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ORIGINAL

Exploring the Synergy: Chinese Female Tennis Players and Chansu-Medicated Serum's Potential in Inhibiting Breast Cancer Cell Proliferation Through Apoptosis and G2 Arrest

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ABSTRACT

This study delves into the synergy between Chinese female tennis players and the potential of Chansu-Medicated Serum in inhibiting breast cancer cell proliferation through apoptosis and G2 arrest. Chinese female tennis players have garnered international recognition for their achievements on the court and their philanthropic endeavors off it. This research investigates the intersection of their impact and the advancement of breast cancer research. Chansu-Medicated Serum, a traditional Chinese medicine derivative, presents promising mechanisms for inhibiting breast cancer cell proliferation, including apoptosis induction and G2 cell cycle arrest. By exploring this conjunction, we aim to shed light on the possible contributions of these athletes and traditional medicine to the ongoing battle against breast cancer.

KEYWORDS: breast cancer; side population; Chansu-medicated serum; apoptosis; G2 arrest; Chinese players, Tennis

1. INTRODUCTION

In the modern world, the intersection of professional sports, healthcare, and philanthropy has provided a fertile ground for extraordinary contributions to society. Chinese female tennis players have risen to prominence not only as elite athletes but also as influential figures beyond the tennis court (Shen et al., 1997) (Garcia, 2016).Their remarkable achievements in international tennis competitions have brought pride to China and inspired countless individuals, while their philanthropic endeavors have addressed critical societal issues. Meanwhile, breast cancer remains a pervasive global health challenge, necessitating continuous advancements in research and treatment modalities. (Liu, Ding, & Wen, 2018; Xia & Mao, 2014). This comprehensive study embarks

on a multifaceted exploration at the intriguing crossroads of these seemingly disparate domains—Chinese female tennis players and the therapeutic potential of Chansu-Medicated Serum in inhibiting the proliferation of breast cancer cells(Y. Chang et al., 2015; Zhao et al., 2015). through apoptosis and G2 arrest(Lan et al., 2019; Li et al., 2015). It is a narrative that delves deep into the lives and impact of these athletes, their dedication to philanthropy, and their unique position as global ambassadors for causes that extend far beyond the tennis court (Huang et al., 2019; McPherson, Cochrane, & Zhu, 2016; Wu, Tsai, Lin, Fu, & Lai, 2018).

Simultaneously, the study shines a spotlight on the promising potential of Chansu-Medicated Serum, a derivative of traditional Chinese medicine, in the context of breast cancer research(Dai et al., 2018; Hong, Chan, & Yeung, 1992).. This serum has emerged as a subject of intense scientific scrutiny, revealing mechanisms that could reshape our approach to combating breast cancer—a disease that affects millions of lives worldwide(Jia et al., 2018; Weidong, Chunsheng, Honggang, Guohong, & Baojin, 2016).The motivation behind this exploration is twofold: first, to comprehensively analyze the multifaceted roles and contributions of Chinese female tennis players, and second, to investigate the potential therapeutic benefits of Chansu-Medicated Serum in the realm of breast cancer(Ferreira et al., 2019). By bridging these diverse spheres, we aim to elucidate how these athletes, with their global reach and dedication to philanthropy, can contribute meaningfully to the ongoing struggle against breast cancer.

Additionally, we seek to uncover how traditional Chinese medicine, embodied in Chansu-Medicated Serum, may hold a pivotal role in shaping the future of breast cancer treatment(Lin, Dong, Oppenheim, & Howard, 2003; Zhou et al., 2014).In this journey of discovery, we intend to not only acknowledge the achievements of these athletes but also to underscore the potential for collective efforts and collaboration across sports, medicine, and philanthropy to effect positive change in breast cancer research and care(Iwama, Amagaya, & Ogihara, 1987). As we embark on this exploration, we hope to lay the foundation for a deeper understanding of the transformative power that such synergies can bring to bear on some of the most pressing challenges of our time.

2. MATERIAL AND METHODS

2.1 Preparation of drug and drug serum

Chansu-medicated serum is extracted from New Zealand white rabbits after gavage with Chansu capsule solution which was obtained from the Chongqing Institute of Chinese medicine. All procedures on animals were approved by The Ethics Committee of Bengbu Medical College (Bengbu, China). New Zealand white rabbits (males and females, 2.0-2.5 kg) from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China) were raised in a climate controlled facility with free access to water at Animal Center of the First Affiliated Hospital of Bengbu Medical College (Anhui, China). Chansu capsule solution was administered once daily via gavage (6 ml/kg) for 3 days and saline solution was used as negative control.

Blood samples were collected via cardiac puncture under chloroform anaesthesia (Sigma-Aldrich, MO, USA) 6 h after last gavage, and were kept overnight at 4℃ before spinning for 10 minutes at 3000 rpm and collecting the serum, which was heated for 30 minutes to 56 ℃ to eliminate potential bioactive compounds. Next, serum was subjected to 0.22 um filtration to remove bacteria, followed by storage - 20℃.

2.1.1 Cell culture

The MDA-MB-453 and MCF-7 cell lines were from the Institute of Cell Research, Chinese Academy of Sciences. Both cells were cultured in RPMI1640 containing 10% FBS and penicillin/streptomycin in at 37 ° C, 5% CO_2, and 95% humidity. When the cells proliferate to 70% confluency, they are digested and passaged with 0.25% trypsin, usually once every 3-4 days.

