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Original Article

Novel cancer cell lines derived from primary breast tumors in Chinese patients

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Abstract: Although many breast cancer cell lines have been used for cancer research during the past several decades, few have originated from primary tumors in Asian patients. Moreover, the incidence of breast cancer has been increasing rapidly in China during this time period. Therefore, it is essential to establish breast cancer cell lines from Chinese patients. Here, we report the establishment of three new breast cancer cell lines, designated BC-023, BC-024, and BC-034, from breast carcinoma tissues of three Chinese patients. These breast cancer cell lines grew as adherent monolayers with characteristic epithelial morphology and were maintained continuously *in vitro* with stable growth rates for at least 20 passages. No bacterial, fungal, or mycoplasma contamination was detected in any of the three cell lines. Additionally, these cells were human epidermal growth factor receptor 2 (C-erbB-2)-positive. All three cell lines had comparable population doubling times of 33-39 h and reproducibly formed colonies in soft agar. Furthermore, these cells displayed aggressive tumorigenicity. Thus, every characteristic of each of these cell lines meets the quality control standards of the American Type Culture Collection (ATCC). We used drug sensitivity testing with growth-inhibition assays and showed that these three lines expressed a wide range of sensitivities to cisplatin (DDP) and adriamycin (ADR). These studies indicate that these three novel cell lines may provide new models for studying cancer biology and for screening new drugs for breast cancer treatment, especially for the Chinese population.

Keywords: Breast cancer, cell lines, drug sensitivity

Introduction

Breast cancer is the most common malignant tumor that affects women [1, 2]. Cell lines are widely used in biological research, and hundreds of different human tumor type-specific cell lines have been employed in experimental cancer research as models for decades. While MCF-7, derived from a pleural effusion in 1973, is the most commonly used breast cancer cell line for *in vitro* studies, quite a few additional cell lines, including MDA-MB-231 and T-47D, have been routinely used as breast cancer models for more than 40 years [3, 4]. However, the phenotype of cells can change during long-term culture, and many of these established cell lines may exhibit phenotypic and genotypic instability, losing some of the original characteristics or eve their tumorigenicity [4, 5]. Obtaining permanent cell lines from primary breast cancer tissues is difficult; thus, it is no surprise that the majority of breast carcinoma cell lines have been initiated from tumor metastases, specifically from malignant pleural effusions [6, 7], while relatively few have been established from primary tumors [8]. Furthermore, most of the routinely used breast cancer cell lines were derived from Caucasians or African Americans and rarely from Asians. In fact, in the more than 70 breast cancer cell lines in the American Type Culture Collection (ATCC), only one of these cell lines was derived from an Asian (Hs 739.T, ATCC NO.: CRL-7477) patient. Thus, few models are available for the study of the molecular and cellular mechanisms of pathogenesis of breast cancer in Asian patients.

China is the world's most populous country, comprising approximately one-quarter of the

Table 1. Origins of the three breast cancer cell lines

Cell line	Species Nationality	Gender	Age	Source	Pathology
BC-023	Human, Chinese	Female	46	Right breast	Medullary carcinoma
BC-024	Human, Chinese	Female	58	Left breast	Invasive ductal carcinoma
BC-034	Human, Chinese	Female	54	Right breast	Invasive ductal carcinoma

world's population. In China, the incidence of breast cancer has increased significantly in recent decades [9, 10], highlighting the need to establish new breast cancer cell lines from xanthoderm to study the pathogenic mechanisms and evaluate novel therapeutic methods. In our study, three new breast cancer cell lines BC-023, BC-024, and BC-034 were established from three Chinese patients and further characterized. BC-024 and BC-034 were established from breast invasive ductal carcinoma tissues, while BC-023 was established from hyperplasia medullary carcinoma. These cell lines, which are all positive for human epidermal growth factor receptor 2 (C-erbB-2), may provide new experimental cell models to study the pathogenic mechanisms of breast cancer and to screen and evaluate new therapeutic strategies for breast cancer, especially for Chinese patients.

Materials and methods

Establishment and maintenance of cell lines

Three tumor samples were obtained from three Chinese female breast cancer patients who underwent primary mammary gland excision at the Zhongnan Hospital of Wuhan University (**Table 1**). Samples were then subjected to pathological examination. All patients provided informed consent for the use of surgical samples for these investigational and analytical studies, and the study was approved by the hospital ethics committee.

Tissue samples were processed within 1-3 h after surgical removal for primary culture according to described previously methods [11-13]. These three cell lines, named BC (breast cancer)-023, BC-024, and BC-034, were deposited in the China Center for Type Culture Collection (CCTCC) with designation numbers CCTCC-GDC0198, CCTCC-GDC0199, and CCTCC-GDC0200, respectively.

Assay of growth characteristics of the cell lines

Cell growth was estimated with the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) method [14-16]. The population doubling times of BC-023, BC-024, and BC-034 cells at passage 20 (P20) were studied. We plated approximately 2×10^3 cells per well in 96-well plates with 7 plates per cell line. One plate of each cell line was assayed every 24 h. MTT (Sigma) was added to each well and incubated with the cells for 4 h. After removal of MTT, 150 μl DMSO was added to the wells. The absorbance was measured with a Microplate Autoreader (Bio-Rad) at 570 nm as the test wavelength and 690 nm as the reference wavelength. The number of cells was calculated by comparison with the cell number on the first day after plating. The growth curves of BC-023, BC-024, and BC-034 were plotted, and the population doubling times were calculated during the exponential growth phase of the cells using online algorithm software (http://www.doubling-time.com).

