Anti-tumor activity of plant extracts against human breast cancer cells are different in monolayer and three-dimensional cell culture screening models: A comparison on 34 extracts

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ABSTRACT
Introduction: The monolayer cell culture model is a popular model for screening anti-tumor activity of plant extracts. However, almost the extracts selected for screening in this model have failed in subsequent animal models. Therefore, there is only about 5% of candidates from the original thousands of drugs that are screened which ultimately reach clinical trial. This study aimed to compare the differences in anti-tumor activity of 34 plant extracts against breast cancer cells in 2 models of monolayer cell culture (2D) and in three-dimensional (3D) cell culture. Methods: Four breast cancer cell lines (MCF-7, CD44+CD24−, MCF-7, VN9, and CD44+CD24− VN9) were used to generate the 2D and 3D models (the 3D model was developed by culturing breast cancer cells in matrigel). The extracts were got from the plant extract library that prepared in the previous study. The anti-tumor activity was evaluated via half inhibitory concentrations (IC50 values). Results: Of the 34 extracts, E12, E7, E5 and E6 of them had an effect on MCF-7, CD44+CD24−, MCF-7, VN9 and CD44+CD24− VN9 cells, respectively. The results indicated 10 potentially strong candidates for future drug development targeting hypoxic areas in breast cancer. Conclusion: The 3D culture model exhibited higher resistance to extracts than the 2D culture model. The CD44+CD24− cell population of both VN9 and MCF-7 cell lines showed higher drug resistance than the original cell lines (VN9 and MCF-7).

Key words: breast cancer, drug screening, natural extract, CD44+CD24−.
neural crest, immature follicles, and zygote). This study used matrigel to create a 3D cell model of breast cancer for the purpose of screening natural compounds that inhibit the growth of breast cancer cell. Modeling of 2D and 3D monolayer cancer cells was carried out in parallel (simultaneously) with the same evaluation agents, including Alarma Blue. The IC\textsubscript{50} (half maximal inhibitory concentration) values were compared between 2D and 3D cancer cell models to evaluate and select the extract which showed different effects in these two models. This study used 34 natural plant extracts and two control drugs (Doxorubicin and Tirapazamine) on 4 cell lines (MCF-7, CD44\textsuperscript{+}CD24\textsuperscript{-}, MCF-7, VN9, and CD44\textsuperscript{+}CD24\textsuperscript{-} VN9 cells).

**METHODS**

**Cell lines**

MCF-7 cell line was obtained from ATCC (Manassas, VA). VN9 cell line was obtained from the Stem Cell Institute, University of Science, VNU-HCM. MCF-7 and VN9 cells were cultured in DMEM/F12 (Sigma-Aldrich, St Louis, MO), 10% fetal bovine serum (FBS) (Sigma-Aldrich), and 1% antibiotic-antimycotic (Sigma-Aldrich, St Louis, MO). The CD44\textsuperscript{+}CD24\textsuperscript{-} cells were sorted from VN9 cells (and termed CD44\textsuperscript{+}CD24\textsuperscript{-} VN9) or from MCF-7 (and termed CD44\textsuperscript{+}CD24\textsuperscript{-} MCF-7) by magnetic-activated cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany), and then expanded in M171 medium (Thermo Fisher Scientific, Waltham, MA) with MEGS supplement (Thermo Fisher Scientific). The CD44\textsuperscript{+}CD24\textsuperscript{-}cells were sorted from VN9 cells (and termed CD44\textsuperscript{+}CD24\textsuperscript{-} VN9) or from MCF-7 (and termed CD44\textsuperscript{+}CD24\textsuperscript{-} MCF-7) by magnetic-activated cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany), and then expanded in M171 medium (Thermo Fisher Scientific, Waltham, MA) with MEGS supplement (Thermo Fisher Scientific). The CD44\textsuperscript{+}CD24\textsuperscript{-} cell lines (MCF-7, CD44\textsuperscript{+}CD24\textsuperscript{-} MCF-7, VN9, and CD44\textsuperscript{+}CD24\textsuperscript{-} VN9 cells).

**Chemicals**

In the research study, the library of the 34 extract (Table 1), which were coded with ‘E’ as the initial label (i.e. E1-E34), were obtained from the Division of Medicinal Chemistry, Faculty of Chemistry, University of Science, Vietnam National University Ho Chi Minh City, Vietnam. Doxorubicin hydrochloride and tirapazamine were purchased from Sigma-Aldrich.

