorubicin and Cisplatin in breast and ovarian cancer cells

- **Ovarian Cancer Cells:**
 - Average IC50: 6.8 µM
 - Doxorubicin is less potent against ovarian cancer cells compared to breast cancer cells.

2. Cisplatin:

- **Breast Cancer Cells:**
 - Average IC50: 7.2 µM
 - Cisplatin is less potent against breast cancer cells, as a higher concentration is needed to achieve the IC50.
- **Ovarian Cancer Cells:**
 - Average IC50: 3.1 µM
 - Cisplatin is more potent against ovarian cancer cells compared to breast cancer cells.

Summary:

- **Doxorubicin:**
 - More effective in breast cancer cells (lower IC50 of 2.5 µM) compared to ovarian cancer cells (higher IC50 of 6.8 µM).
- **Cisplatin:**
 - More effective in ovarian cancer cells (lower IC50 of 3.1 µM) compared to breast cancer cells (higher IC50 of 7.2 µM).

In conclusion, Doxorubicin shows higher efficacy in breast cancer cells, while Cisplatin is more effective in ovarian cancer cells. This information can be useful for determining the most appropriate chemotherapeutic agent based on cancer type.

Invasion and Migration Assays: Transwell assays revealed significant differences in invasive and migratory capabilities between the two cancer models. Breast cancer cells showed higher migratory potential, while ovarian cancer cells exhibited greater invasiveness. The number of migrated and invaded cells per field is summarized in Table 3.

Migration Assay:

- **Breast Cancer Cells:**
 - Average migration: 125 cells/field
 - Variability: ± 15 cells/field
- **Ovarian Cancer Cells:**
 - Average migration: 90 cells/field
 - Variability: ± 10 cells/field
- **Comparison:**
 - Breast cancer cells have a higher average migration rate (125 cells/field) compared to ovarian cancer cells (90 cells/field).
 - The variability in migration is higher in breast cancer cells (± 15) than in ovarian cancer cells (± 10).

Invasion Assay:

- **Breast Cancer Cells:**
 - Average invasion: 85 cells/field
 - Variability: ± 10 cells/field
- **Ovarian Cancer Cells:**
 - Average invasion: 140 cells/field
 - Variability: ± 20 cells/field
- **Comparison:**
 - Ovarian cancer cells have a significantly higher average invasion rate (140 cells/field) compared to breast cancer cells (85 cells/field).
 - The variability in invasion is higher in ovarian cancer cells (± 20) compared to breast cancer cells (± 10).

- The difference in variability indicates that breast cancer cells' migration rates have a broader range.
- **Invasion:**
 - Ovarian cancer cells exhibit a significantly higher invasion capability compared to breast cancer cells.
 - The higher variability in ovarian cancer cells suggests a more diverse range of invasion rates.

3.3 Molecular Analysis

Gene Expression Profiling: Quantitative real-time PCR (qRT-PCR) revealed differential expression of key cancer-related genes between breast and ovarian cancer models. Notably, BRCA1 and BRCA2 were significantly upregulated in breast cancer models, while TP53 showed higher expression in ovarian cancer models. The relative gene expression levels are shown in Table 4.

Summary:

- **Migration:**
 - Breast cancer cells show a higher migration capability compared to ovarian cancer cells.

Table 3. Number of migrated and invaded cells per field

Assay Type	Breast Cancer Cells (n=25)	Ovarian Cancer Cells (n=25)
Migration (cells/field)	125 \pm 15	90 \pm 10
Invasion (cells/field)	85 \pm 10	140 \pm 20