2.1.2 MTT assay

Trypsin was used to harvest cells, and then they were plated ($5x10^3$/well) into 96-well plates (Corning, NY, USA) overnight. Media was then exchanged for fresh media supplemented with Chansu-medicated serum (%v/v=1.25, 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0%) for 72 h.

Negative control serum is obtained from rabbit without Chansu treatment. 10mM Bufalin is used as quality control. Next, each well was treated using 20 μL of MTT (Sigma-Aldrich; 5 mg/mL in PBS), and an additional incubation at 37°C was conducted for 4 h. DMSO (Sigma-Aldrich) was then used to dissolve formazan crystals by adding 100 uL/well following supernatant removal. A microplate reader (Bio-Rad, CA, USA) was then used to assess absorbance (OD) at 490 nm. The percentage of cell viability was: cell growth inhibition rate = (1- OD of treatment group / OD of control group) ×100%.

2.1.3 Colony formation analysis

Cells that were growing logarithmically were collected as above, after which 400 cells in 2 mL were added into wells of 6-well plates (Corning). Plates were incubated for 15 days, after which 10% formalin (Sigma-Aldrich) was used for fixation and 0.1% crystal violet (Sigma-Aldrich) was utilized to stain the fixed cells. Plates were then washed, dried in air, and colonies were counted via microscopy (Olympus, Tokyo, Japan).

2.1.4 Xenograft study

To evaluate the tumorigenicity and proliferation property of SP cells and NSP cells in vivo, we used SP and NSP-derived murine xenograft models. The Ethical Commission of the First Affiliated Hospital of Bengbu Medical College (Bengbu, China) approved this analyses.

Nude BALB/c mice (male; five weeks) were reared under specific pathogen-free (SPF) conditions. After one week, $1×10^6$ of DA-MB-453-SP, MCF-7-SP，DA-MB-453-NSP, and MCF-7-NSP cells injected subcutaneously to the right of each mouse. respectively.

Tumor xenografts started to form in mice two or three weeks after inoculation and all mice were sacrificed 45 days after inoculation. The xenograft sizes were measured with calipers, and the tumor volume was: Tumor volume = 1/2(length × width^2).

2.1.5 Assessment of cell apoptosis

Annexin V / propyl iodide (PI) determination was made based on provided directions (BD Pharmingen, California, USA). First, 1×10^6 of cells were incubated for 48 h with Chansu-medicated serum (1.25% and 20%).

Next, cells were harvested, subjected to a cold PBS wash, spun downw, resuscitated using 400μL of binding buffer supplemented with 5μL of FITC-annexin-V and 10 L of PI, and allowed to incubate for 15 minutes while at 4°C protected for light. A flow cytometer (BD Biosciences) was then used to acquire 10,000 or more events per sample.

2.1.6 SP cell identificaiton

Cells were cultured to 70% confluence, harvested via trypsinizaiton, collected, and spun down for 5 minutes at 500 xg. They were then resuspended at 1x10^6 cells/mL in media that contained 2% FBS, to which 5 μg/ml Hoechst 33342 (Biotium Inc., CA, USA) was added along with 0 or 100μg/mL of the ABC transporter inhibitor verapamil (Sigma-Aldrich) as a means of assessing whether this altered the fluorescent efflux effect.

Cells were allowed to rest for 90 minutes at 37 ° C in a water bath, with gentle shaking once every 15 minutes. Cold PBS was then used to wash cells twice, after which dead cells were stained by resuspension in PBS containing 2% FBS and 1 μg/ml PI. SP profiling and cell sorting was then conducted via MoFloTM XDP high-performance cell sorter (Beckman Coulter, CA, USA). SP cells exhibited lower intensity Hoechst signal. SP and non-SP (NSP) cells were classified for downstream experiments. The Summit v.5.2 program (Beckman Coulter) was usd for data and image acquisition.

2.1.7 Cancer Stem cell (CSC) marker examination

Western blotting was employed for detecting the total expression of ABC superfamily G member 2 (ABCG2), CD44 and CD133 and other CSC markers in sorted cells.

2.1.8 Measurement of ROS

The lipophilic cationic membrane potential-sensitive dye JC-1 was used to assess mitochondrial membrane potential alterations within live cells. JC-1 produces fluorescent red J aggregates in the mitochondrial matrix of healthy live cells, whereas if mitochondrial membrane potential is lost this dye can leak into the cytosol wherein it exists in a monomeric form that fluoresces green. Cells were incubated with Chansu-medicated serum (1.25% and 20%) for 12 h.

5× 10^5 cells were collected and stained with 0.5 ml JC-1 dye. The dye was diluted using complete growth media before use, and was used to stain

incubating cells for 20 min at 37 ˚C. This media was then removed, and cells were washed two time using fresh growth media before adding back additional growth media. Flow cytometry was then used to analyze fluorescence.

2.1.9 Western blotting

Cells were treated with Chansu-medicated serum (1.25% and 20%) for 48 h and a Cell Mitochondria Isolation Kit (Beyotime, Shanghai, China) was next employed for mitochondrial isolation. Protein levels were then assessed via BCA assay (Beyotime).

Protein samples were separated via SDS-PAGE, and transferred to nitrocellulose membrane (Millipore, MA, USA), which were blocked for 1 h with 5% skim milk prior to overnight incubation with appropriate primary antibodies at 4˚C, followed by 1 h incubation with HRP-linked secondary antibodies (Santa Cruz Biotechnology) A Supersignal chemiluminescence detection kit (Pierce, IL, USA) was then employed for protein detection. IV Cox and GAPDH served to normalize mitochondrial and cytosolic protein levels, respectively.