Contamination tests

Testing for bacteria and fungi: Detection of bacterial and fungal contaminants in cell lines was performed according to the standard procedures of the ATCC [17]. Cells at P20 were cultured in the abovementioned medium without the addition of antibiotics for 5 d and examined as described previously [11].

Testing for mycoplasma: In order to investigate potential mycoplasma contamination, we used both direct and indirect assays [17]. Vero (CCTCC GDC0029) and B6yH4 (CCTCC GDC0017) cells obtained from CCTCC were used as negative and positive controls, respectively. Cultures were examined microscopically for typical mycoplasma colonies each week for at least 3 weeks. We performed indirect assays as described previously [11]. The cells were observed under a fluorescence microscope. Extracellular fluorescence indicated mycoplasma contamination.

Isoenzyme analysis

To verify that the three breast cancer cell lines were derived from humans without cross-con-

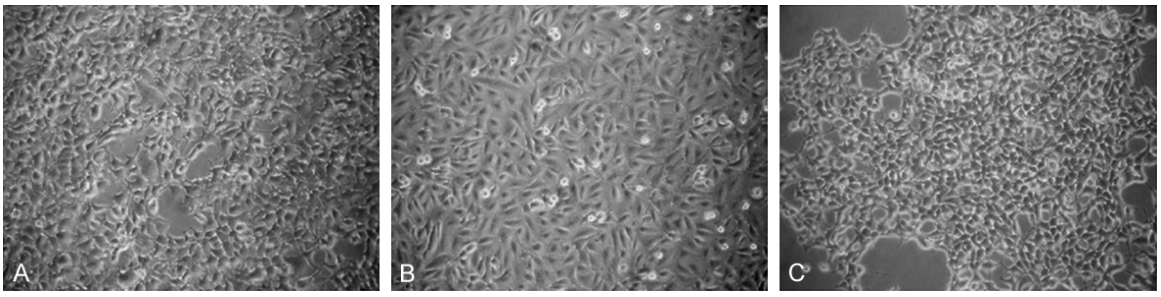

Figure 1. Morphology of the three breast cancer cell lines. A: BC-023 cell line at passage 22 (× 100); B: BC-024 cell line at passage 34 (× 100); C: BC-034 cell line at passage 27 (× 100).

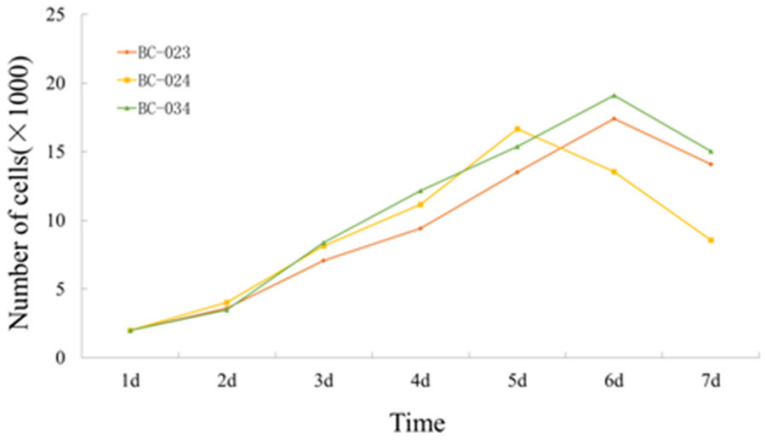

Figure 2. Growth curves of the three breast cancer cell lines. The cells were seeded at 2 × 10^3 cells per well, and growth was measured by MTT every 24 h for 7 d.

tamination from other species, we compared the profiles of lactate dehydrogenase (LD) and glucose-6-phosphate dehydrogenase (G6PD) in the breast cancer cell lines. LD and G6PD were analyzed using AuthentiKit™ (Innovative Chemistry) following the manufacturer instructions. We used HeLa cells and mouse fibroblast L929 cells as standard references.

Immunocytochemistry analysis

Immunocytochemistry methods were used to analyze the expression of estrogen receptor (ER), progesterone receptor (PR), and C-erbB-2, which are commonly used tumor markers for breast cancer in clinical diagnosis [18, 19]. The expression of ER, PR, and C-erbB-2 was detected using SuperRmEPC™ Breast Cancer Detection Kit (Maxim Biotechnology Development Co., Ltd.). Cells were cultured on slides for 24 h, washed three times with PBS, and then fixed for 10 min at room temperature. The MRC-5 cell line (ATCC CCL-171) and MCF-7 cell line (ATCC HTB-22) were used as negative and positive controls, respectively.

Anchorage-independent growth capacity analysis

Colony formation in soft agar was used to study the malignant phenotype of these cell lines. Anchorage-independent cell growth was measured in a soft-agar colony assay as described previously [20]. Briefly, 6-well plates were first covered with an agar layer consisting of 2 ml MEM with 0.5% agar (Sigma) and 4% FBS (Sijiqing). The middle layer contained 2 × 10^3 cells in 0.5 ml MEM with 0.33% agar and 4% FBS. The top layer, consisting of 2 ml MEM, was added to prevent drying of the agar. The plates were then incubated at 37°C in 5% CO_2 for 14 d. Cultures were evaluated and photographed. The number of colonies, defined as >50 cells/colony, were counted.