**Cell culture in monolayer (2D) and three-dimensional (3D) culture**

For 2D models, single cells (MCF-7, CD44\textsuperscript{+}CD24\textsuperscript{-} MCF-7, VN9 or CD44\textsuperscript{+}CD24\textsuperscript{-} VN9) were harvested and seeded in 96-well plates at a final density of 1000 cells per well, and grown for 5 days. Fresh medium was replenished every two days. Cancer cells were cultured in DMEM/F12, 10% FBS (Sigma-Aldrich), and 1% antibiotic-antimycotic (Sigma-Aldrich). CD44\textsuperscript{+}CD24\textsuperscript{-} cancer cells were cultured in M171 medium (Thermo Fisher Scientific) with MEGS supplement (Thermo Fisher Scientific).

**RESULTS**

**IC\textsubscript{50} values of extracts are different on MCF-7 2D and 3D models**

The IC\textsubscript{50} results of doxorubicin and tirapazamine showed that both 2D and 3D models were successfully established for anti-tumor activity evaluation (Table 2). The IC\textsubscript{50} results of the 34 plant extracts on MCF-7 breast cancer cells in 2D and 3D models are summarized in Table 3. There were 12/34 extracts which showed effects on both 2D and 3D culture models. These 12 extracts were: E4, E10, E11, E12, E17, E18, E20, E21, E22, E27,
Table 1: List of 34 natural extracts used in this study

| Code of extract | Plant (solvent)                      | Code of extract | Plant (solvent)                      |
|-----------------|--------------------------------------|-----------------|--------------------------------------|
| E4              | Buchanania Latifolia – (CH$_3$OH)    | E26             | Anisoptera costata – (CH$_3$OH)      |
| E7              | M. Camptosperma – (CH$_3$OH)         | E27             | Anisoptera costata – (CH$_3$OH)      |
| E8              | D. Dyeri – (CH$_3$OH)                | E28             | Willughbeia cochinchinensis – (CH$_3$OH) |
| E9              | H. recpeoi – (CH$_3$OH)              | E30             | Strebus ilicifolius – (CH$_3$OH)     |
| E10             | H. recpeoi – (CH$_3$OH)              | E31             | B. pandurate – (CH$_3$OH)            |
| E11             | S. thoreli – (CH$_3$OH)              | E32             | Paramignya trimera – (CH$_3$OH)      |
| E12             | S. thoreli – (CH$_3$OH)              | E35             | Mangifera mekongiensis – (CH$_3$OH)  |
| E13             | D. turbinatus – (CH$_3$OH)           | E36             | Embelia ribes – (CH$_3$OH)           |
| E14             | D. turbinatus – (CH$_3$OH)           | E37             | Willughbeia cochinchinensis – (C$_4$H$_8$O$_2$) |
| E15             | D. costatus – (CH$_3$OH)             | E38             | Artocarpus heterophyllus – (C$_4$H$_9$O$_2$) |
| E16             | D. costatus – (CH$_3$OH)             | E39             | Mangifera mekongiensis – (C$_4$H$_5$O$_2$) |
| E17             | Hopea odorata – (CH$_3$OH)           | E40             | Taxus wallichiana – (CH$_2$Cl$_2$)    |
| E19             | Vatica odorata – (CH$_3$OH)          | E41             | Caesalpinia sappan – (CH$_3$Cl$_2$)   |
| E20             | Vatica odorata – (CH$_3$OH)          | E42             | Trigona minor – (Hexan)               |
| E21             | Dipterocarpus alatus – (CH$_3$OH)    | E43             | B. pandurate - (Chloroform)           |
| E22             | Shorea roxburghii – (CH$_3$OH)       | E45             | Swintonia floribunda – (CH$_3$OH)     |
| E25             | K. laurifolia – (CH$_3$OH)           | E46             | Mangifera reba Pierre 1897 – (CH$_3$OH) |