Antibodies used herein were rabbit monoclonal antibodies from abcam (MA, USA) unless otherwise stated: anti-CD44; anti-CD133 (Miltenyi Biotec, CA, USA); Goat polyclonal anti-ABCG2 (Santa Cruz Biotechnology); Mouse monoclonal anti-GAPDH (Santa Cruz Biotechnology). anti-CD252c; anti-CD252 (phospho S216) anti-active + pro caspase 3; Rabbit polyclonal anti-active Caspase 9; anti-Cytochrome C; anti-bax; anti-bcl-2; Mouse monoclonal anti-COX.

2.2 Statistical analysis

Data are means±standard deviations (m±SD) from triplicate assays. SPSS v22.0 was used for statistical testing. The significance of differences was determined via Student's t-tests or One-way ANOVAs, with $P<0.05$ as the significance threshold.

3. RESULTS

3.1 Effect of Chansu-medicated serum on the viability and apoptosis of breast cancer cell lines

The effects of Chansu-medicated serum on the in vitro growth of MDA-MB-453 and MCF-7 cells were tested. As is shown in Figure 1A, Chansu-medicated serum induced a concentration-dependent drop in the viability of both cell lines ($p<0.05$), as analyzed by MTT assay. We then assessed the impact of Chansu-medicated serum on apoptosis of these breast cancer cells via annexin V/PI staining, revealing a marked increase in apoptosis rate was obvious in both cell lines after Chansu-medicated serum treatment compared with control cells (Fig 1B-C). We found that the proportion of apoptotic cells in both cell lines was significantly increased following treatment with 20% and 40% Chansu medicated serum. ($p<0.05$).

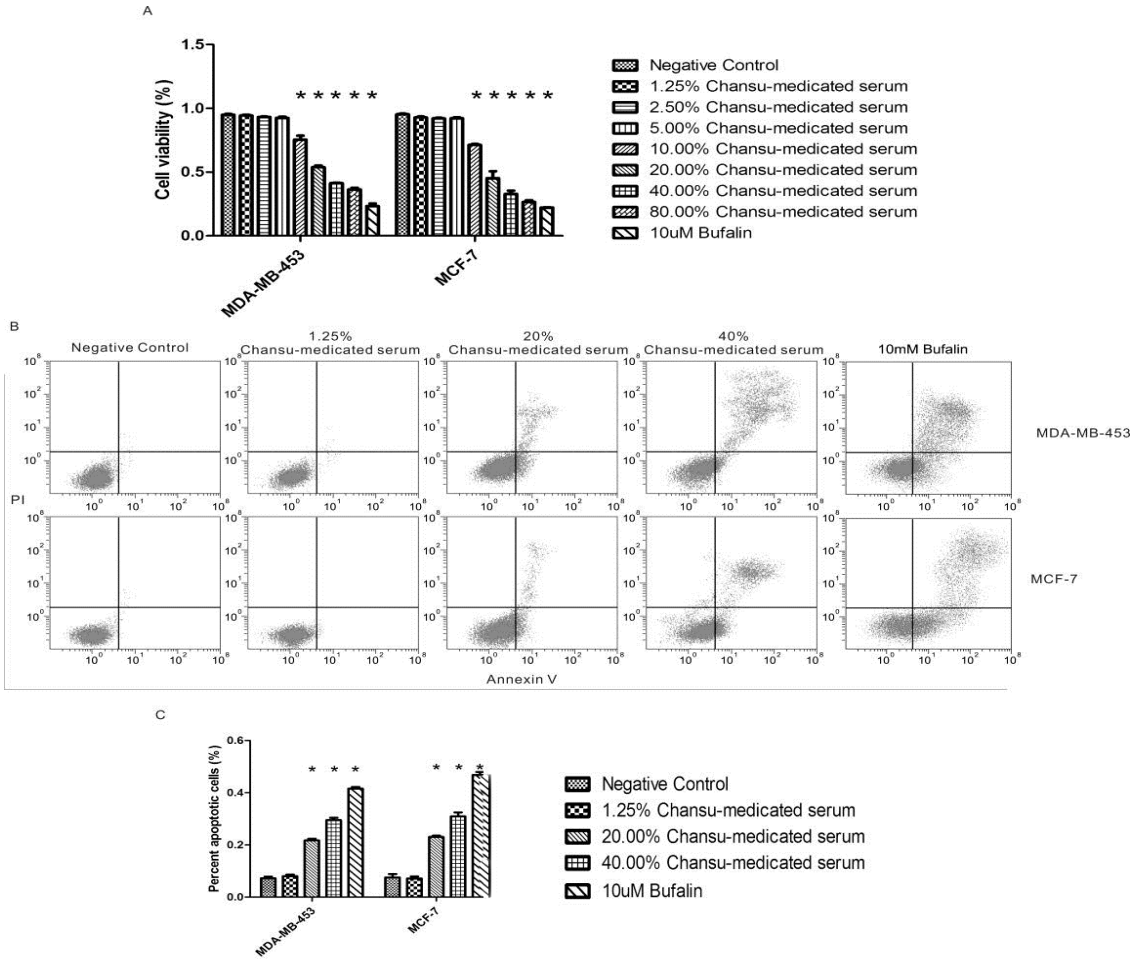

Figure 1. Effects of Chansu-medicated serum on growth and apoptosis of breast cancer cells.

