Efficacy of Resveratrol Against Advanced Breast Cancer-Derived Organoids: A comparison with that of Clinically Relevant Drugs

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Abstract

Background: The lack of reliable drugs is a therapeutic challenge of advanced breast cancers (ABCs). Resveratrol (Res) exerts inhibitory effects on breast cancer cell lines and animal models, while its efficacy against individual breast cancer cases remain unknown. This study aims to address this issues using ABC-derived organoids (ABCOs) as the ex vivo therapeutic model.

Methods: The ABCOs derived from 24 ABC patients were treated with 100 mM resveratrol for 96 hours. Paclitaxel, gemcitabine and fulvestrant were cited as the efficacy controls. Trypan blue staining and Calcein-AM/PI double staining were conducted to elucidate the cell viability, and EdU proliferation assay to evaluate ABCO growth activity. Reactive oxygen species (ROS) levels in Res-treated ABCOs were determined by peroxide-dependent DCFH-DA oxidation assay and the statuses of pSTAT3-mediated signaling was examined by immunofluorescent and immunohistochemical labeling.

Results: ABCOs were established from 6 surgical specimen, 14 needle biopsies, 3 cancerous pleural effusion and 1 cancerous ascites. Immunohischemical staining confirmed that the ABCOs maintained ER, PR, HER2 and Ki67 expression patterns of their original tumors. ABCO proliferation and viability tests showed > 50% cell death rates in 79.2% (19/24) of Res-treated, 28.6% (2/7) fulvestrant-treated, 66.7% (4/6) paclitaxel-treated and 66.7% (6/9) gemcitabine-treated ABCOs. The increased ROS levels were found in Res-sensitive ABCOs. pSTAT3 nuclear translocation were more frequent in Res-sensitive (14/19; 73.7%) than that (1/5; 20%) of Res-insensitive ABCOs, which were suppressed upon Res treatment. Statistical analysis revealed close correlation of STAT3 activation with the efficacy of Res.

Conclusions: We demonstrate for the first time the effectiveness and broader range of Res against different subtypes of ABCOs in comparison with that of conventional anti-breast cancer drugs, providing an alternative approach for better management of breast cancers, especially those at advanced stage.

Background

Breast cancer (BC) is the commonest female malignancy, accounting for 15% of the total cancer mortality in women [1]. Advanced breast cancer (ABCs) includes local spreading and distant metastatic breast cancers and metastases are the leading cause of the death. Because few third-line chemotherapy drugs have been so far available, first-line and second-line anti-BC drugs have to be used to treat BC patients at the advanced stage [2, 3]. Nevertheless, the therapeutic effects of those drugs on ABCs are usually unsatisfactory with intolerable adverse effects such as bone marrow suppression, liver and kidney malfunction and cardiotoxicity [4]. Although a panel of targeted drugs have been developed for BC treatment, such as the ones oriented to HER2, CDK4/6 or mTOR [5, 6], about 30% of early BC cases eventually reaches to the advanced stage, and 90% of them acquires multidrug resistance (MDR) [7]. Therefore, it is worthwhile to explore alternative agent(s) to efficiently treat advanced BCs without further damaging Patients' health.