Figure 1: The 3D cell culture method using matrigel. The matrigel and the cells were seeded with density of 1000 cells/well. The matrigel was established on the edge of the well after 30 mins in 37°C which has crescent shape. After 5 days in progress, the drug testing was processed in 48 hours.
### Table 2: The IC$_{50}$ of doxorubicin and tirapazamin on cell lines

| Cell lines       | Models | IC$_{50}$ DOX (ng/mL) | IC$_{50}$ TPZ (µg/mL) |
|------------------|--------|-----------------------|-----------------------|
| VN9              | 2D     | 1476                  | 292                   |
|                  | 3D     | 1868                  | 128                   |
| CD44$^+$/CD24$^-$ VN9 | 2D   | 98.52                 | 315.2                 |
|                  | 3D     | 1711                  | 105.4                 |
| MCF-7            | 2D     | 1674                  | 159.4                 |
|                  | 3D     | 2354                  | 68.14                 |
| CD44$^+$/CD24$^-$ MCF-7 | 2D | 278.3                | 174.9                 |
|                  | 3D     | 3131                  | 147                   |

**Abbreviations:** DOX: doxorubicin, TPZ: tirapazamin, IC$_{50}$: half inhibitory concentration

### Table 3: The IC$_{50}$ values of 34 extracts on MCF-7 breast cancer cell line

| Extracts | 2D model | 3D model | Extracts | 2D model | 3D model |
|----------|----------|----------|----------|----------|----------|
| E4       | 187.5    | 383.7    | E25      | 597.4    | 870.8    |
| E7       | 248.2    | 332.8    | E27      | 165.3    | 242.5    |
| E8       | 478.7    | 533.1    | E28      | 299.7    | 673.3    |
| E9       | 701.4    | 653.5    | E30      | 1476     | 794      |
| E10      | 310      | 154.7    | E31      | 257.3    | 308.5    |
| E11      | 342.9    | 198.3    | E32      | 235.2    | 225.1    |
| E12      | 303.5    | 160.4    | E35      | 4450     | 615.9    |
| E13      | 1779     | 1061     | E36      | 2187     | 575.5    |
| E14      | 348.6    | 593      | E37      | 326.1    | 308.8    |
| E15      | 1106     | 639.8    | E38      | 368.1    | 692.2    |
| E16      | 316.8    | 361.9    | E39      | 345.6    | 270.6    |
| E17      | 159.4    | 232.4    | E40      | 70       | 1419     |
| E18      | 112.4    | 230      | E41      | 526.2    | 2063     |
| E19      | 489.5    | 621.8    | E42      | 499.7    | 359.6    |
| E20      | **86.42**| 168.4    | E43      | 306      | 620.4    |
| E21      | **57.67**| 71.97    | E45      | 155      | 361.9    |
| E22      | **83.58**| 87.92    | E46      | 135.4    | 387.6    |
Table 4: The IC\textsubscript{50} values of 34 extracts on CD44\textsuperscript{+}CD24\textsuperscript{-} MCF-7 breast cancer cell line

| Extracts | IC\textsubscript{50} values (\(\mu\text{g/mL}\)) | Extracts | IC\textsubscript{50} values (\(\mu\text{g/mL}\)) |
|----------|--------------------------------|----------|--------------------------------|
|          | 2D model | 3D model | 2D model | 3D model |
| E4       | 66.2     | 360.3    | E25      | 173.6    | 587.8    |
| E7       | 69.42    | 307.7    | E27      | 80.45    | 214.1    |
| E8       | 103.2    | 937.8    | E28      | 134.9    | 481.6    |
| E9       | 153      | 887.8    | E30      | 508.4    | 935.9    |
| E10      | 50.48    | 162.2    | E31      | 85.04    | 253.5    |
| E11      | 58.14    | 217.5    | E32      | 56.65    | 223      |
| E12      | 61.95    | 162      | E35      | 73.95    | 624.6    |
| E13      | 262.4    | 1243     | E36      | 103.5    | 322.7    |
| E14      | 74.52    | 375.2    | E37      | 229.8    | 980.4    |
| E15      | 258.3    | 699.7    | E38      | 70.91    | 386.6    |
| E16      | 20.31    | 56.97    | E39      | 62.65    | 386.6    |
| E17      | 35.6     | 89.56    | E40      | 227.4    | 1648     |
| E18      | 31.88    | 227.4    | E41      | 303.3    | 1257     |
| E19      | 145      | 39.89    | E42      | 102.3    | 293.9    |
| E20      | 20.91    | 110.4    | E43      | 60.97    | 449.6    |
| E21      | 20.31    | 56.97    | E44      | 32.16    | 295.5    |
| E22      | 809.5    | 110.4    | E45      | 71.98    | 459.6    |

Abbreviation: IC\textsubscript{50}: half inhibitory concentration

E45, and E46. However, most of the extracts predominantly had effects on the 2D model. In fact, 27 extracts on the 3D models were correlated with increased resistance by the cancer cells as compared to the resistance on the 2D models. Specifically, there were 7 extracts that had an IC\textsubscript{50} values in the 3D model which were lower than in the 2D culture model. The 7 extracts were: E10, E12, E15, E30, E35, E36, and E42 (Figure 2). Thus, they are potential candidates for further use in the 3D culture model of MCF-7 breast cancer.