*A: Effects of various concentrations of Chansu-medicated serum (%v/v=1.25%, 2.5%, 5.0%, 10.0%, 20.0%, 40.0% and 80.0%) on growth of MDA-MB-453 cells and MCF-7 cells. 10mM is used as quality control. B: MDA-MB-453 and MCF-7 cells were incubated with Chansu medicated serum, followed by staining with Annexin-V/PI. The left lower quadrant (annexin V−/PI−), right lower quadrant (annexin V+/PI−) and upper right quadrant (annexin V+/PI+) correspond to live cells, cells in early stages of apoptotic death, and cells in late stages of apoptotic death, respectively. C: The apoptotic cell frequency increased significantly after treatment with 20% and 40% Chansu-medicated serum in MDA-MB-453 and MCF-7 cells, while 1.25% Chansu-medicated serum did not induce apparent apopotosis. *P<0.05.*

3.2 Isolation and identification of SP cells

A flow cytometric discrimination approach was next used to identify the SP fractions of MDA-MB-453 and MCF-7 cell lines (Fig. 2A). These cell lines had respective SP percentages of 4.37%±0.29% and 2.91%±0.24%, while they were significantly decreased to 1.41%±0.21% and 1.12%±0.15% after treatment with verapamil (Figure 2B). Subsequently, CSC marker expression was assessed. SPs expressed higher levels of CSC markers including CD44, CD133, and ABCG2 than did NSP cells (Figure 2C) (P<0.05).

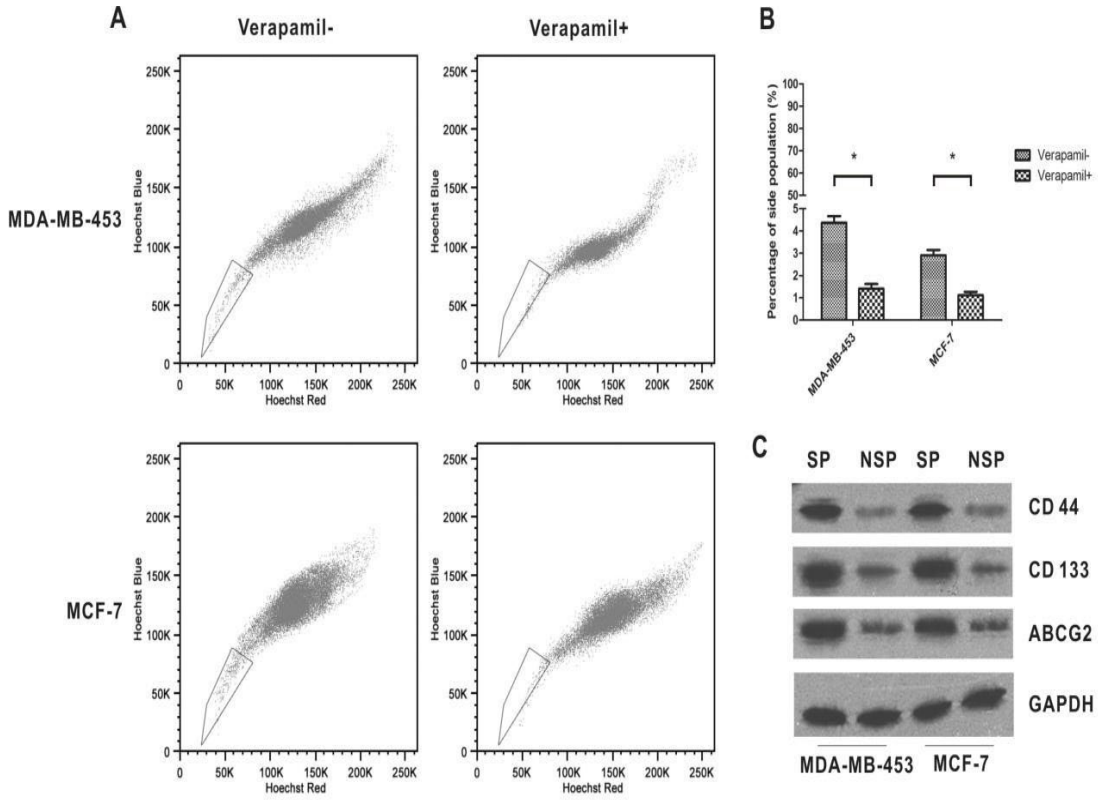

Figure 2. SP cell sorting from breast cancer cell lines.

A. *Scatter-blot analysis of SP cells in MDA-MB-453 and MCF-7 cell lines following Hoechst 33342 staining.* ***B*** *The percentages of SP cells in both cell lines were decreased after treatment with verapamil.* ***C*** *The protein expressions of CSC markers, CD34, CD133 and ABCG2 in SP and NSP cells were assessed via western blot. *P<0.05.*

3.3 Biological characteristics of SP cells

To examine the difference in the biological behaviour of SP cells and NSP cells, their colony formation ability, tumorigenicity and drug resistance to Chansu-medicated serum were evaluated using colony formation assay and MTT assay. As is show in (Figure 3A and B), the number of colonies forming by SP cells is much larger than those by NSP cells, indicated that SP cells can better form colonies than can NSP cells ($p<0.05$).

The tumorigenicities of SP cells and NSP cells were determined in mouse xenograft tumor models. During observation, the rate of tumor formation is 100% (8/8) in nude mice inoculated with SP cells, while it is only 50% (4/8) in those inoculated with NSP cells, indicating that SP cells possess higher capacity for tumorigenicity than NSP cells. 45 days after inoculation, xenograft tumors formed by SP cells have larger size and weight than those formed by NSP cells (Figure 3C and D), which indicates that SP cells possess greater proliferative activity than NSP cells in vivo.