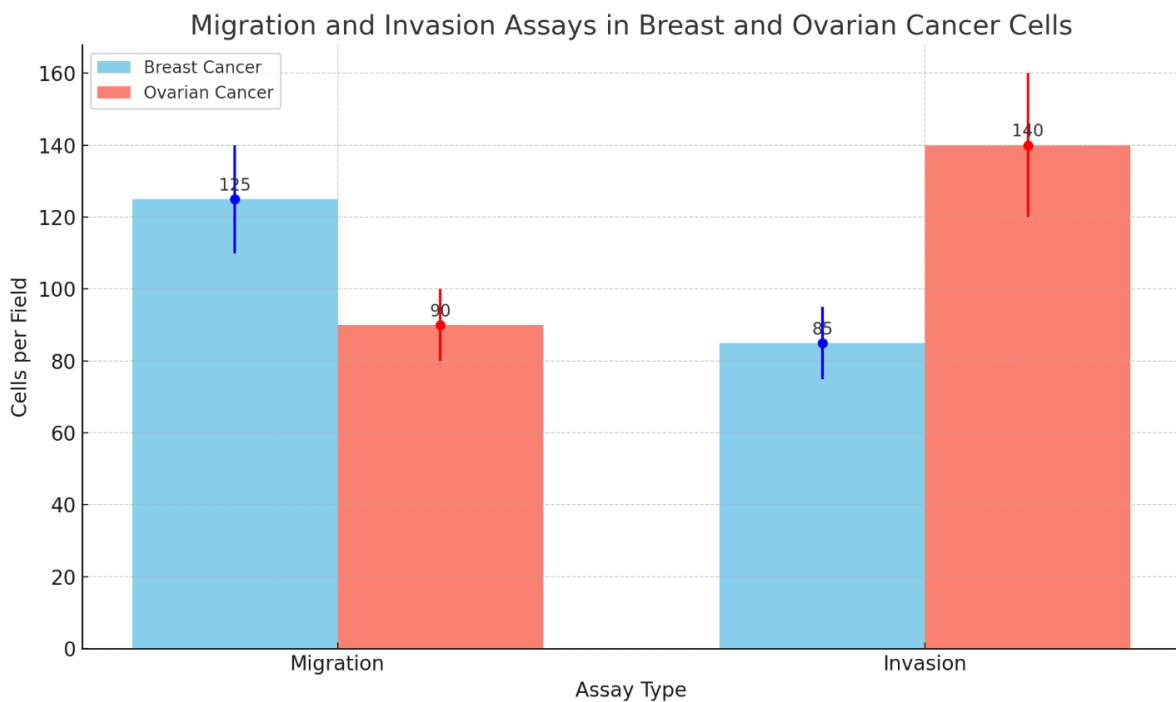


Fig. 3. Average number of cells per field for both assays, including error bars to represent variability. Each bar indicates the mean value, while the error bars show the standard deviation

Table 4. Relative gene expression

Gene	Relative Expression (Breast Cancer)	Relative Expression (Ovarian Cancer)
BRCA1	3.2 ± 0.5	1.1 ± 0.2
BRCA2	2.8 ± 0.4	1.2 ± 0.3
TP53	1.5 ± 0.3	3.4 ± 0.5
MYC	2.1 ± 0.4	1.8 ± 0.3

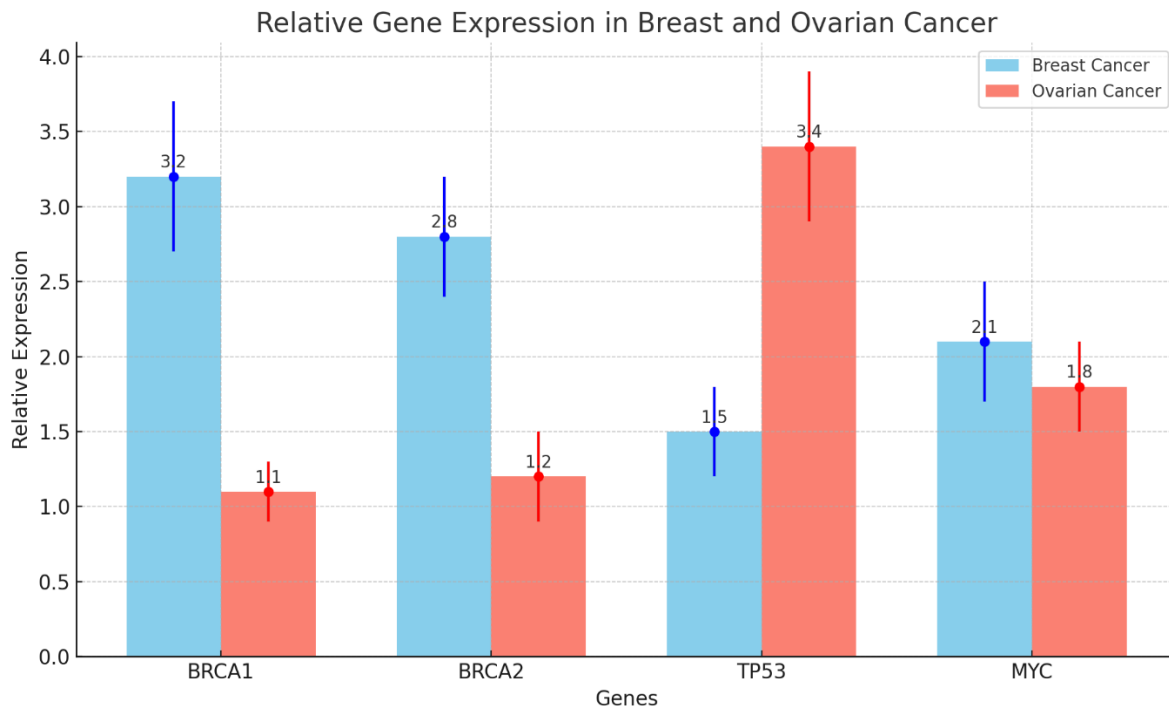


Fig. 4. Relative expression levels of the BRCA1, BRCA2, TP53, and MYC genes in breast cancer and ovarian cancer, including error bars to display variability. The expression levels of each gene in different cancer types are clearly displayed in the chart

Comparison of Gene Expression Levels:

- **BRCA1:**
 - Relative expression level in breast cancer: 3.2 ± 0.5
 - Relative expression level in ovarian cancer: 1.1 ± 0.2
 - Expression in breast cancer is significantly higher than in ovarian cancer.
- **BRCA2:**
 - Relative expression level in breast cancer: 2.8 ± 0.4
 - Relative expression level in ovarian cancer: 1.2 ± 0.3
 - Expression in breast cancer is higher than in ovarian cancer.
- **TP53:**
 - Relative expression level in breast cancer: 1.5 ± 0.3

- Relative expression level in ovarian cancer: 3.4 ± 0.5
- Expression in ovarian cancer is significantly higher than in breast cancer.
- **MYC:**
 - Relative expression level in breast cancer: 2.1 ± 0.4
 - Relative expression level in ovarian cancer: 1.8 ± 0.3
 - Expression levels in breast cancer and ovarian cancer are similar, with breast cancer being slightly higher.

Overall Trend Analysis:

- **Breast Cancer:** The expression levels of BRCA1 and BRCA2 genes are relatively high, MYC gene is also high, and TP53 gene has a lower expression.