The results of hit extracts on CD44\textsuperscript{+}CD24\textsuperscript{-} MCF-7 in 2D and 3D models

There were 7/34 extracts that had effects on both 2D and 3D culture models. These 7 extracts were: E7, E10, E12, E17, E18, E19, E21, and E45. However, the majority of the extracts predominantly showed effects on the 2D model (Table 5). As seen in Table 5, cells grown in the 3D model showed more resistance to the effects of the 32 extracts than the cells grown in the 2D model. In particular, there were 2 extracts which had IC\textsubscript{50} values in the 3D model that were lower than the values in the 2D model; those 2 extracts were E26 and E22 (Figure 3). Therefore, they are potential candidates for further research in the 3D culture model of the MCF-7 breast cancer stem cell (CSC). Comparing with the results on the MCF-7 cell line, it was observed that the CD44\textsuperscript{+}CD24\textsuperscript{-} sub-population of MCF-7 cells has a much higher resistance to the same extracts tested.

The results of hit extracts on VN9 cultured in 2D and 3D models

There were 5/34 extracts which showed effects on both 2D and 3D culture models. The 5 extracts were: E4, E7, E20, E21, and E45. However, most of the extracts had predominant effects on the 2D models (Table 6). As Table 6 demonstrates, 29 extracts on the 3D models were correlated with increased resistance by the cancer cells, as compared to their resistance on the 2D models. In particular, 5 extracts had IC\textsubscript{50} values in the 3D model that were lower than the values in the 2D model. The 5 extracts were: E15, E18, E22, E25, and E30 (Figure 4). Therefore, these are potential...
Table 5: Summary of hit extracts on each cell types and models

| Cells          | 2D model       | 3D model       | The extracts more sensitive on 3D than 2D |
|----------------|----------------|----------------|------------------------------------------|
| MCF-7          | E20, E21, E22, E40 | -              | E10, E12, E15, E30, E35, E36, E42        |
| CD44⁺ CD24⁻ MCF-7 | E4, E7, E10, E11, E12, E14, E16, E17, E19, E20, E21, E27, E31, E32, E35, E39, E43, E45, E46 | E17, E18, E20 | E26, E22 |
| VN9            | -              | -              | E15, E18, E22, E30                       |
| CD44⁺ CD24⁻ VN9 | E4, E7, E10, E11, E12, E14, E16, E17, E19, E20, E21, E31, E32, E35, E39, E45, E47 | E7, E21 | E18, E22 |

Table 6: The IC₅₀ values of 34 extracts on VN9 breast cancer cell line

| Extracts | IC₅₀ values (μg/mL) | Extracts | IC₅₀ values (μg/mL) |
|----------|---------------------|----------|---------------------|
|          | 2D model            | 3D model            | 2D model            | 3D model            |
| E4       | 238.9               | 518.2               | E25                 | 4681                | 722.9               |
| E7       | 345.6               | 297                 | E27                 | 497.8               | 345.1               |
| E8       | 1287                | 588.2               | E28                 | 1806                | 613.5               |
| E9       | 916.8               | 535.7               | E30                 | 4976                | 1568                |
| E10      | 712.6               | 270.9               | E31                 | 293.5               | 463.9               |
| E11      | 559.2               | 686.4               | E32                 | 403.6               | 347                 |
| E12      | 635.9               | 496.2               | E35                 | 5799                | 3004                |
| E13      | 7756                | 2706                | E36                 | 7437                | 3005                |
| E14      | 531.4               | 638.3               | E37                 | 559.3               | 977.5               |
| E15      | 5744                | 1088                | E38                 | 3158                | 2260                |
| E16      | 377.7               | 987.1               | E39                 | 697.3               | 847.6               |
| E17      | 211                 | 431                 | E40                 | 267.2               | 1212                |
| E18      | 2055                | 430.6               | E41                 | 1083                | 871.1               |
| E19      | 357.5               | 654                 | E42                 | 2136                | 963.8               |
| E20      | 103.8               | 122.6               | E43                 | 247.5               | 504.1               |
| E21      | 304.2               | 146.1               | E45                 | 196.1               | 262                 |
| E22      | 2964                | 324.1               | E46                 | 1080                | 417.3               |