Tumorigenicity in nude mice

Tumorigenicity studies were performed using athymic male nude mice (BALB/c). Large-scale cultures were prepared at P20 for BC-023, P23 for BC-024, and P24 for BC-034. The cultured cells (1 × 10^7) were washed, resuspended in 0.2 mL PBS, and injected subcutaneously into the left groin of 4-week-old BALB/c (nu/nu) nude mice. The same procedure was performed with MRC-5 and MCF-7 cells to serve as negative and positive controls, respectively. Each

Table 2. Tests for detecting bacterial and fungal contaminants

Test medium	Temperature (°C)	Gas phase	Observation time (d)	Results
Brain heart infusion broth	37	Aerobic	14	Negative
	26			Negative
Blood agar plates	37	Aerobic	14	Negative
	37	Anaerobic		Negative
Trypticase soy broth	37	Aerobic	14	Negative
	26			Negative
Thioglycollate broth	37	Aerobic	14	Negative
	26			Negative
Sabouraud broth	37	Aerobic	21	Negative
	26			Negative
YM broth	37	Aerobic	21	Negative
	26			Negative
Martin modified medium	37	Aerobic	21	Negative
	26			Negative
Nutrient broth incline planes	37	Aerobic	21	Negative
	26			Negative

cell line was injected into 10 mice. All the mice were examined for development of palpable tumors every week for 8 weeks [21-23]. At the end of the experiment, the animals were euthanized, and the tumors were excised.

Assay of drug sensitivity

The drug sensitivity of BC-023, BC-024, and BC-034 cells was determined with the MTT assay as described previously [15, 24]. We tested the antitumor drugs adriamycin (ADR) and cisplatin (DDP). Each drug was diluted in MEM to the therapeutic peak plasma concentration (Cmax × 1) that is achieved by intravenous administration of clinical doses [25]. ADR was tested at 1.0 μg/ml, while DDP was tested at 10 μg/ml. The 10 × equivalents for each drug were also examined (Cmax × 10). The MEM with non-essential amino acids was supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. All drugs were obtained from the Zhongnan Hospital of Wuhan University and stored at -70°C solubilized in 100% DMSO.

Briefly, rapidly growing cells were harvested and counted and 5×10^3 cells were then added to wells of a 96-well flat-bottomed microtiter plate. Plates were incubated at 37°C in a humidified 5% CO_2 atmosphere for 96 h. The chemosensitivity assay was performed in triplicate. In addition, triplicate wells containing 5×10^3 cells suspended in complete medium were used as controls for cell viability, and triplicate wells containing only complete medium were used as controls for nonspecific MTT dye reduction. After 24 h, drugs were applied to triplicate culture wells, and cultures were incubated for 6 d at 37°C. One plate of each cell line was assayed every 24 h. The absorbance was measured after 4 h of incubation with MTT in a microtiter plate reader at 570 nm as the test wavelength and 690 nm as the reference wavelength. The number of cells was determined as a comparison of the MTT levels compared with the original cell number.

The control wells that did not contain cells had an OD_{570} of <0.005, and in comparison, samples with an OD_{570} value >0.1 were accepted for analysis. The background of the wells containing cells (including dead cells) without addition of MTT had an OD_{570} of <0.012 after 96 h of incubation, and the influence of dead cells could therefore be ignored in the present study. We used ANOVA and post-hoc Tukey HSD (honest significant difference) test to analysis the data. The viability of tumor cells was maintained at 75-90% during the 96-h incubation and the OD_{570} values before and after the 96-h incubation were 0.36 ± 0.17 and 0.33 ± 0.14, respectively, with the background subtracted out.

Results

Establishment of cell lines

Three breast cancer cell lines BC-023, BC-024, and BC-034 were established from breast carcinoma tissues from three different Chinese female patients. Within 6 d of primary culture, all the epithelial cells grew gradually from explants and formed several sparse colonies. Around day 15, the first subcultures of the new cell lines were performed, and an additional

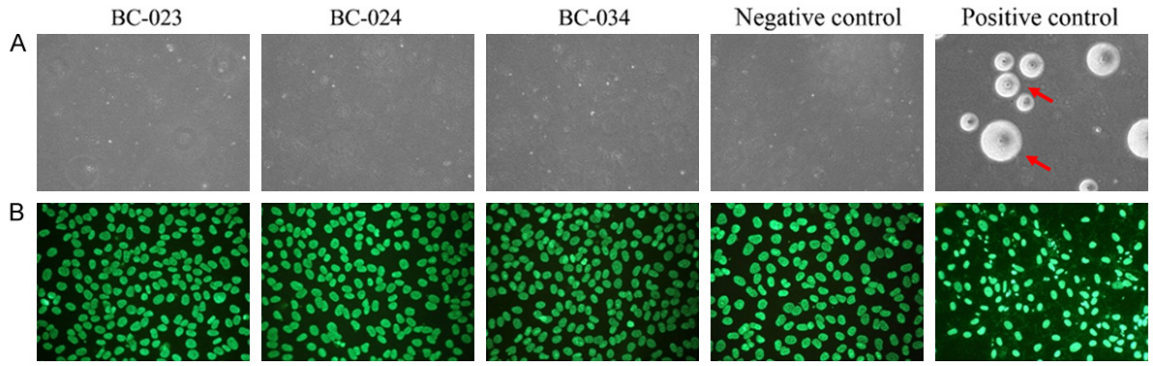

Figure 3. Mycoplasma analysis in breast cancer cell line cultures. A: Mycoplasma colonies were observed as fried-egg morphology in the positive control culture. B: Hoechst 33258 staining of cell line cultures. Mycoplasma contamination was observed as small extracellular fluorescent particles (red arrows indicate the positive control).