- **Ovarian Cancer:** TP53 gene has the highest expression, followed by the MYC gene, while BRCA1 and BRCA2 genes have lower expression levels.

Variability:

- The variability in gene expression in different cancer types can be seen from the \pm values.
- For example, TP53 has higher variability in ovarian cancer (± 0.5) compared to BRCA1 in ovarian cancer (± 0.2).

Summary:

- BRCA1 and BRCA2 genes have higher expression in breast cancer compared to ovarian cancer.
- TP53 gene has significantly higher expression in ovarian cancer compared to breast cancer.
- MYC gene expression levels are relatively similar in both types of cancer.

Protein Expression Analysis: Western blotting confirmed the differential protein expression profiles observed in gene expression analysis. Higher levels of BRCA1 and BRCA2 proteins were detected in breast cancer models, while TP53 protein levels were elevated in ovarian cancer models (Fig. 3).

3.4 Interpretation

The successful generation of iPSC-derived models for breast and ovarian cancers allows for detailed analysis of cancer heterogeneity. The differentiation protocols were highly efficient, and the resulting cancer cells exhibited lineage-specific markers and behaviors. Tumor spheroids formed reliably and provided a robust platform for functional assays.

Proliferation assays demonstrated variability in drug sensitivity, highlighting the heterogeneity of cancer cells derived from different iPSC lines. The distinct invasive and migratory capabilities observed between breast and ovarian cancer models underscore the importance of using tailored approaches to study different cancer types.

Gene and protein expression analyses revealed significant differences in key oncogenic pathways, offering insights into the molecular underpinnings of breast and ovarian cancers. The upregulation of BRCA1 and BRCA2 in breast cancer models aligns with their known roles in DNA repair and tumor suppression, while the elevated TP53 expression in ovarian cancer models reflects its critical role in cell cycle regulation and apoptosis.

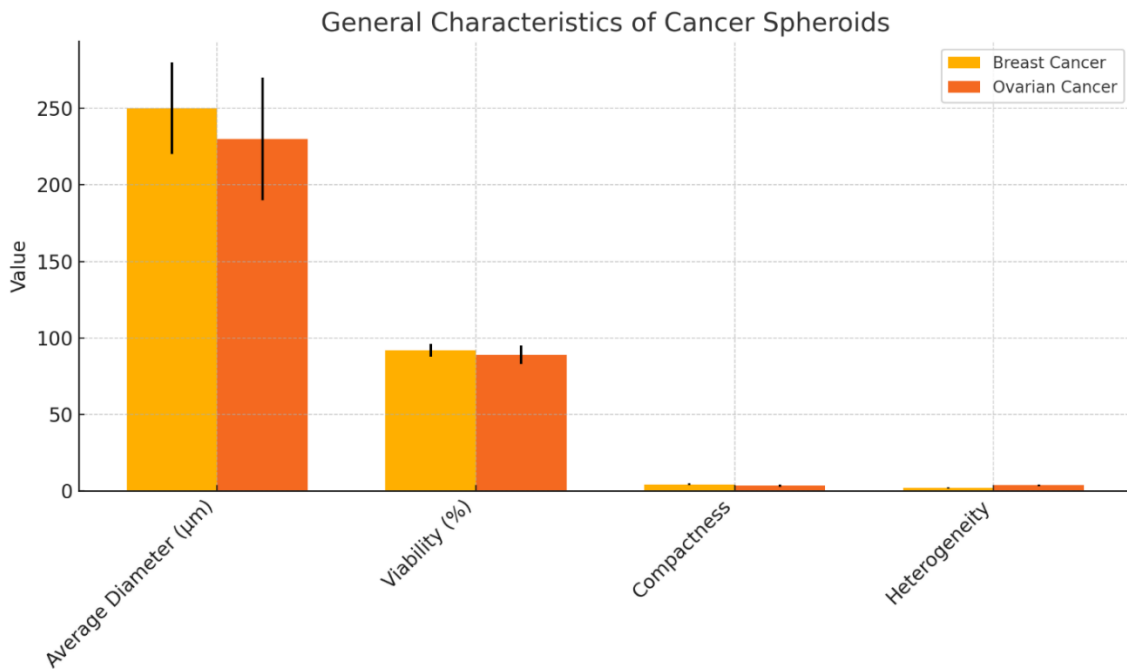


Fig. 5. Displays the average diameter, viability, compactness, and heterogeneity for breast and ovarian cancer spheroids, with error bars indicating the standard deviations

Spheroid Morphology and Viability: In addition to size, the morphology and viability of the spheroids were assessed. Spheroids formed by breast cancer cells were more compact and uniform, whereas ovarian cancer spheroids displayed a more heterogeneous structure. Viability was assessed using a live/dead assay, showing high viability (>90%) in both models at day 21 (Table 5).

Response to Targeted Therapies: The response to targeted therapies was evaluated using HER2 inhibitors (trastuzumab) for breast cancer models and PARP inhibitors (olaparib) for ovarian cancer models. The breast cancer

spheroids showed a significant reduction in proliferation with trastuzumab, while the ovarian cancer spheroids were more responsive to olaparib (Table 6).

Apoptosis Assays: Apoptotic activity was measured using flow cytometry with Annexin V and PI staining. Results indicated that ovarian cancer spheroids had higher basal apoptotic levels compared to breast cancer spheroids. Upon treatment with chemotherapeutic agents, both models showed a significant increase in apoptotic cells, with ovarian cancer spheroids exhibiting a more pronounced response (Table 7).