Moreover, SP and NSP cell drug resistance against Chansu-medicated serum was determined by MTT assay. SP cells were more resistance to Chansu-medicated serum compared with NSP cells, 40% Chansu-medicated serum inhibited the proliferation of SP cells (Figure 3E, $p<0.05$).

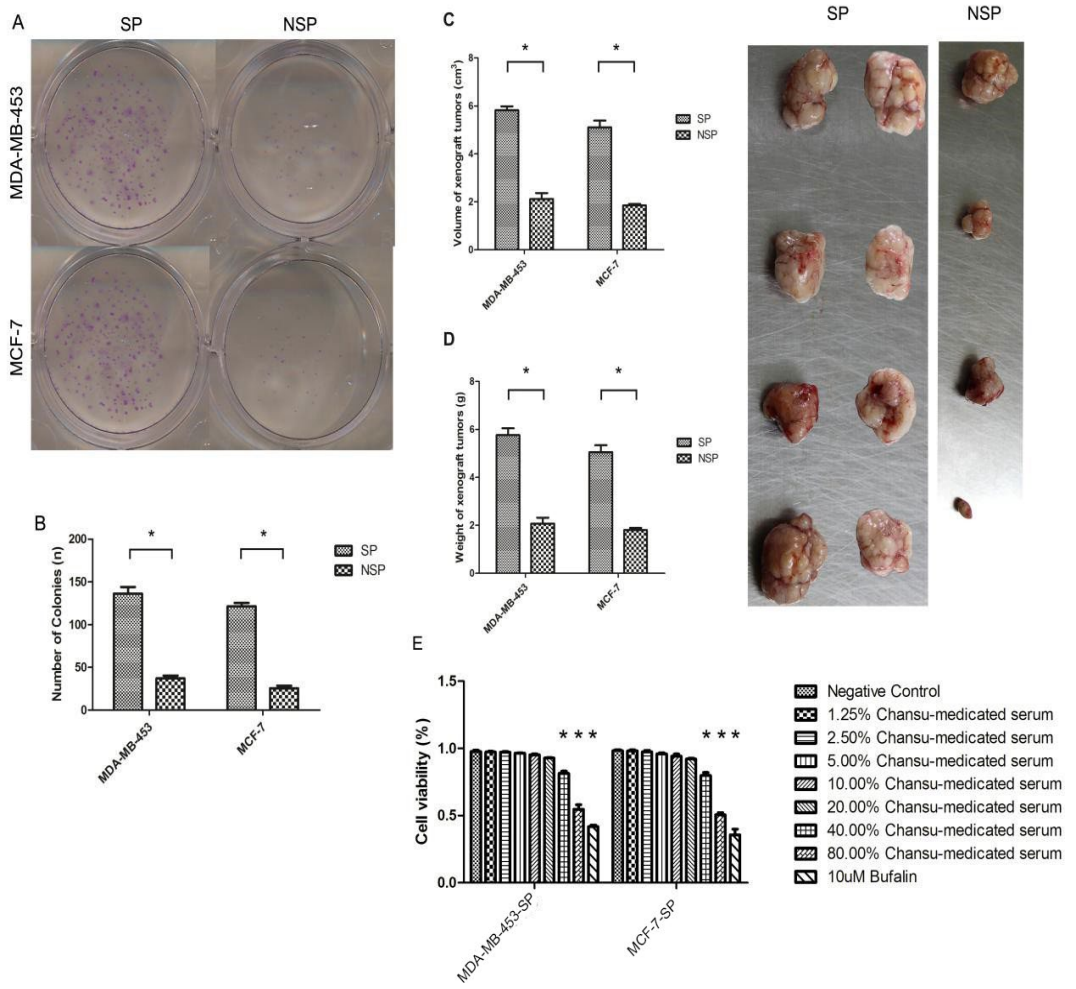

Figure 3. Effect of Chansu medicated serum on growth and apoptosis of SP cells in MDA-MB-453 and MCF-7 cell lines.

A *Colony formation of SP and NSP cells stained with crystal violet. The images shown are representative images of three indepedant experiments.* ***B*** *The number of colonies formed by SP cells is much larger than that formed by NSP cells. The volume* ***(C)*** *and weight* ***(D)*** *of tumor xenografts 45 days after inoculation. The xenografts were harvested after euthanasia.* ***E*** *Effects of 1.25%, 2.5%, 5.0%, 10%, 20%, 40% and 80% Chansu-medicated serum on proliferation of SP cells in MDA-MB-453 cells and MCF-7 cells by MTT assay. *P<0.05.*

Above results indicated that SP cells in both tested cell lines have more aggressive biologic characteristics than NSP cells, which is close to CSCs characteristics.

3.4 Effect of Chansu-medicated serum on cell cycle distribution and apoptosis of SP cells in MDA-MB-453 and MCF-7 cell lines

As shown in Table 1, a marked rise in cells in the G2 phase was obvious in SP cells in both tested cel lines after treatment with 40% Chansu-medicated serum. To further explore the mechanisms underlying G2 arrest induced by Chansu-medicated serum, we detected the expression of CDC25c, a critical regulator during G2/M transition, after treatment with Chansu-medicated serum by western blot. The CDC25c and p-CDC25c protein levels were markedly

decreased after treatment with 40% Chansu-medicated serum (Figure 4 A, $p<0.05$).

Table 1. Cell cycle analysis after treatments.