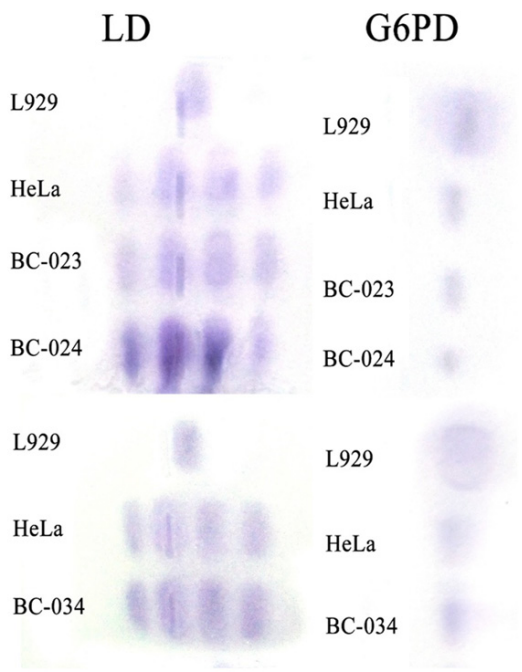

Figure 4. Isoenzymology analysis of lactate dehydrogenase (LD) and glucose-6-phosphate dehydrogenase (G6PD) in the three breast cancer cell lines. L929 is the mouse control, and HeLa is the human control.

subculture was then performed 4 d later. Cells were steadily subcultured at a split ratio of 1:3 every 3-4 d after 5 passages. All three breast cancer cells lines have been maintained in culture continuously for more than 6 months since initiation, with a total of more than 20 passages (BC-023: 23 passages; BC-024: 34 passages; BC-034: 27 passages). As seen in **Figure 1**, all three of the cell lines grew as an adherent monolayer with characteristic epithelial morphological features. These cell lines maintained a consistent morphology from the primary culture to the later passages and were robustly viable upon re-establishment from the cryopreserved state.

Growth characteristics of cell lines

The growth curves of BC-023, BC-024, and BC-034 are shown in **Figure 2**. The population doubling time of BC-023 cells at passage 23, BC-024 cells at passage 34, and BC-034 cells at passage 27 were 35, 39, and 33 h, respectively. These doubling times are quite similar to MCF-7 cells (29 h), according to the ATCC (https://atcc.org/Products/All/HTB-22.aspx#specifications).

Contamination tests

In order to test for bacterial, fungal, and mycoplasma contaminants in the cell cultures, we performed contamination tests. Despite the use of eight different growth medium compositions, no bacterial or fungal contaminants were detected in the three breast cancer cell lines (**Table 2**). Also, mycoplasma agar culture and Hoechst 33258 fluorescence staining failed to detect any mycoplasma in the three new cell lines (**Figure 3**). These results demonstrate that the three breast cancer cell lines were free of bacterial, fungal, and mycoplasma contaminants.

Isoenzyme analysis

The profiles of LD and G6PD from the three breast cancer cell lines were compared with those of HeLa and mouse fibroblast L929 cells