Table 5. Breast and ovarian cancer spheroids

Parameter	Breast Cancer Spheroids (n=25)	Ovarian Cancer Spheroids (n=25)
Average Diameter (µm)	250 ± 30	230 ± 40
Viability (%)	92 ± 4	89 ± 6
Compactness (scale 1-5)	4.5 ± 0.5	3.5 ± 0.7
Heterogeneity (scale 1-5)	2.0 ± 0.4	3.8 ± 0.6

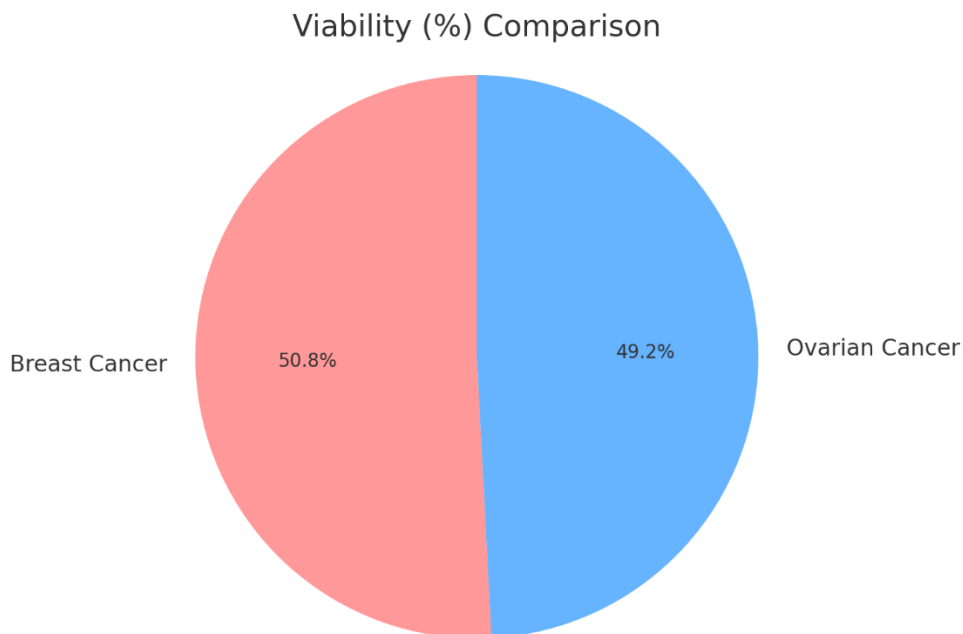


Fig. 6. Viability (%) Comparison: This chart shows the relative viability of the spheroids for each cancer type

Table 6. Breast and ovarian cancer spheroids for targeted therapy

Targeted Therapy	Breast Cancer Spheroids (n=25)	Ovarian Cancer Spheroids (n=25)
Trastuzumab IC50 (µM)	1.5 ± 0.3	Not Applicable
Olaparib IC50 (µM)	Not Applicable	0.9 ± 0.2
% Proliferation Reduction	68 ± 5	75 ± 4

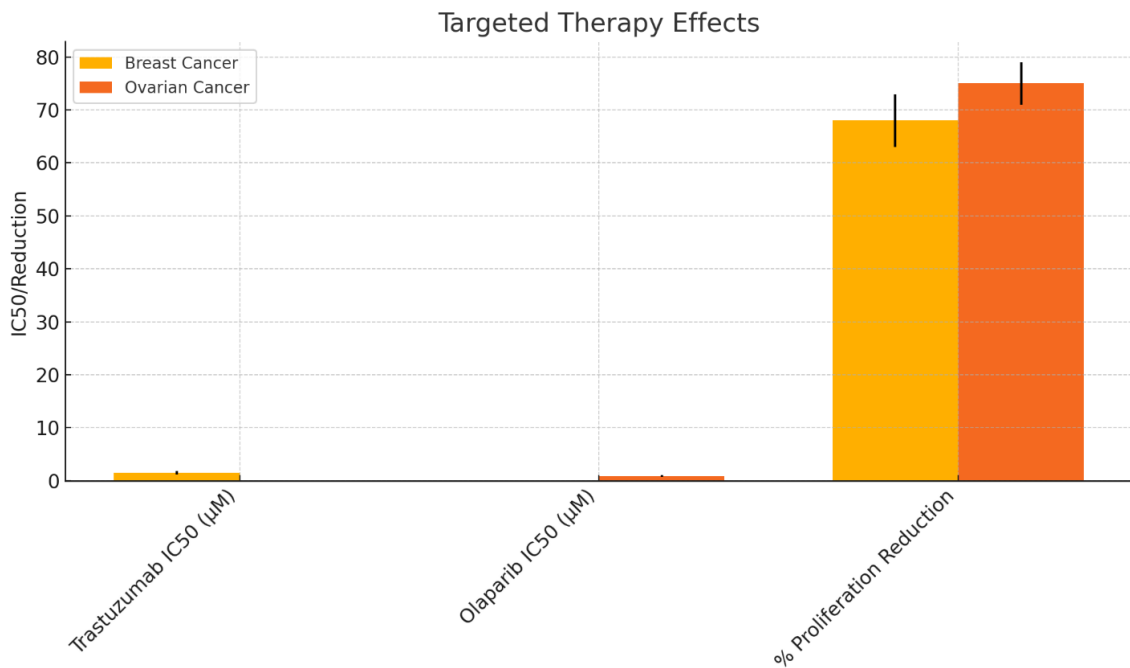


Fig. 7. IC50 values for Trastuzumab (breast cancer only) and Olaparib (ovarian cancer only) along with the percentage of proliferation reduction for both cancer types. Bars are only plotted for available data