Resveratrol (Res), as a kind of natural polyphenol compound, exhibits inhibitory effects on many kinds of cancers [2, 8] via induction of oxidative stress [9, 10] and inactivation of STAT3 signaling [11, 12]. In the case of BCs, Res is able to suppress the lung metastasis in vitro and in vivo [13], and to improve the sensitivity of BC cells to clinically relevant anti-BC drugs [14–17]. More importantly, Res has little cytotoxicity to normal breast epithelial cells [10]. However, the above data are largely obtained from the established BC cell lines and their transplanted animal models, which may not reflect the real in vivo situation of individual ABC patients because of certain disadvantages of these two experimental models such as passage variation, genetic instability and species difference [18, 19]. On the other hand, some clinical trials have shown that Res can change breast promoter hypermethylation in women at high BC risk [20] and has positive effect on sex steroid hormone binding globulin (SHBG) and estrogen metabolism in postmenopausal women [21]. Those trials thus prove the potential of Res in the BC prevention and its safety in clinical application, while the efficacy of Res against BCs especially the advanced ones still remains uncertain. To validate whether the promising anti-BC effects of Res are also hold true for individual ABC patients, it is necessary to develop a more reliable experimental therapeutic model which is closer to the real situation of BC patients. Patient-derived tumor organoid (PDO) is a three-dimensional structure composed of cell components in the original specimen [22]. A body of evidence demonstrates that PDOs well maintain the basic phenotypic and biological features of their original tumors including the response manners to different drugs [18, 19]. Unlike animal tumor models, species difference can be avoided in PDO experimental system [18, 19]. PDOs are therefore regarded as an ideal in vitro model for personalized drug sensitivity assay as well as exploring new therapeutic agents [23–25]. Even though, no report has been so far available concerning the effect of resveratrol on PDOs derived from different subtypes of ABCs and its comparative advantage with other conventional anti-BC drugs. The current study aims to address the above issues by the use of PDOs of 24 advanced BC cases.

Materials And Methods

1. Ethic issue

This study was conducted after the proposal had been approved by the medical ethics committee of Guangdong Provincial People's Hospital, Guangzhou, China (No. KY-Q-2021-97029-3). All procedures were carried out in accordance with the Helsinki Declaration. The sources of the BC samples are shown in Table 1.

2. Sample collection and organoid culture

Fresh BC tissue or metastatic samples were washed 3-5 times in PBS (Gibco, NY, USA) containing 500 U/mL Penicillin (Gibco, NY, USA), 500 mg / mL Streptomycin (Gibco, NY, USA) and 50 mg / mL Nystatin (Sangon Biotech, Shanghai, China). The tumor tissues were cut into small pieces (about 0.5-1 mm in diameter) and incubated with 1 ml TrypLE (Gibco, NY, USA) at 37 ° C for 45 minutes. After digestion, the mixture was used to remove tissue fragments through a 70 mm cell filter, centrifuged for 300 rpm for 5

minutes, and then the isolated cells were re-suspended in standard BC culture medium and mixed with a 1:2 volume of Matrigel matrix (Corning, NY, USA). 20 ml droplets were added to the preheated 48-well plate. After the droplets were solidified, 200 ml of the standard ABCO medium was added to each well and incubated under 37 °C, 5% CO₂ and 90% humidity condition. The medium was renewed in two-day intervals. The standard BC organoid culture medium was composed of heparin (Stemcell technologies, Vancouver, Canada), cortisone (Stemcell technologies, Vancouver, Canada) and mammoCult™ Human Medium KIT (Stemcell technologies, Vancouver, Canada), 100 U / ml penicillin (Gibco, NY, USA), 100 mg / ml streptomycin (Gibco, NY, USA), 20 mg / ml of nystatin ((Sangon Biotech, Shanghai, China).

3. Histological and immunohistochemical staining

In order to show the three-dimensional structure of ABCO, 1ml cell recovery solution (Corning, NY, USA) was added to the ABCO containing well and incubated at 4°C for 1h. After being diluted with 1ml/well cold PBS, the solution was centrifuged (4000 rpm, 4 min, 4 °C) and then fixed in 4% paraformaldehyde for 1h. The fixed ABCOs were mixed with 2% warm agarose and embedded in paraffin. Paraffin sections and standard HE staining were performed on all ABCOs and tissues. The antibodies used were as follows: mouse anti- Bcl-2 (Proteintech, Chicago, USA), rabbit anti-Ki67 (Abcam, Cambridge, MA, USA), rabbit anti-pSTAT3 (Abcam, Cambridge, MA, USA) , rabbit anti-ER (Abcam, Cambridge, MA, USA), rabbit anti-PR (Abcam, Cambridge, MA, USA), rabbit anti-HER2 (Abcam, Cambridge, MA, USA), Coralite488-conjugated affinipure goat anti-rabbit IgG (Proteintech, Chicago, USA) and Coralite594-conjugated goat anti-mouse IgG (Proteintech, Chicago, USA). The experimental procedures of immunofluorescence and immunohistochemistry refer to the previously published experimental scheme[26]. The images were collected with Nikon immunofluorescent microscope (Nikon, ECLIPSE Ci-L, Tokyo, Japan).