Abbreviation: IC₅₀: half inhibitory concentration
Figure 2: Comparing the IC\textsubscript{50} values of 34 extracts, doxorubicin and tirapazamine on MCF-7 breast cancer cell line. Scale 1: Red corresponds to sensitivity, green corresponds to high resistance. Scale 2: Black corresponds to ratio of 2D/3D concentration is greater than 1. Gray white corresponds to ratio of 2D/3D concentration is less than 1. Abbreviations: TPZ: tirapazamine, 2D: mononuclear cell culture, 3D: three-dimensional cell culture model, IC\textsubscript{50}: half inhibitory concentration.

Figure 3: Comparing the IC\textsubscript{50} values of 34 extracts, Dox and TPZ on CD44\textsuperscript{+}CD24\textsuperscript{−} MCF-7 breast cancer cell line. Scale 1: Red corresponds to sensitivity, green corresponds to high resistance. Scale 2: Black corresponds to ratio of 2D/3D concentration is greater than 1. Gray white corresponds to ratio of 2D/3D concentration is less than 1. Abbreviations: Dox: doxorubicin, TPZ: tirapazamine, 2D: mononuclear cell culture, 3D: three-dimensional cell culture model.
Figure 4: Comparing the IC_{50} values of 34 extracts, Dox and TPZ on VN9 breast cancer cell line. Scale 1: Red corresponds to sensitivity, green corresponds to high resistance. Scale 2: Black corresponds to ratio of 2D/3D concentration is greater than 1. Gray white corresponds to ratio of 2D/3D concentration is less than 1. Abbreviations: Dox: doxorubicin, TPZ: tirapazamine, 2D: mononuclear cell culture, 3D: three-dimensional cell culture model.

Figure 5: Comparing the IC_{50} values of 34 extracts, Dox and TPZ on CD44^+CD24^- VN9 breast cancer cell line. Scale 1: Red corresponds to sensitivity, green corresponds to high resistance. Scale 2: Black corresponds to ratio of 2D/3D concentration is greater than 1. Gray white corresponds to ratio of 2D/3D concentration is less than 1. Abbreviations: Dox: doxorubicin, TPZ: tirapazamine, 2D: mononuclear cell culture, 3D: three-dimensional cell culture model.
candidates for further studies in the 3D culture model of VN9 breast cancer.

**Results of hit extracts on CD44+CD24- VN9 cultured in 2D and 3D**

There were 6/34 extracts affected both the 2D and 3D culture models: E4, E7, E10, E12, E18, and E45. Most of the extracts, however, mainly affected the 2D models (Table 7). As shown in Table 7, 32 extracts on the 3D models were correlated with increased resistance by the cancer cells, as compared to their resistance on the 2D models. In particular, there were 2 extracts which had IC50 values in the 3D model that were lower than those in the 2D culture model; these extracts were E18 and E22 (Figure 5). Thus, they are potential candidates for further studies in the 3D VN9 breast CSC model. Comparison of screening results of VN9 with CD44+CD24- phenotype versus the original VN9 demonstrated that the CSC cell line (CD44+CD24- VN9) was more resistant to the extracts in the 3D culture model. Therefore, in this study, the number of extracts tested that showed an effect on this cell line was 2, indicating that VN9 CSC can carry more resistant characteristics than normal cells.

**DISCUSSION**

The use of bio-matrix substrates (such as matrigel) to create 3D culture models is very convenient for drug screening. Use of a gel forming method- that contains the cells on the side of the culture well in a 96-well plate- facilitates easy manipulation without disrupting the gel structure or limiting cell growth in the form of single layer in the center of the well. This method also allows the creation of a 3D cell mass with a size of 100 μm within 5 days of culture. The drug test is conducted in 48 hours such that the entire drug testing procedure can be summarized in 7 days. In order to minimize errors when comparing 2D and 3D models, we conducted all experiments with both models in parallel. For both 2D and 3D models, the threshold of extracting effect was lower than 200 μg/mL.