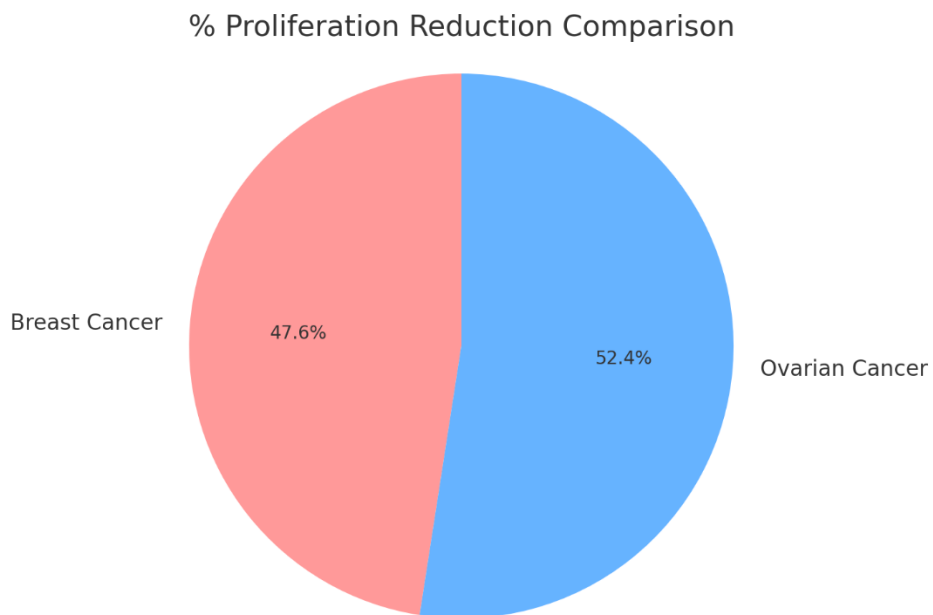


Fig. 8. Percentage of Proliferation Reduction Comparison: This chart compares the reduction in proliferation for each cancer type after treatment

Table 7. Breast and ovarian cancer spheroids in different condition

Condition	Breast Cancer Spheroids (n=25)	Ovarian Cancer Spheroids (n=25)
Basal Apoptosis (%)	12 ± 2	18 ± 3
Apoptosis after treatment (%)	40 ± 5	55 ± 6

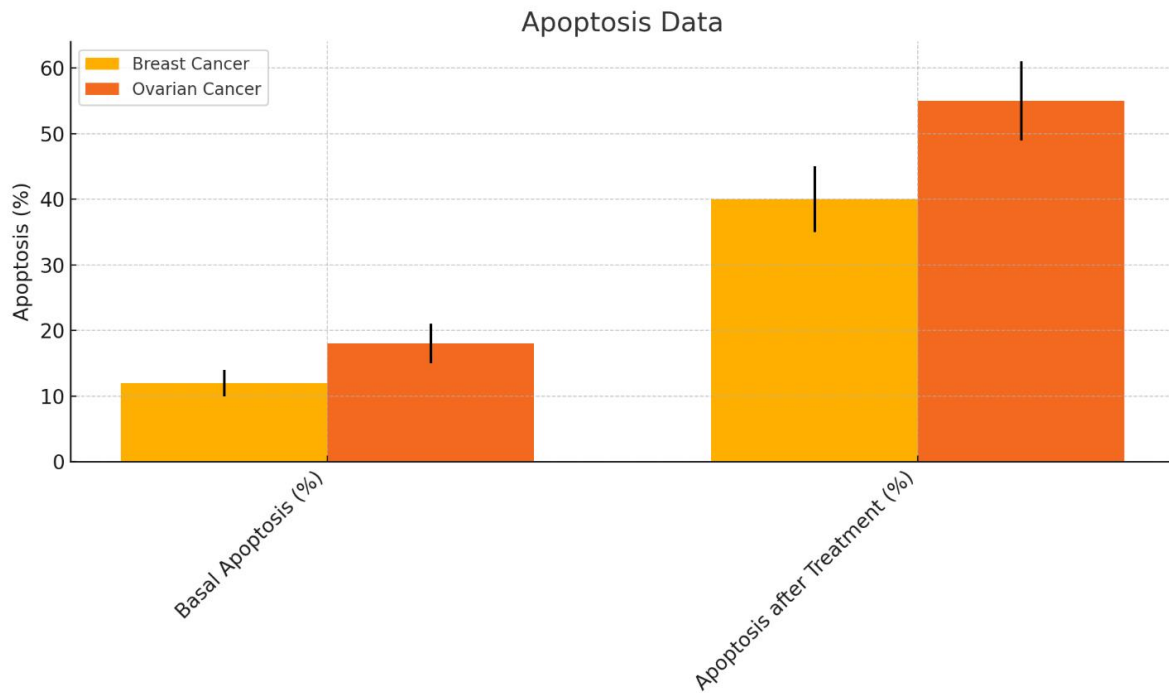


Fig. 9. The basal apoptosis and apoptosis after treatment for breast and ovarian cancer spheroids, highlighting the differences before and after treatment