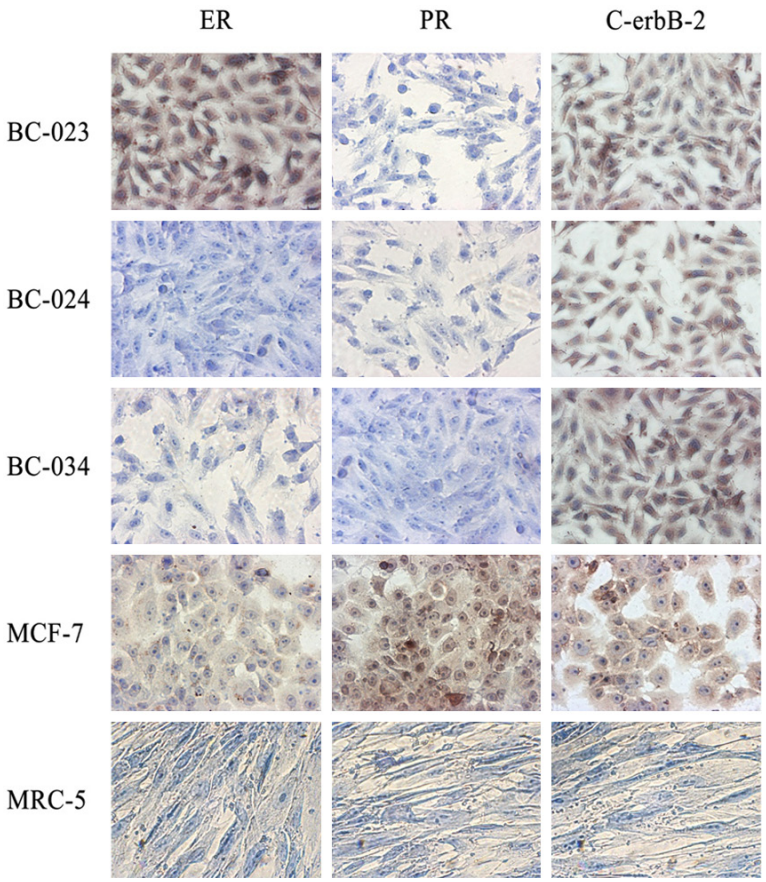

Figure 5. Immunocytochemical analysis of the three breast cancer cell lines. The expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (C-erbB-2) were detected using the SuperRmEPC™ Breast Cancer Detection Kit.

(**Figure 4**). The profiles of the LD and G6PD in the breast cancer cell lines were distinct from those of mouse fibroblast cell line L929 but identical to those from HeLa cells. These results indicate that the origin of these three cell lines was solely human tissue.

Expression of ER, PR, and C-erbB-2 markers

Using immunocytochemistry, we examined the expression of ER, PR, and C-erbB-2 in the three breast cancer cell lines. BC-023, BC-024, and BC-034 were all positive for C-erbB-2 (**Figure 5**). The expression level of C-erbB-2 in these three cell lines was similar to that of MCF-7. All three new breast cancer cell lines were negative for PR expression. Furthermore, BC-023 was positive for ER expression, while BC-024 and BC-034 were negative. The negative control cell line MRC-5 did not express ER, PR, or C-erbB-2 in our assay.

Anchorage-independent growth capacity

To assess anchorage-independent growth, a soft-agar assay was performed by seeding 2 × 10^3 cells per well in 6-well culture plates with 6 wells per group. After 14 d, visible foci were observed with control MCF-7 cells (**Figure 6**). The BC-023, BC-024, and BC-034 lines showed a similar capacity for formation of colonies, which were defined as >50 cells in soft agar. Each of the three cell lines yielded >60 colonies per well. As a result, we concluded that the BC-023, BC-024, and BC-034 cell lines have high capacity for forming clones in soft agar, indicating high malignant potential.

Tumorigenicity of the cell lines in nude mice

In order to study the *in vivo* tumorigenicity of the BC-023, BC-024, and BC-034 cell lines, we performed a xenograft transplantation to nude mice. Within 14 d post-injection of the breast cancer cells, visible subcutaneous tumors appeared in all injected nude mice at the site of inoculation, while tumors in MCF-7-injected nude mice did not appear until 21 d post-injection. The dimensions of these tumors ranged from 1.5 to 3.5 cm within 5 weeks post-injection (**Figure 7**). The negative control cell line MRC-5 did not induce tumors in any of the 10 inoculated nude mice (**Figure 7A**). To our surprise, the size of the tumors in the nude mice injected with BC-034 cells reached 3.5 × 2 × 1.5 cm by 32 d post-injection, which was much bigger than that of MCF-7. Furthermore, BC-024 cells caused bilateral tumors >1.5 cm in one mouse (**Figure 7D**), although the cells were only injected into the left groin. No metastasis was observed in mice injected with MCF-7 cells. Altogether, these findings indicate that BC-023, BC-024, and BC-034 are all highly tumorigenic cell lines.