4. Drug treatment

The ABCOs were cultured in ABCO medium containing 100 mM resveratrol (Sigma-Aldrich, USA) [27], 1 mg/ml paclitaxel (MedChemExpress, NJ, USA) [28], 1mM gemcitabine (MedChemExpress, NJ, USA) [29] or 1 mM fulvestrant (MedChemExpress, NJ, USA) [30], respectively. The treatments were last for 96h and the drug-containing medium was changed every 48 hours. After 96h of the treatments, the ABCOs were analyzed by cell viability/death test and EdU cell proliferation assay. The drug use scheme of drug sensitivity test from each patient is provided by clinicians to promote the principle of individualized treatment.

5. Cell viability assay

Cell viability was elucidated by trypan blue (Invitrogen, Carlsbad, CA, USA) discrimination staining and Calcein-AM/PI double staining kit (Beyotime, Shanghai, China) according to manufacture' instructions. Briefly, the ABCO cells were stained with 0.4% trypan blue, and the stained nonviable cells and unstained

viable cells were counted with blood cell counting plate and cell death rate was determined by the stained cell number/the total cell number. In Calcein-AM/PI double staining test, the living cells with intact membrane and esterase activity are distinguished by strong and uniform green fluorescence produced by enzymatic hydrolysis of Calcein-AM, while the dead cells with damaged membrane emit bright red fluorescence due to the combination of propidium iodide and nucleic acid. The cell labeling patterns of ABCO population were captured under fluorescent microscope (Nikon, ECLIPSE Ni-U, Tokyo, Japan), followed by calculation of the dead cell fraction. In order to compare the cell death rate caused by the same drug in ABCO populations from different patients, adjusted mortality (%) was calculated via removing the natural mortality of ABCOs (the death rates of normally cultured ABCOs) from the total one by the formular shown below. All the experiments were repeated for three times. The mean adjusted mortality was the mean of the adjusted mortality over three replicates.

Adjusted mortality (%) = (treatment mortality - control mortality) / (1 - control mortality) * 100%

6. Cell proliferation assay

For EdU cell proliferation assay, the ABCOs were cultured in 48 well plates and then treated with different anticancer drugs selected for test for 96 hours. The proliferation activity of ABCOs was determined by EdU Kit (Beyotime, Shanghai, China), following the manufacturer's instruction. Briefly, the ABCOs in each group were labeled with 5-acetyl-2'-deoxyuracil (EdU) for 8h, and then incubated with Click additive solution for 30 minutes in darkness at room temperature. 5 mg/ml Hoechst 33342 (Beyotime, Shanghai, China) was used for nuclear contrast staining. The images of Hoechst 33342 and EdU staining were captured by fluorescence microscope (Nikon, ECLIPSE Ni-U, Tokyo, Japan) respectively and merged thereafter.

7. Determination of intracellular reactive oxygen species (ROS) levels

2'-7'-dichlorodihydrofluorescein diacetate (DCFH-DA) is a non fluorescent intracellular compound, which is cleaved by cytolactonase and then converted to fluorescent compound under the oxidation of ROS. Intracellular ROS levels were measured by hydrogen peroxide dependent DCFH-DA oxidation (Beyotime, Shanghai, China). ABCOs were treated with 100 mM Res for 0h, 2h, 4h and 6h, and washed at each time point by DMEM/F12 (Gibco, NY, USA). The ABCOs were incubated with 10 mM DCFH-DA in DMEM/F12 (Gibco, NY, USA) for 20 min at 37°C in the darkness, followed by three washes with DMEM/F12. The ABCO-bearing coverslips were collected at 0, 2, 4 and 6 hour time points and stained with DCFH-DA in situ. The cells were observed and photographed with a fluorescence microscope (Nikon, ECLIPSE Ni-U, Tokyo, Japan).