A number of published studies have show that 3D breast cell culture better reflect the histological, biological, and molecular features of primary tumors than the same cells cultured using traditional 2D techniques15. In a study by Imamura et al., on a 3D breast cancer model, the breast cell mass was found to have the presence of a hypoxic cell population7; it is for this reason that the cell mass becomes sensitive to tiparazamine. In our study, we show that 10 extracts have the same effect as tiparazamine on breast cancer cells, and that they might be suitable candidates for hypoxia-targeted drug development (Table 5). Furthermore, in their study, Imamura and colleagues also showed that expression of Ki-67 was less in 3D breast cancer cell mass than in 2D, suggesting that the greater G0-dormant subpopulation was responsible for drug resistance in 3D culture.

Many studies have show that the breast cancer cell population with phenotype CD44+CD24- possesses higher tolerability to chemotherapy, hormone therapy, and radiotherapy16-21. Thus, for the drug screening in our study, these 4 breast cancer cell lines were suitable for our evaluations: MCF-7, CD44+CD24-MCF-7, VN9, CD44+CD24- VN9. Moreover, a promising outcome from our study is the identification of 10 extracts which have a more sensitive effect on the 3D culture model than the 2D culture model. These 10 extracts include: E10, E12, E15, E18, E22, E26, E30, E35, E36, and E42. These could be suitable candidates for the next steps towards developing drugs that target the hypoxic region in breast cancer. Therapies targeting cancer cells in areas of hypoxia and studies to discern mechanisms have garnered increased interest for cancer treatment. Hypoxia-related mechanisms such as overexpression of hypoxia-inducible factor (HIF) are also important avenues of research. Inhibiting HIF activity and changing the molecules involved in HIF offer hope for identifying molecular target to inhibit tumor growth or even completely halt growth22. HIF-1 also induces an increase in adenosine 2B receptor expression, thereby promoting the enrichment of breast cancer stem cells by activating protein kinase C-β23. Therefore, in the study herein, it was shown that use of a 3D model of breast cancer cell culture for drug screening reflects a huge difference in drug resistance and drug sensitivity when compared to the 2D culture model. The matrigel 3D culture model is significant for screening compounds related to hypoxia-based therapy for breast cancer.

**CONCLUSION**

Medium-throughput screening on breast cancer cell models MCF-7, CD44+CD24- MCF-7, VN9, and CD44+CD24- VN9, in 2D and 3D culture, with 34 extracts showed that resistance to these extracts occurred when cancer cells were cultured in 3D. Resistance to extracts also manifested in the CD44+CD24- cell populations (i.e. CSC populations). There were 12/34 and 7/34 extracts which affected MCF-7 and CD44+CD24- MCF-7 cells, respectively. For the Vietnamese breast cancer cell line...
(VN9), there were 5/34 and 6/34 extracts which affected the VN9 and CD44+CD24+ VN9 cells, respectively. Overall, our study results indicated 10 potential candidates for future drug development targeting hypoxia in breast cancer.

**ABBREVIATIONS**

Dox: Doxorubicin  
HIF: Hypoxia-Inducible Factor  
TPZ: Tirapazamine  
VN9: Vietnamese breast cancer cell line #9

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interests regarding the publication of this article.

**AUTHOR CONTRIBUTIONS**

Nhan Phan designed the project and carried out the experiments. Khuong Pham contributed to feasibility experiments. Mai Nguyen provided the extract. Nhan Phan analyzed the data and wrote the paper with contributions from all authors. Phuc Pham, Kiet Truong and Ngoc Phan suggested the idea, corrected the scientific matters, english wording and review all paper.

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**REFERENCES**

1. Futamura Y, Yamamoto K, Osada H. Phenotypic screening meets natural products in drug discovery. Biosci Biotechnol Biochem. 2017;81(1):28–31. PMID:27885937. Available from: https://doi.org/10.1080/09168451.2016.1248365.

2. Alexandrov V, Brunner D, Hanania T, Leahy E. High-throughput analysis of behavior for drug discovery. Eur J Pharmacol. 2015;750(89):82. PMID:25592319. Available from: https://doi.org/10.1016/j.ejphar.2014.11.047.

3. Mullard A. Microfluidics platform lowers barrier to drug combination screening. Nat Rev Drug Discov. 2018;17(10):691–692. PMID:25592319. Available from: https://doi.org/10.1038/nrd.2018.161.