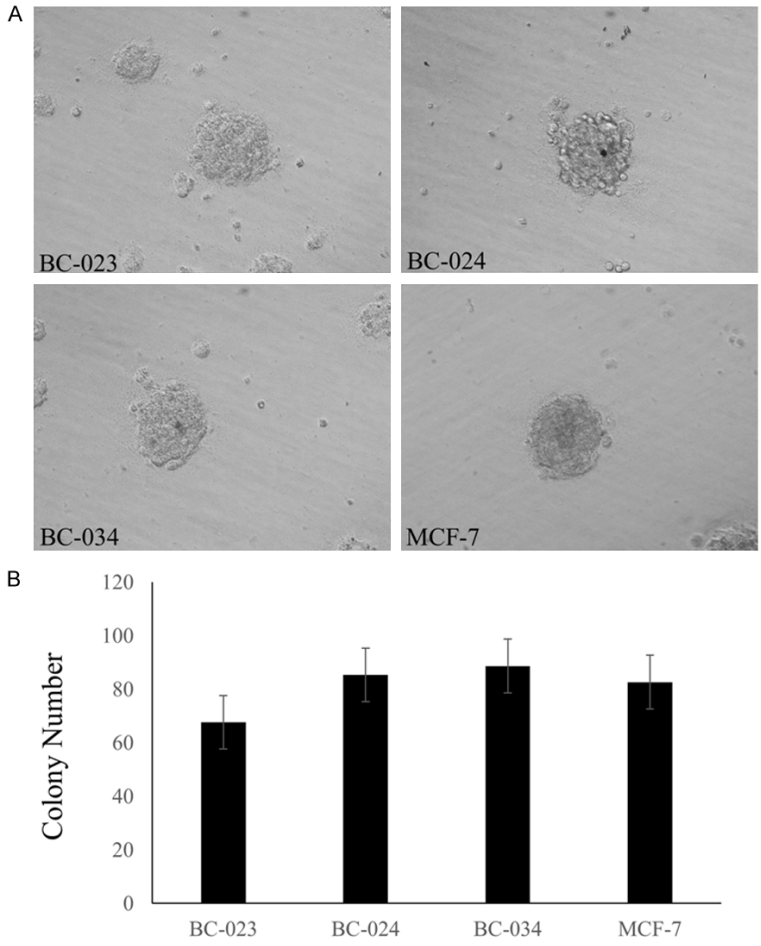

Figure 6. Anchorage-independent growth capacity analysis. A: Representative images for each cell line after 14 d of growth. B: Colony formation assays. Cells were seeded at 2 × 10^3 cells per well in 6-well culture plates. Values in B represent means ± SEM for triplicate wells.

Drug sensitivity analysis

We also performed the MTT assay to assess the drug sensitivity of these cell lines. We used ANOVA and post-hoc Tukey HSD (honest significant difference) test to compare the cell numbers among BC-023, BC-024, and BC-034 cells with those from the control groups. As shown in **Figure 8**, at day 4, the cell number of the control group was 11942, which is significantly higher than that of the BC-023 ADR group (value = 742). After 48 h of incubation with the drugs, the inhibition rates of BC-023, BC-024, and BC-034 cells for DDP and ADR were both higher than those for MCF-7 (for all comparisons, $P<0.005$; **Figure 8**). Thus, all three of the cell lines are sensitive to DDP and ADR. Interestingly, for ADR, the curve of the inhibition of BC-024 cells is very similar to that of MCF-7 cells (**Figure 8B**). Overall, the reduction in cell viability in the presence of the drugs was concentration-dependent.

Discussion

In this report, we established and characterized three breast cancer cell lines (BC-023, BC-024, and BC-034) derived from breast carcinoma tissues of Chinese female patients. Morphology studies revealed that the newly established breast cancer cell lines grew as adherent monolayers with epithelial characteristics. As these lines have be cultured continuously for more than 6 months and have undergone more than 20 passages, BC-023, BC-024, and BC-034 appear to be permanent cell lines with consistent morphology that was maintained from primary culture through extensive passaging. In addition, after recovery from cryopreservation, all three of these new breast cancer cell lines continued to grow with consistent characteristics. The population doubling time of BC-023, BC-024 and BC-034 were 35, 39, and 33 h, respectively, which are quite similar to that of MCF-7 cells (29 h). Unlike MCF-7 and T-47D cells that require additional insulin, all three of these new breast cancer cell lines grew rapidly in MEM without any extra elements [3, 26]. Taken together, these findings provide us important information about the morphological and growth characteristics of these new breast cancer cell lines.

Contamination by bacteria, fungi, and mycoplasma can be very harmful to cell cultures. Among them, mycoplasma contamination is often the most difficult to detect because the contaminated culture grows well and appears normal by ordinary light microscopy [27]. Moreover, mycoplasma is highly contagious and can spread rapidly through cell stocks. The possible consequences of mycoplasma infec-

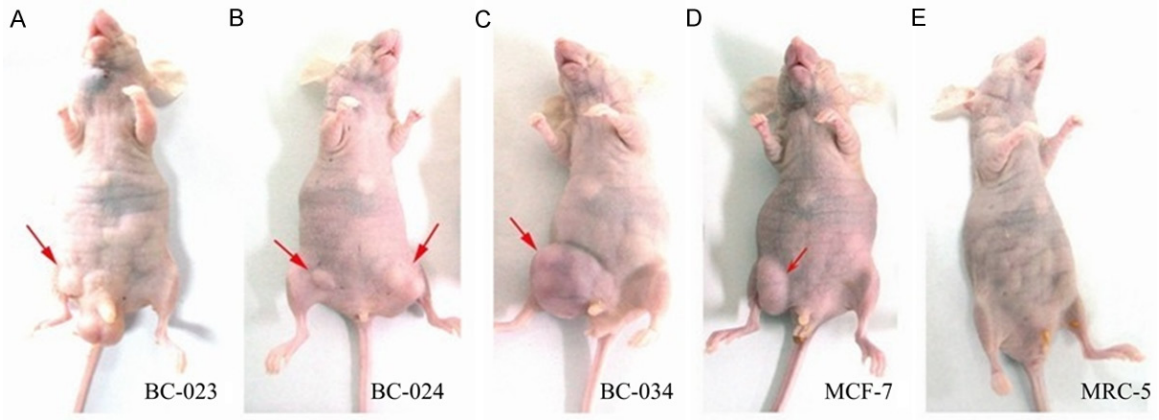

Figure 7. Tumorigenicity test of breast cancer cells in nude mice. Red arrows indicate the tumors in the nude mice. Nude mouse 30 d post-injection with (A) MRC-5 cells (negative control), (B) MCF-7 cells (positive control), (C) BC-023 cells, (D) BC-024 cells (note the bilateral tumors), and (E) BC-034 cells.

tion for host cells are numerous and variable, ranging from no apparent effect to extensive changes, such as inhibition of cell proliferation, induction of apoptosis, and malignant transformation [28, 29]. Therefore, we assessed the three new cell lines for contamination but did not detect any bacteria, fungi, or mycoplasma in any of the cell line cultures. Additionally, isoenzyme analysis indicated that the origin of the three cell lines was identical to that of the HeLa cell line, demonstrating that the three breast cancer cell lines were derived from human tissues without any cross-contamination from other species during establishment of the cultures. Thus, the three new cell lines were free from any detectable contamination, supporting their use in further biological studies.