8. Statistical analyses

All the experiments were repeated at least three times. Calcein/PI test data were analyzed by Student's t-test. Chi-square test was used to analyze the relationship between the efficacy of Res and the expression of pSTAT3. The data were analyzed by SPSS software (version 26.0; SPSS, Chicago, IL, USA). If necessary, P values are illustrated in the figure legend.

Results

1. Successful establishment of ABCO models

After obtaining the informed consent of the patients and their immediate family members, the tumor samples were collected from 24 patients with advanced BCs in the forms of surgical specimen (6), biopsies (14), cancerous pleural effusion (3), and cancerous ascites (1) as summarized in Table 1. All of those samples were successfully cultured to form PDOs. HE staining revealed that the PDOs retained the basic features of their original tumors in terms of the similar morphology, imbalanced plasma:nucleus ratio, nuclear atypia (Figure 1A and 1B) and ER, PR, HER2 and Ki67 expression patterns (Figure 1B).

2. Resveratrol suppressed proliferation of ABCOs

Res were used to treat all ABC cases and clinically relevant drugs were used selectively according to ER, PR, HER2 and Ki67 expression patterns of the individual cases by the procedure shown in Figure 2A (see Figure 2B-D and Table 1 for clinical information). After 96h Res treatment, phenotypic changes such as structural fragmentation, cell fragmentation, lack of clear edges and dullness of PDOs are considered to be the signs of Res-caused cell death (Figure 3A; BC23). In accordance, EdU cell proliferation assay showed that EdU-positive cells were frequently detected in most of the ABCOs in the untreated group, indicating the active proliferation status of the ABCOs; in contrast, distinct reduction and even absence of EdU-labeled cells was evidenced in 96h Res-treated ABCO populations such as BC23 cases shown in Figure 3A. In contrast, RES-insensitive ABCOs showed the clear organoid boundary and EdU-nuclear labeling after 96 hours of RES treatment (Figure 3A; BC24).

3. Significant increase of nonviable cell fraction in resveratrol-treated ABCO population

Based on trypan blue cell discrimination staining and Calcein/PI fluorescent labeling, the proportion of dead and living cells in ABCOs without (N) and with 96 hours resveratrol (Res) treatment were calculated. It was found that of 24 cases so far studied, 5 cases had less than 50% mean adjusted mortality (15.23% to 44.42%) and judged as Res-insensitive, 1 had 50-60% mean adjusted mortality as Res low sensitive, 11 had 60-80% mean adjusted mortality as Res sensitive and 7 had more than 80% mean adjusted mortality as Res highly sensitive cases (Table 2; Figure 3A-D). Calcein/PI fluorescent staining showed that

Res-sensitive ABCOs such as BC23 had a high proportion of dead cells than that of Res-insensitive BC24 (Figure 3A).

4. Broader anti-ABC range of resveratrol

The relevance of receptor expression patterns and pathological classification of BC patients with their ABCO sensitivity to Res was analyzed (Figure 3 B-D). The results revealed that the majority of ABCOs with different receptor expression patterns (ER, PR and HER2), histologic grades (I, II and III) or histological subtypes (IDC, ILC, IMPC) well responded to resveratrol in terms of reduction of EdU-labeling frequency, increased PI-labeled cells, suggesting the broad anti-BC range of Res irrespective to molecular and pathological classifications (Figure 3 B-D).