CELL LINE	CONCENTRATIONS OF CHANSU-MEDICATED SERUM	CELL CYCLE DISTRIBUTION (G1, S, G2)		
		G1 phase (%)	S phase (%)	G2 phase (%)
MDA-MB-453-SP	0%	54.56%±1.66%	29.11%±1.32%	16.36%±0.55%
	1.25%	55.64%±1.47%	27.15%±2.13%	17.22±1.01%
	20%	51.15%±2.32%	28.31%±1.56%	20.55±1.29%
	40%	38.82%±2.38%	16.67%±1.05%	48.51%±1.57%*
MCF-7-SP	0%	52.38%±1.53%	28.79%±2.21%	18.83%±1.33%
	1.25%	57.75%±2.25%	20.88%±1.11%	21.40%±1.38%
	20%	51.62%±3.54%	30.87%±1.76%	17.51%±0.98%
	40%	32.39%±0.66%	14.05%±0.35%	53.56%±3.63%*

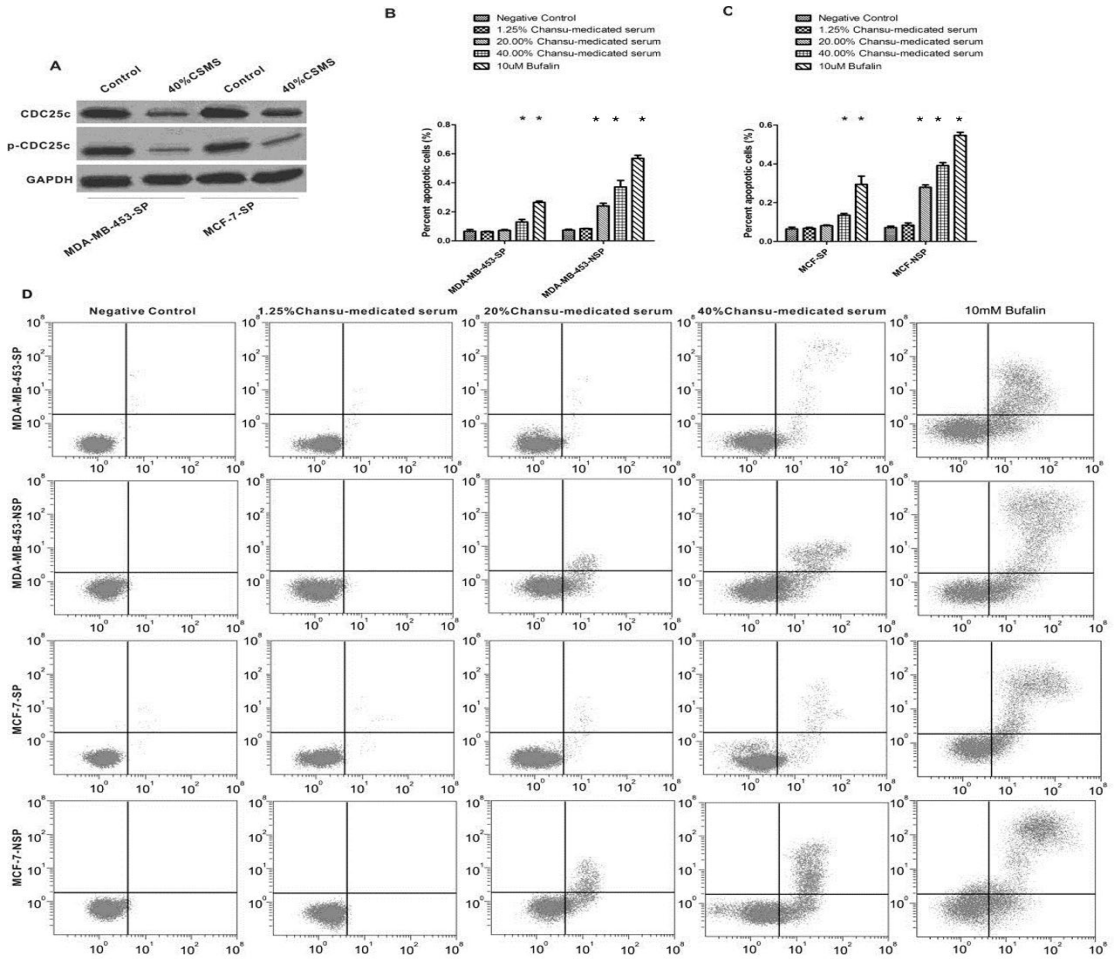

Figure 4. Effects of Chansu-medicated serum on cell cycle progression and apoptosis of SP cells in MDA-MB-453 and MCF-7.

***A** The protein levels of CDC25c and p-CDC25c (phospho S216) were significantly decreased after treatment with 40% Chansu-medicated serum. **B** and **C** The proportion of apoptotic cells increased significantly after treatment with 40% Chansu-medicated serum in MDA-MB-453-SP and MCF-7-SP cells, while 1.25% and 20% Chansu-medicated serum did not induce apparent apopotosis. **D** Annexin V/PI double staining analysis by Flow cytometry. X axis: Intensity of Annexin V fluorescence, Y axis: Intensity of PI fluorescence. *P<0.05.*

Moreover, the apoptosis of SP cells were also examined after treatment with Chansu-medicated serum. And as shown in Figure 4 B-D, the proportions of apoptotic NSP cells are significantly increased after treatment with 20% Chansu-medicated serum ($p<0.05$), while the proportions of SP cells are significantly increased following treatment with 40% Chansu-medicated serum ($p<0.05$). Chansu-medicated serum promoted ROS generation, mitochondria-mediated apoptosis in SP cells. The intracellular ROS levels were measured by the intensity of DCF-fluorescence. Our data showed that 40% Chansu-medicated serum significantly induced ROS production (Figure 5A and B), indicating that high concentration of Chansu-medicated serum may promote ROS production in SP cells of MDA-MB-453 and MCF-7 cells.