Biological markers, such as ER, PR, and C-erbB-2 are useful for prediction of medical treatment response and patient prognosis. Among these markers, C-erbB-2 is linked with poor prognosis in breast cancer in association with shorter disease-free intervals, increased risk of metastasis, and resistance to many types of therapy [30]. Overexpression of human C-erbB-2, present in 15-30% of breast cancers, has been associated with a more aggressive clinical phenotype and a poor prognosis [31, 32]. As this marker is expressed at low levels in normal adult tissues, C-erbB-2 is an ideal target for therapy. Coexpression of C-erbB-2 receptors and ligands leads to stimulation of tumor cell proliferation and apoptosis resistance through PI3K/AKT signaling pathways [33-35]. Meanwhile, both clinical and experimental evidence suggests a link between C-erbB-2 and not only tumor growth but also breast cancer progression, including acquisition of a metastatic phenotype and some types of drug resistance [36]. Moreover, ER-positive, C-erbB-2-positive status has been found to be accompanied by shorter survival time than ER-positive, C-erbB-2-negative status in patients [37, 38]. Therefore, C-erbB-2 expression may be a better predicator of response to hormonal therapy than ER expression. In our studies, all three cell lines were positive for the expression of C-erbB-2, implying a potential common pathway involved in Chinese breast carcinomas and providing us with a good cell model for new therapeutic reagents directed against C-erbB-2 in breast cancer.

Moreover, the three breast cancer cell lines grew rapidly and had highly malignant characteristics. When injected into nude mice, the cell-induced tumorigenicity was even greater than that of MCF-7 cells. Metastasis was occasionally observed in nude mice injected with BC-024 cells but not in mice injected with BC-023, BC-034, or MCF-7 cells. In our study, all three cell lines formed obvious tumors in nude mice with 100% incidence within 2 weeks. The size of the tumor formed by these new cells was much larger than that formed by MCF-7 cells under the same conditions, and the maximum dimension of these tumors (formed by BC-034) reached as large as 2.5 cm within 5 weeks post-injection. Furthermore, the high frequency of colony formation in soft agar indicated strong malignant growth properties of the