5. Comparative anti-ABCO advantage of resveratrol

According to the actual situation of individual patients, the conventional anti-BC drugs that were suitable for individual cases were selected as the therapeutic controls. The drug sensitivity test was carried out and the anti-ABCO efficacy of Res was compared with that of paclitaxel, gemcitabine and fulvestrant (Table 2). It was found that the > 50% cell killing rate of Res was achieved in 79.2% (19/24) of ABC cases, while that of fulvestrant was 28.6% (2/7), paclitaxel was 66.7% (4/6) and gemcitabine was 66.7% (6/9). Res exerted similar and even better inhibitory effects on 5 cases of ABCOs that were suitable for paclitaxel, gemcitabine and fulvestrant treatments (Figure 4A). The results of EdU cell proliferation assay were in accordance with the above findings (Figure 4B). BC3 patients had been treated by fulvestrant but the disease kept progress; 1 mM fulvestrant[30] caused 38.5% cell death rate of her ABCOs and 100 mM resveratrol was 75.8% (Figure 4A and 4B).

6. STAT3 inactivation in resveratrol-suppressed ABCOs

pSTAT3-oriented immunohistochemical staining revealed that pSTAT3 expressed was found in 75% (18/24) of ABCO cases, of which 17 cases were sensitive and 1 cases was insensitive to Res. Alternatively, of 25% (6/24) ABCO cases without pSTAT3 expressed, 2 cases were sensitive and 4 were insensitive to Res (Table 3). As shown in Figure 5A, the mean adjusted mortality of Res-treated BC9, BC17 and BC19 was 77.35%, 34.17% and 44.42% respectively and their pSTAT3 labeling intensities were from high to low. After 24 hours of Res treatment, the content of pSTAT3 and the frequency of nuclear translocation in BC9 decreased significantly, accompanied by the decrease of Bcl-2. Statistical analysis revealed a close correlation (Table 3; $P=0.006$) of pSTAT3 activation with resveratrol sensitivity of the ABCOs.

7. Increased ROS production in Res-sensitive ABCOs

Res has been shown to increase reactive oxygen species and cause mitochondrial damage in breast cancer cells that are sensitive to it [9]. ROS levels reached to the top at 2h time point in Res-sensitive ABCOs and then gradually decreased with time, suggesting that Res pro-oxidative stress is an early biological event (Figure 5B). In contrast, no elevated ROS generation was induced by Res in Res-insensitive ABCOs (Figure 5B).

Discussion

Patient-derived organoid was nominated as the annual technology in the field of life sciences in 2017 [31], because it well maintains the basic biological features of its parent tumor including the intratumoral heterogeneity, histological phenotypes, genomic alterations, transcriptome patterns and therefore the respond manner to the anticancer drugs [23, 32, 33]. It has been reported that positive predictive value of PDO-based drug screening is 88% and negative predictive value is 100% in forecasting the response to targeted agents or chemotherapy in vivo [34]. In the later case, drug-resistant PDOs can be used to explore the new therapeutic agents and their underlying mechanism [34, 35]. Currently, targeted therapy for breast cancer patients is largely based on the presence or absence of hormone receptors (ER, PR and HER2) [3]. In this study, we find that the organoids derived from advanced breast cancers (ABCOs) show both similar phenotypic features and hormone receptor expression patterns of their original counterparts. Therefore, the experimental therapeutic results obtained from those ABCOs would reflect the actual situation of the patients [23, 25, 36, 37]. For instance, BC3 organoids were the insensitivity to fulvestrant because this case had been treated by fulvestrant for 10 months but the disease kept progress and spread to the liver and the brain. Consequently, PDOs from patients with ABCs would be an ideal model for screening potential effective drugs. The effectiveness of Res against breast cancers has been proved in vitro and in animal models [9, 10, 13–17]. In addition, a number of clinical trials on women at high risk of breast cancers have proved that this polyphenol compound has the potential values in preventing breast cancer formation [20, 21]. For instance, the resveratrol metabolites could be found in the surgical cancer specimens of the breast cancer patients who had taken resveratrol seven days before operation, suggesting the long-term chemopreventive effect of this polyphenol compound without associated adverse effect [38, 39]. Even though, no pre-clinical data has been so far available concerning the efficacy of Res against different subtypes of breast cancers, especially the advanced ones with multidrug resistance and distal metastases. In order to evaluate the practical values of Res and to explore alternative approach for ABC management, Res was used to treat the ABCOs derived from 24 advanced BC cases. Meanwhile, the anti-BC spectrum and comparative advantages of Res with conventional drugs relevant with individual cases were investigated. The current chemotherapeutic drugs for ABCs include anti-mitotic taxanes and anti-metabolic gemcitabine, but they inevitably have multiple side-effects [4]. Fulvestrant as the first-line endocrine drug for postmenopausal women with ABC has less side effects, while some ABCs may eventually exhibit resistance to it [40]. In this study, only 2 out of 7 suitable cases (28.6%) are sensitive to fulvestrant in terms of > 50% cell killing rates. Therefore, the more reliable anticancer agent(s) with low toxicity is required to treat those patients, and Res would be an ideal candidate. Therefore, the efficacy of Res against advanced BC derived organoids (ABCOs) was