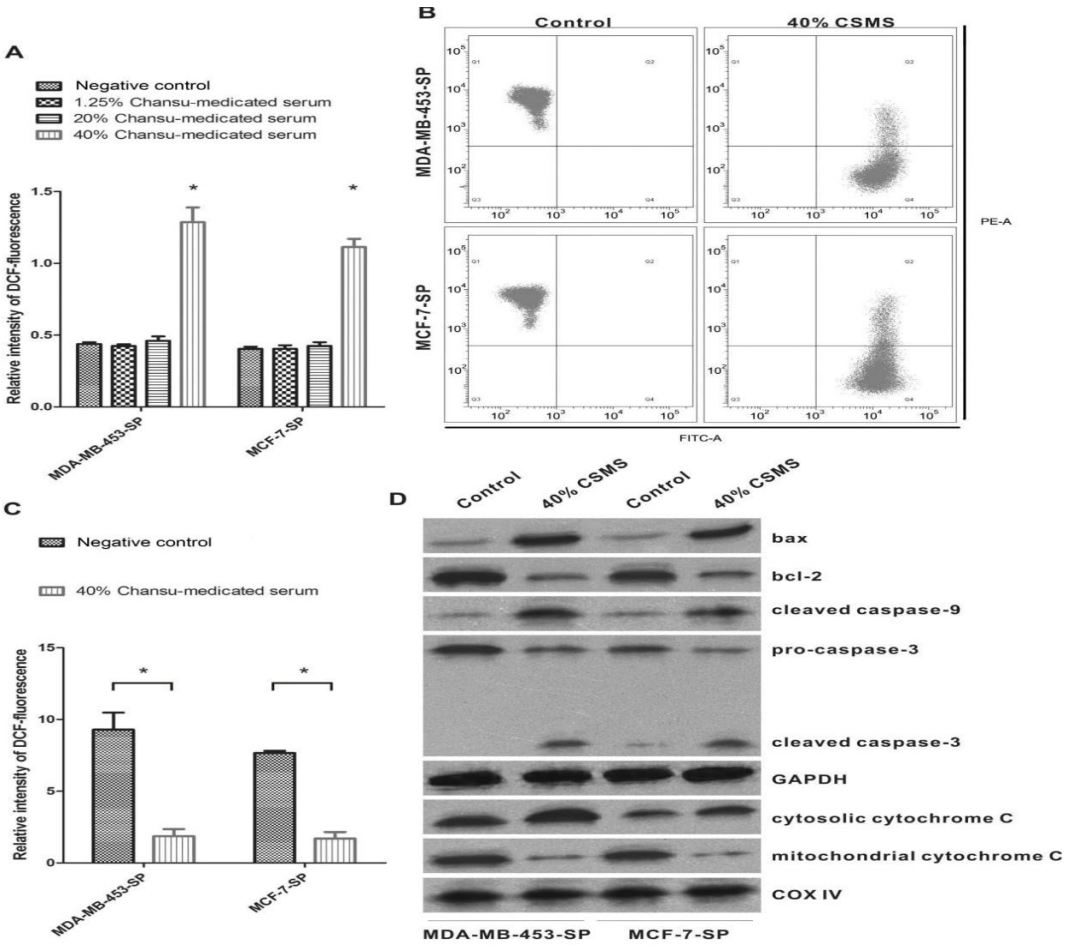

Figure 5. ROS generation, loss of mitochondrial membrane potential and alteration in expression levels of apoptosis-associated proteins after treatment with Chansu-medicated serum.

***A** The intensity of DCF-fluorescence was increased markedly after treatment with 40% Chansu-medicated serum in MDA-MB-453-SP and MCF-7-SP cells. **B** Effects of Chansu-medicated serum on mitochondrial membrane potential was determined by staining with JC-1 dye. JC-1 monomer with green fluorescence (X axis) is increased whereas J-aggregates with red fluorescence (Y axis) is decreased indicating depolarization of mitochondrial membrane. **C** The ratio of red fluorescence/ green fluorescence after treatment with Chansu-medicated serum. **D** The protein levels of Bax, Bcl-2, cytosolic cytochrome c, mitochondrial cytochrome c, cleaved caspase-9, pro-caspase-3 and cleaved caspase-3 after treatment with 40% Chansu-medicated serum were examined by western blot. GAPDH and COX IX served as loading control of cytoplasmic protein and mitochondrial protein respectively. *P<0.05.*

Changes in MMP were evident following Chansu-medicated serum treatment, as shown by a change from red to green JC-1 fluorescence as this dye was released from the mithochondrial matrix into the cytosol (Fig 5C-D). These observations suggested that the exposure of MDA-MB-453-SP and MCF-7-SP cells to 40% Chansu-medicated serum leads to mitochondrial dependent apoptosis induction and cell death eventually. Subsequenctly, we further examined the expressions of several members downstream of mitochondrial apoptotic pathway in this context. We examined the variations of Bax, Bcl-2, cytochrome c, caspase-9 and caspase-3 in protein levels after treatment with Chansu-medicated serum by western blotting (Figure 5E). It turned out that Bax, cleaved caspase-9, and -3 levels were increased, whereas Bcl-2 and pro-caspase-3 levels were decreased. Cytosolic cytochrome c protein levels were markedly increased, however, the protein level of that in mitochondria was decreased, which indicated the mitochondrial cytochrome c was released into the cytosol.