4. Verjans ET, Doijen J, Luyten W, Landuyt B, Schoofs L. Three-dimensional cell culture models for anticancer drug screening: Worth the effort? J Cell Physiol. 2018;233(4):2993–3003. PMID:28618001. Available from: https://doi.org/10.1002/jcp.26052.

5. Haycock JW. 3D cell culture: a review of current approaches and techniques. Methods Mol Biol. 2011;2011(695):1–15.
PMID: 21042962. Available from: https://doi.org/10.1007/978-1-60761-984-0_1.

6. Breslin S, O’Driscoll L. Three-dimensional cell culture: the missing link in drug discovery. Drug Discov Today. 2013;18(5-6):240–249. PMID: 23073387. Available from: https://doi.org/10.1016/j.drudis.2012.10.005.

7. Imamura Y, Mukohara Y, Shimono T, Funakoshi Y, Chayahara Y, Toyoda N, et al. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol Rep. 2015;33(4):1837–1843. PMID: 25634491. Available from: https://doi.org/10.3892/or.2015.3767.

8. Abe-Fukasawa N, Otsuka K, Aihara A, Itasaki N, Nishino T. Novel 3D Liquid Cell Culture Method for Anchorage-independent Cell Growth, Cell Imaging and Automated Drug Screening. SciRep. 2018;8(1):3627. PMID: 29483620. Available from: https://doi.org/10.1038/s41598-018-21950-5.

9. Mittler F, Obeïd P, Rulina AV, Haguet V, Gidrol X, Balakirev MY. High-Content Monitoring of Drug Effects in a 3D Spheroid Model. Front Oncol. 2017;(7):293. PMID: 28722778. Available from: https://doi.org/10.3389/fonc.2017.00293.

10. Shin HS, Hong HJ, Koh WG, Lim JY. Organotypic 3D Culture in Nanoscaffold Microwells Supports Salivary Gland Stem-Cell-Based Organization. ACS Biomater Sci Eng. 2015;2(12):4311–4320. PMID: 30591951. Available from: https://doi.org/10.1021/acsbiomaterials.8b00894.

11. Dolega ME, Abeille F, Picollet-D’hahan N, Gidrol X. Controlled 3D culture in Matrigel microbeads to analyze clonal acinar development. Biomaterials. 2015;35:347–357. PMID: 25818441. Available from: https://doi.org/10.1016/j.biomaterials.2015.02.042.

12. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Seminars in cancer biology. 2005;15(5):378–386. PMID: 15975825. Available from: https://doi.org/10.1016/j.semcancer.2005.05.004.

13. Lee JM, Mhawech-Fauceglia P, Lee N, Parsanian LC, Lin YG, Gayther SA, et al. A three-dimensional microenvironment alters protein expression and chemosensitivity of epithelial ovarian cancer cells in vitro. Lab Invest. 2013;93(5):528–42. PMID: 23459371. Available from: https://doi.org/10.1038/lab.2013.41.

14. Palomeras S, Ruiz-Martinez S, Puig T. Targeting Breast Cancer Stem Cells to Overcome Treatment Resistance. Molecules. 2018;23(9). PMID: 30202622. Available from: https://doi.org/10.3390/molecules23092193.

15. Sun H, Jia J, Wang X, Ma B, Di L, Song G, et al. CD44+/CD24-breast cancer cells isolated from MCF-7 cultures exhibit enhanced angiogenic properties. Clin Transl Oncol. 2013;15(1). PMID: 22855175. Available from: https://doi.org/10.1007/s12094-012-0891-2.

16. Deng X, Apple S, Zhao H, Song J, Lee M, Luo W, et al. CD24 Expression and differential resistance to chemotherapy in triple-negative breast cancer. Oncotarget. 2017;8(24):38294–38308. PMID: 28418843. Available from: https://doi.org/10.18632/oncotarget.16203.

17. Rodriguez CE, Berardi DE, Abrigo M, Todaro LB, de Kier Joffé ED, Fiszman GL. Breast cancer stem cells are involved in Trastuzumab resistance through the HER2 modulation in 3D culture. J Cell Biochem. 2018;119(2):1381–1391. PMID: 28722778. Available from: https://doi.org/10.1002/jcb.26298.

18. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Seminars in cancer biology. 2005;15(5):378–386. PMID: 15975825. Available from: https://doi.org/10.1016/j.semcancer.2005.05.004.