scrutinized and then compared with that of paclitaxel, gemcitabine and fulvestrant. The results showed that the total effective rate (> 50% cell death in organoids) of Res (79.17%) was significantly higher than that of paclitaxel (66.7%), gemcitabine (66.7%) and fulvestrant (28.6%). It was also noticed that the inhibitory effect of Res on ABCOs is not related with receptor expression patterns, clinicopathological classification as well as the histological grade of ABCs. These findings thus demonstrate for the first time that comparing with other three first-line anti-BC drugs, resveratrol has higher overall anti-ABCO efficacy and exerts similar or even better therapeutic effects on the individual ABCO cases suitable for paclitaxel, gemcitabine or fulvestrant treatments. The safety is another advantage of Res [9, 10, 41]. Clinical trials in colorectal cancer show that oral administration of 0.5 or 1.0g resveratrol per day is sufficient to suppress tumor cell growth without adverse reaction in vivo [42]. Given the above evidence and commercial availability of resveratrol as functional product and health-care agent, it would be reasonable to propose that this polyphenol compound may be suitable for ABC patients at least as an adjunct therapy.

Signal transducer and activator of transcription 3 (STAT3) is constitutively activated in many kinds of cancers, including more than 40% of BCs [43]. Because STAT3 constitutes a key oncogenic pathway and promotes BC metastasis [44], its activation is known as a critical factor for BC stem cell self-renewal and chemoresistance [45]. For these reasons, the activated STAT3 signaling is considered as potential target in BC management [46]. Res has multifaceted biological effects on cancer cells, of which suppression of STAT3 transcription and signal transduction is regarded as one of the crucial events [26, 47, 48]. Because the anti-ABCO efficacy of Res was unrelated to pathological subtypes, tumor growth sites and hormone receptor expression patterns, the influence of Res in the STAT3 signaling activation and its downstream Bcl-2 expression of ABCOs was investigated. The results revealed that pSTAT3 expression was observed in 18 out of 24 ABCO cases (75%). Because the ABCOs so far checked are derived from advanced BCs, their higher rate of STAT3 activation may suggest the important roles of STAT3 signaling in promoting BC progression. It is reasonable to consider that the ABCOs with STAT3 activation is targetable by Res and therefore sensitive to it. This notion is further supported by the findings from the five Res-resistant ABCO cases in which pSTAT3 levels are low or undetectable and thus without this critical target of Res. Alternatively, the above phenomena may indicate the presence of STAT3-dependent and STAT3-independent BC subtypes that would respond to Res differently. In this context, Res should be selectively used according to the status of STAT3 signaling of individual cases.