4. DISCUSSION

Breast cancer is the deadliest cancer among women, with an estimated global incidence about 2.09 million cases accounting for approximately 11.6% of all newly diagnosed female cancers and approximately 0.63million deaths per year accounting for 6.6% of all female deaths. Although surgery remains the mainstay of treatment for operable breast cancer, recurrence and metastasis post-operation are very common. At a global scale, the combination of gemcitabine and cisplatin is the treatment regimen that is most typically used to treat patients suffering from advanced/metastatic and recurrent breast cancer. Recent research has increasingly explored the potential utility of natural TCM compounds owing to their potential anti-cancer properties. Due to mild treatment and less side effect, TCM emerged as effective and safe second-line therapeutics for breast cancer, which may prolong disease-free survival, improve quality of life (QOL) and reduce the risk of recurrence and metastasis.

In recent years, the inhibitory effects of Chansu on growth of many tumors such as bone, prostate, lung, and liver cancerhave been documented. Chansu is extracted from toad glandular secretions and dozens of active ingredients have been identified, some of which are found to exert inhibitory activities against breast cancer growth. Bufadienolides, purified from Chansu, exhibited the significant antitumor activities against breast cancer cells with triple-negative or HER2-positive status via induction of apoptosis. In this study, Chansu-medicated serum is extracted using the method of serum pharmacology, which seems to be a more reasonable and simple way to test the biological activites of a drug with complex components such as Chansu.

CSCs represent a cancer cell sub-population possessing a range of stem-lije properties including a capacity for self-renewal. These CSCs are increasingly thought to be present in a range of different cancers, wherein they may drive oncogenesis, tumor maintenance, and eventual recurrence even after patients have been treated. And conventional chemotherapeutics for cancers are not able to kill the CSCs completely. However, due to the lack of specific markers, how to identify, isolate and purify CSCs is an urgent problem to be solved. In recent years, there has been increasing focus on the SP tumor

cell subset that was first detected via flow cytometry and was demonstrated to have CSCs characteristics. Generally, the drug transporter protein ABCG2 is highly expressed in SP cells, which may help SP cells to exclude drugs and agents including Hoechst 33342. SP cells have been shown to exist in many kinds of tumors and they play significant roles in tumorigenesis and cancer therapy. Previous studies have also shown SP cells to exist breast cancer cell lines, and CD133, CD44 and ABCG are characteristics markers of breast CSCs. In this study we also found a certain proportion of SP cells in MDA-MB-453 and MCF-7 cell lines, and the protein expressions of CD133, CD44 and ABCG2 of SP cells were significantly higher than those of other tumor cells. In addition, SP cells showed more aggressive biological behaviour in tumorigenicity, growth and drug resistance which may be also viewed as CSCs characteristics.

As is known, the abnormality of cell cycle and apoptosis are important factors that control the growth of tumor cells and most effective strategies for treat tumors are to induce cell cycle arrest and cell death. Previous work has indicated that bufalin, an effective component of Chansu, can induce G2 / M phase arrest in leukemia cells. Our study observed same result in MDA-MB-453-SP and MCF-7-SP cells following Chansu-medicated serum treatment, and the expression levels of CDC25c and p-CDC25c were markedly reduced, indicating that Chansu-medicated serum may induce G2/M arrest through inhibition of CDC25c expression and activation.

Apoptosis, also termed programmed cell death, may be caused by physiological or pathological stimulator. Several signaling pathways participate in the process of apoptosis, including the ER, death receptor, and mitochondrial apoptosis pathways. Reactive oxygen species (ROS) serve as key drivers of intracellular signaling, but when they are produced in excess this can result in oxidative damage, mitochondrial membrane potential collapse, and ultimately apoptosis or necrosis. We found that Chansu-medicated serum induced apoptosis in SP cells of breast cancer cell lines via inducing ROS generation, leading to increased Bax levels, decreased protein level of bcl-2, disrupted MMP, and cytosolic cytochrome c release that resulted in caspase-9 and caspase-3 cascade activation.

5. Conclusion

This exploration of the intersection between Chinese female tennis players and the potential of Chansu-Medicated Serum in inhibiting breast cancer cell proliferation through apoptosis and G2 arrest highlights the promising synergy between sports and medicine. Chinese female tennis players, renowned for their success on the global stage, have the potential to contribute significantly to breast cancer awareness and research. Meanwhile, Chansu-Medicated Serum, derived from traditional Chinese medicine, represents a promising avenue in the pursuit of effective breast cancer treatments. As we navigate the challenges posed by breast cancer, it is imperative to recognize the value of collaborations that bridge diverse fields, from professional sports to medical research. The advocacy and philanthropic efforts of these athletes can amplify the voices of breast cancer patients and survivors, raising awareness and funding for crucial research initiatives. Simultaneously, the potential of Chansu-Medicated Serum in inhibiting breast

cancer cell proliferation underscores the importance of exploring traditional medicines for innovative solutions in cancer treatment. Moving forward, continued research and collaboration in both the sports and medical domains hold the promise of advancing breast cancer research and care. By harnessing the dedication of Chinese female tennis players and the potential of Chansu-Medicated Serum, we can aspire to a future where breast cancer is more effectively understood, treated, and ultimately conquered.

DECLARATIONS

Ethics approval and consent to participate

The present study was granted ethical approval by the Institutional Review Board of Bengbu Medical College. Written informed consent was obtained from all participants involved in the study.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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