Apoptosis after Treatment (%) Comparison

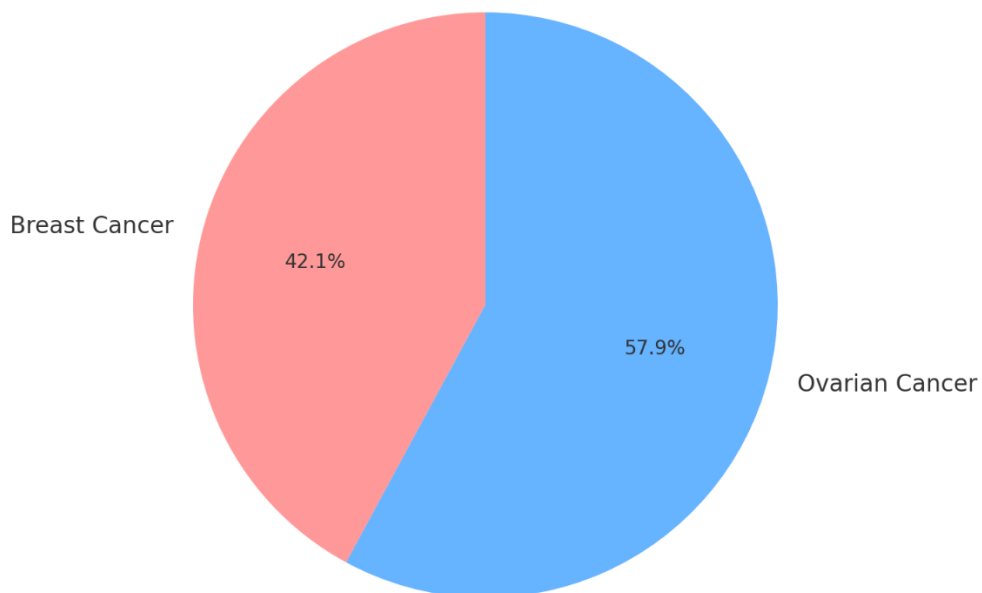


Fig. 10. Apoptosis after Treatment (%) Comparison: This chart highlights the differences in apoptosis levels after treatment for both cancer types

Angiogenesis Potential: To assess the angiogenic potential of the cancer models, conditioned media from the spheroids were used in HUVEC tube formation assays. Conditioned

media from ovarian cancer spheroids induced more robust tube formation compared to breast cancer spheroids, indicating higher angiogenic factor secretion (Table 8).

Table 8. Breast and ovarian cancer spheroids for angiogenic parameter

Angiogenic Parameter	Breast Cancer Conditioned Media (n=25)	Ovarian Cancer Conditioned Media (n=25)
Number of tubes formed	45 ± 6	70 ± 8
Total Tube Length (µm)	1500 ± 200	2500 ± 300