The ability of Res to cause DNA fragmentation and apoptosis of BC cells in vitro has been well documented [49, 50]. Res is known as an antioxidant agent, while accumulating data prove that high concentration of Res can promote ROS production rather than suppress oxidative stress [9, 50, 51]. Because ROS can mediate DNA fragmentation, the pro-oxidative effect may be another mechanism of Res-induced apoptosis of cancer cells as found in anaplastic thyroid cancer [52]. Additionally, the involvement of ROS is considered to be an early event during the treatment of high concentration of resveratrol [9]. However, similar datum from patient-derive ABCOs remains unavailable. In this study, we found that the ROS production of Res-sensitive ABCOs reached the highest level as early as 2h after Res treatment and gradually decreased to the baseline level at 6h time point, while ROS level in the Res-insensitive ABCOs remains as similar as that before Res treatment. These results may be explained as

the higher Res bioavailability in Res-sensitive BC cells with poorly operated resveratrol metabolic system, which may result in ROS accumulation and oxidative damage [52]. On the other hand, the higher antioxidant enzyme (superoxide dismutase 2 and catalase) levels produced in Res-resistant cancer cells may prevent Res-sensitive cancer cells from apoptosis [52, 53]. It would be therefore possible that the ability of Res to cause oxidative stress may depend upon the intrinsic state of antioxidant machinery of the involved ABCOs, which may be another parameter to predict the respond manners of BC cells and organoids to Res and other pro-oxidation drugs.

Conclusions

This study confirms for the first time the efficacy of Res against the organoids derived from different subtypes of the advanced breast cancers and demonstrates the comparative advantages of Res with clinically relevant anticancer drugs in terms of its therapeutic efficiency for individual ABCO case and its broader range against different types of BCs. Our study indicates that STAT3 signaling pathway is the key molecular target and the personalized therapeutic biomarker of Res. The statuses of intracellular antioxidant system would be another element to determine the respond manners of BC cells and organoids to Res. Because Res has been used to prevent breast cancer in high risk population, it may be also applicable to the treatment of the advanced breast cancers with multidrug resistance, STAT3 activation and malfunctioned antioxidant machinery.

Abbreviations

ABC: Advanced breast cancers; ABCOs: ABC-derived organoids; Bcl-2: B-cell lymphoma-2; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; IDC: Invasive ductal carcinomas; ILC: Invasive lobular carcinomas; IMPC: Infiltrating micropapillary carcinoma; MDR: Multidrug resistance; PDO: Patient-derived tumor organoid; PR: Progesterone receptor; pSTAT3: phospho-STAT3; Res: Resveratrol; ROS: Reactive oxygen species; SHBG: Sex steroid hormone binding globulin; STAT3: transducer and activator of transcription 3.

Declarations

Ethics approval and consent to participate

The use and storage of human material have been approved by the medical ethics committee of Guangdong Provincial People's Hospital, Guangzhou, China (No. KY-Q-2021-97029-3).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The samples received informed consent from the donor.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

H.L., and J.L., conceived and designed the experiments; K.W., and H.G., recruited the patients, provided the resected tissues; H.L., performed the pathological analysis of the mammary tissues and organoids; H.Y.,J.N.,M.L., and T.L., performed the experiments , analyzed the data and generated the figures; H.Y. wrote the manuscript; All the authors critically reviewed the manuscript.

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Tables

Table 1

Clinical information of 24 breast cancer cases

Specimen source	Manner	Patient code	Age
Breast	surgery	BC6	63
		BC19	51
		BC23	48
		BC24	47
	biopsy	BC7	46
		BC9	50
		BC10	38
		BC11	50
		BC13	49
		BC15	37
		BC21	43
Chest wall	surgery	BC5	61
		BC8	40
Bone	biopsy	BC12	57
		BC14	38
Liver	biopsy	BC3	58
		BC4	67
		BC17	47
		BC22	43
Pleural effusion	biopsy	BC2	50
		BC16	76
		BC18	56
Ascite	biopsy	BC20	52
Gluteus	biopsy	BC1	51