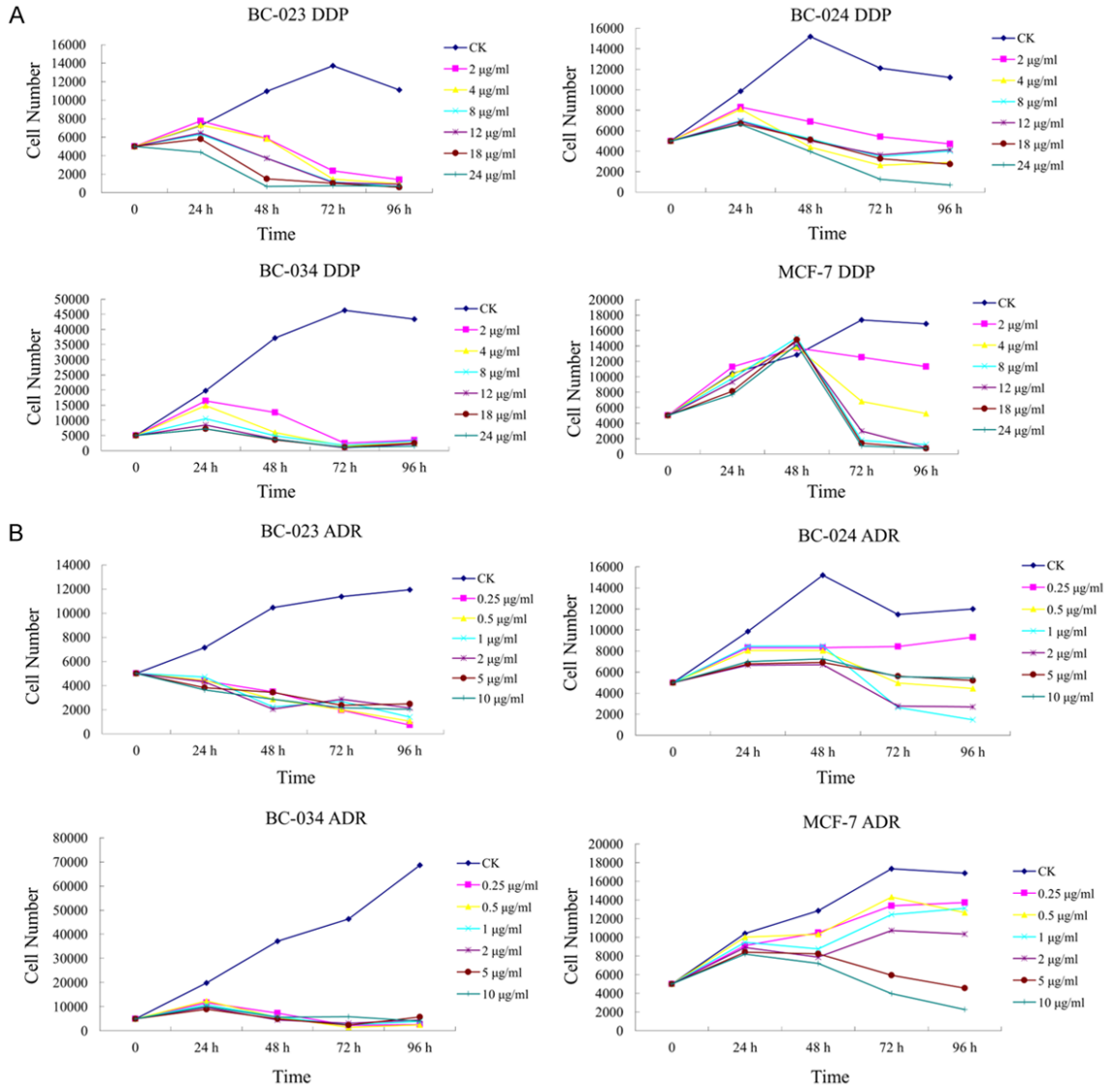

Figure 8. Drug sensitivity assays. Drug sensitivity curves show cell viability of BC-023, BC-024, BC-034, and MCF-7 cells treated with the indicated concentrations of cisplatin (DDP; A) or adriamycin (ADR; B). For all comparisons, $P<0.005$.

BC-023, BC-024, and BC-034 cell lines. Based on our characterization, we propose that these newly established breast cancer cell lines will serve as useful experimental models for studying the molecular mechanism of breast cancer.

Various drug sensitivity assays have been reported using primary or secondary passage tumor cells in the past [39]; however, most of the tumors consisted of solid areas and nests of tumor cells as well as anastomosing cords within a lymphoplasmocytic stroma. Based on these outcomes, these assays are often not accurate due to contamination of the cultures with many other types of cells, such as fibroblasts. Human cancer cell lines represent a mainstay of tumor biology and drug discovery due to the ease of experimental manipulation and applicability to high-throughput applications. In 1991, a "disease-oriented" drug screening approach using human cancer cell lines derived from different types of cancer was first used [40]. This approach facilitates high-throughput screening of large numbers of drugs to select only a limited number of drugs for further preclinical testing in xenograft models. Although progress has been made in breast

cancer diagnosis and treatment, there remains an absolute requirement to establish *in vitro* model systems to study the complex multistep process of breast cancer at various stages as well as to develop new strategies to treat cancer cell growth and progression [41].

Both DDP and ADR have significant antitumor effects and are common anticancer drugs used for the treatment of a variety of advanced cancers, including breast, ovarian, and lung cancer. Drug resistance is an important obstacle to the success of chemotherapy and is caused by various reasons, including inhibited drug transport, target alterations, and metabolic changes [42]. In our studies, DDP and ADR clearly inhibited the proliferation of all three of the new breast cancer cell lines. Compared to MCF-7 cells, BC-023, BC-024, and BC-034 cells appeared more sensitive to DDP and ADR and may be suitable models for characterizing the molecular mechanisms of ADR- and DDP-sensitive tumor cell lines and for additional drug targeting studies for breast cancer.

Numerous clinical, epidemiological, and molecular studies have indicated considerable differences in various aspects of breast cancer between Asians and patients from Western countries. Even among Asians, racial differences have been reported for clinical and histopathological aspects of breast cancer [43, 44]. Most published studies of breast cancer employed cell lines derived from Caucasians, limiting their applicability to the study of pathogenic mechanisms of breast cancer among Asian patients. Furthermore, most previously established breast cancer cell lines used in research were derived not from primary breast tumors but from tumor metastases, especially from ascites or pleural effusions. Due to these characteristics of the most commonly used breast cancer cell lines, there is an identified need for additional breast cancer cell lines derived from Chinese patients and for cell lines derived from primary breast cancer tumors. The generation of such cell lines, as described here, will allow for the study of racial differences in breast cancer, provide a novel experimental tumor model, increase our understanding of breast carcinogenesis and cell biology, and facilitate the identification and testing of novel anticancer drugs.

In conclusion, we report three new human breast cancer cell lines (BC-023, BC-024, and BC-034) from three Chinese patients were successfully established and characterized. BC-023 and BC-034 are defined as immortalized human breast cancer cell lines with high tumorigenicity, while BC-024 is a breast cancer cell line with high malignancy potential. Importantly, all three cell lines were isolated directly from the primary tumor site, and detailed pathology is available for these tumors to allow the characteristics of the culture cells to be compared with those of the original tumor. The BC-023, BC-024, and BC-034 cell lines described here may be helpful for dissecting the genetic and epigenetic influences that trigger the progression of breast cancer from localized disease to metastatic disease and to investigate the potential inter-racial differences in malignancy-associated phenotypes in breast cancer.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

ADR, adriamycin; ATCC, American Type Culture Collection; CCTCC, China Center for Type Culture Collection; C-erbB-2, human epidermal growth factor receptor 2; DDP, cisplatin; DMSO, dimethyl sulfoxide; ER, estrogen receptor; FBS, fetal bovine serum; G6PD, glucose-6-phosphate dehydrogenase; LD, lactate dehydrogenase; MEM, minimal essential medium; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; PR, progesterone receptor.

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