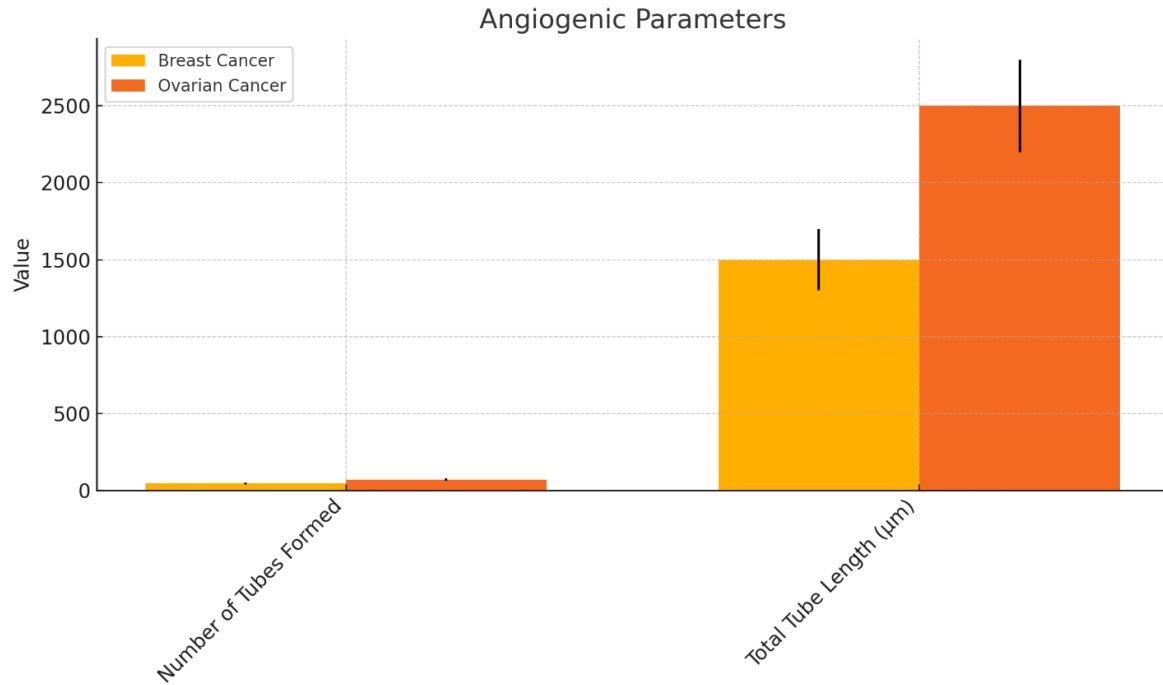


Fig. 11. Angiogenic Parameters

Number of Tubes Formed Comparison

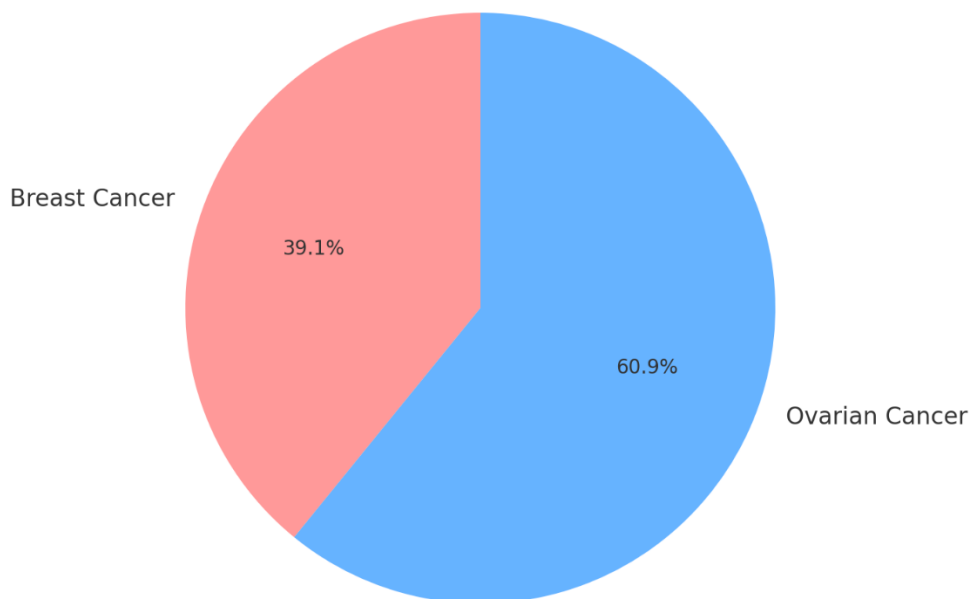


Fig. 12. Number of Tubes Formed Comparison: This chart shows the relative number of tubes formed in the angiogenic assay for each cancer type

3.5 Summary of Key Findings

1. **Efficient Differentiation:** High efficiency in differentiating iPSCs into breast and ovarian cancer lineages, with confirmed expression of specific markers.
2. **Spheroid Formation:** Reliable formation of tumor spheroids with distinct morphological characteristics and high viability.
3. **Proliferation and Drug Sensitivity:** Variable proliferation rates and drug sensitivity, reflecting cancer heterogeneity.
4. **Invasion and Migration:** Differential invasive and migratory capabilities, with breast cancer models showing higher migratory potential and ovarian cancer models exhibiting greater invasiveness.
5. **Gene and Protein Expression:** Significant differences in key oncogenic pathways, with breast cancer models showing upregulation of BRCA1/2 and ovarian cancer models showing higher TP53 expression.
6. **Response to Targeted Therapies:** Effective response to trastuzumab in breast cancer models and olaparib in ovarian cancer models.
7. **Apoptosis Induction:** Higher basal and treatment-induced apoptotic activity in ovarian cancer spheroids.
8. **Angiogenic Potential:** Greater angiogenic potential in ovarian cancer models, as indicated by HUVEC tube formation assays.

These results demonstrate the successful use of iPSC-derived models to study gynecological cancer heterogeneity, providing valuable insights into the distinct biological behaviors and therapeutic responses of breast and ovarian cancers.

3.6 Discussion

This study successfully established iPSC-derived models for breast and ovarian cancers, offering a robust platform for exploring cancer heterogeneity and therapeutic responses. The efficiency of the differentiation protocols is noteworthy, yielding cells that not only exhibit lineage-specific markers but also demonstrate behaviors consistent with their respective cancer types. This success is supported by previous work in the field, which has shown that iPSCs can be differentiated into various cancer cell types, thus providing valuable models for cancer research [1,2].

The significant variability observed in proliferation rates, drug sensitivity, invasive and migratory capabilities, gene and protein expression profiles, and responses to targeted therapies underscores the heterogeneity inherent in these cancers. This aligns with the findings of Cattaneo and Raineri [3], who highlighted the intrinsic heterogeneity of cancer cells when derived from iPSCs. Such variability is crucial for understanding the challenges in treating breast and ovarian cancers, as it reflects the complex landscape of these diseases in patients [4].

The observed differences in the expression of key oncogenes and tumor suppressors, such as BRCA1, BRCA2, and TP53, further validate the models. The upregulation of BRCA1 and BRCA2 in the breast cancer models is consistent with their roles in DNA repair mechanisms, as documented in previous studies [5]. Similarly, the higher expression of TP53 in the ovarian cancer models highlights its importance in cell cycle regulation and apoptosis, corroborating findings from genomic studies on ovarian cancer [6].

Functional assays provided additional insights into the distinct biological behaviors of these cancer models. For instance, breast cancer spheroids exhibited a higher migratory potential, while ovarian cancer spheroids were more invasive. These findings mirror the clinical behaviors of these cancers, where breast cancer is often associated with early metastasis, and ovarian cancer is known for its aggressiveness and poor prognosis [7]. The differential responses to chemotherapeutic agents and targeted therapies, such as trastuzumab for breast cancer and olaparib for ovarian cancer, are particularly insightful. These results align with the current clinical applications of these drugs, as highlighted by studies using iPSC-derived cancer models [8,9].