Table 2. Comparison of anti-BCO efficacy of Res and three conventional drugs

Table 3

Correlative analysis of STAT3 phosphorylation statuses and resveratrol anti-ABCO efficacy

pSTAT3 nuclear labeling			
Res efficacy	+	-	Sum
Sensitive	17	2	19
Resistance	1	4	5
Sum	18	6	24
Fisher exact test: P = 0.006<0.05			

Figures

Figure 1

Figure 1

ABCOs well maintain the biological characteristics of primary tumor tissue. A. HE staining (Left) and bright field imaging (Right) demonstrate the similar morphology of ABCOs and their original tumors (Scale bar, 50 µm). The insets are the corresponding organoids derived from the same tumors. B. ABCOs and their original tumors share the same receptor expression patterns (BC24, Scale bar, 25 µm).

Figure 2

A

B

C

D

Figure 2

Experimental scheme and clinical information of the advanced breast cancer specimens for ABCO culture

A. Overview of the experimental procedures to elucidating the efficacy and molecular effects of resveratrol against ABCO organoids. The clinically relevant drugs are cited as the controls. B-D. Classification of the ABCOs according to the breast cancer-associated receptor statuses (B), the histological subtypes (C) and histologic grades (D) of their original tumors. Blank: no reported.

Figure 3

Figure 3

Effects of resveratrol on different subtypes of ABCOs A. The images of brightfield phenotype (Scale bar, 50 μ m), Calcein-AM/PI viable (green)/nonviable (red) cell staining (Scale bar, 25 μ m) and EdU proliferating cell labeling (Scale bar, 25 μ m) of the ABCOs with or without 100 μ M Res treatment for 96 hours. B-D. The adjusted cell mortality of ABCO subtypes classified by their breast cancer-associated receptor statuses (B), the histological subtypes (C) and histologic grades (D). All data represent the means $\pm$ SD of three independent experiments.

Figure4

Figure 4

Comparison of the anti-ABCO efficacy of Res and the three clinically relevant anticancer drugs A. Cell mortality in ABCOs treated by Res, paciltaxol, fulvestrant and gemcitabine. *, $P < 0.05$ and **, $P < 0.01$ in comparison with the Control group at the experimental condition. B. Images of EdU cell proliferation assay (Scale bar, $10\text{ }\mu\text{m}$) of ABCOs treated for 96 hours by $100\text{ }\mu\text{M}$ Res, $1\text{ }\mu\text{g/ml}$ paciltaxol, $1\text{ }\mu\text{M}$ fulvestrant and $1\text{ }\mu\text{M}$ gemcitabine. C. The proportions of the average corrected cell mortality of ABCOs treated by $100\text{ }\mu\text{M}$ Res, $1\text{ }\mu\text{g/ml}$ paciltaxol, $1\text{ }\mu\text{M}$ fulvestrant and $1\text{ }\mu\text{M}$ gemcitabine for 96 hours. $> 80\%$ of average corrected mortality of ABCOs is indicated in blue, $60\text{-}80\%$ in red, $50\% - 60\%$ in green and $< 50\%$ in purple.

Figure 5

Figure 5

Inactivated STAT3 signaling and increased ROS production in Res sensitive but not insensitive ABCOs A. Reduction of pSTAT3 nuclear translocation and downregulation of Bcl-2 expression in Res-sensitive ABCOs of BC9 case and undetectable pSTAT3 nuclear translocation and Bcl-2 expression in Res-insensitive ABCOs of BC19 and BC17 cases. Scale bar, 25 μ m. B. Sequential assessment of ROS levels in Res-sensitive (BC11) and Res-insensitive ABCOs (BC24) after 100 μ M resveratrol treatment for 0h, 2h, 4h and 6h, respectively. ROS accumulation occurs and reaches to highest level at 2h time point. ROS was undetectable in BC24 organoids irrespective to Res treatment. Scale bar, 25 μ m.