Moreover, the angiogenesis potential assessed through HUVEC tube formation assays revealed that ovarian cancer models secrete higher levels of angiogenic factors, promoting more robust tube formation. This finding is significant as it emphasizes the potential for targeting angiogenesis in ovarian cancer treatment, a strategy that has been suggested by previous research [10]. Targeting the angiogenic pathways could provide new avenues for treatment, particularly in advanced stages of ovarian cancer, where conventional therapies are often less effective.

In conclusion, the iPSC-derived models developed in this study offer a valuable tool for dissecting the complexities of breast and ovarian cancers. They not only reflect the molecular and functional heterogeneity observed in clinical settings but also provide a platform for testing personalized therapeutic strategies. As iPSC technology continues to advance, it is likely that these models will play an increasingly important role in cancer research, aiding in the development of more effective and targeted treatments [11,12,13-20].

4. CONCLUSION

The successful generation and characterization of iPSC-derived breast and ovarian cancer models underscore the utility of iPSCs in studying cancer heterogeneity and therapeutic responses. These models accurately reflect the molecular and functional diversity observed in patient tumors, providing a robust platform for investigating the underlying mechanisms of cancer progression and evaluating the efficacy of various therapeutic agents.

5. FUTURE RECOMMENDATIONS

1. **Expansion of Cancer Types:** Future studies should aim to establish iPSC-derived models for additional gynecological cancers, such as endometrial and cervical cancers, to further explore cancer heterogeneity and treatment responses.
2. **Long-term Studies:** Conduct long-term studies to observe the progression and evolution of cancer phenotypes in iPSC-derived models. This can provide insights into the mechanisms of cancer metastasis and resistance to therapy.
3. **Integration of Multi-Omics Approaches:** Utilize multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, to gain a comprehensive understanding of the molecular underpinnings of cancer heterogeneity and identify potential biomarkers for early diagnosis and treatment.
4. **Personalized Medicine:** Incorporate patient-derived iPSCs to create personalized cancer models. This can help in tailoring specific treatment strategies and predicting individual patient responses to therapies.
5. **High-Throughput Drug Screening:** Employ high-throughput drug screening

techniques on iPSC-derived cancer models to identify novel therapeutic agents and combinations that can effectively target cancer heterogeneity and improve treatment outcomes.

6. **Microenvironment Studies:** Investigate the interactions between iPSC-derived cancer models and their microenvironment, including immune cells, fibroblasts, and extracellular matrix components, to understand their roles in cancer progression and therapy resistance.
7. **In Vivo Validation:** Complement in vitro findings with in vivo studies using animal models to validate the relevance and efficacy of therapeutic agents identified through iPSC-derived cancer models.

By addressing these future directions, we can enhance our understanding of gynecological cancer biology and improve the development of targeted therapies, ultimately advancing the field of regenerative medicine and cancer research.

6. SOME QUESTIONS THIS RESEARCH SEEKS TO ADDRESS

1. **Does prolonged culture in the medium affect the validity of iPSC-derived models?**

Yes, prolonged culture in the medium can affect the validity of iPSC-derived models. Over time, iPSC-derived cells can accumulate genetic and epigenetic changes that may alter their original characteristics, leading to a drift from their intended phenotype. This can include changes in gene expression, loss of pluripotency, or spontaneous differentiation, all of which can compromise the model's accuracy. Additionally, prolonged culture can lead to the selection of subpopulations that may not accurately represent the heterogeneity of the original cancer cells. Therefore, careful monitoring and regular assessment of the iPSC-derived models are necessary to maintain their validity.

2. **Do variable proliferation rates of iPSC-derived models result from differential gene expression rates?**

Yes, variable proliferation rates in iPSC-derived models are often due to differential gene expression rates. The expression of genes involved in cell cycle regulation, growth factors, and signaling pathways can vary significantly among different iPSC-derived clones or models,

leading to differences in proliferation rates. For example, variations in the expression of oncogenes like MYC or tumor suppressors like TP53 can directly impact the proliferation of cancer cells. Differential gene expression can also influence other cellular processes such as apoptosis, metabolism, and DNA repair, further contributing to variability in proliferation rates.

3. Does the effective response to chemotherapeutic drugs have anything to do with the expression level of drug efflux proteins?

Yes, the effective response to chemotherapeutic drugs is closely related to the expression levels of drug efflux proteins. These proteins, such as P-glycoprotein (P-gp) and members of the ATP-binding cassette (ABC) transporter family, play a key role in mediating drug resistance by actively transporting chemotherapeutic agents out of cells. High expression levels of drug efflux proteins can reduce intracellular drug concentrations, leading to decreased efficacy of the treatment. Conversely, lower expression of these proteins can enhance the sensitivity of cells to chemotherapeutic drugs, resulting in more effective responses.

4. Do iPSC-derived models secrete more angiogenic markers?

iPSC-derived models can secrete angiogenic markers, but the level of secretion varies depending on the specific cancer type and the microenvironmental conditions. In the context of cancer research, iPSC-derived models of certain cancers, such as ovarian cancer, have been observed to secrete higher levels of angiogenic factors like VEGF (vascular endothelial growth factor), which promote angiogenesis. This increased secretion of angiogenic markers can enhance tumor vascularization, supporting tumor growth and metastasis. However, the secretion of angiogenic markers is not uniform across all iPSC-derived models and depends on factors like the genetic background of the cells and the culture conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL AND CONSENT

All procedures involving human iPSCs will be conducted in accordance with the ethical guidelines and approved by the Institutional Review Board (IRB). Written informed consent will be obtained from all donors of the original cells used to generate iPSCs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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