Tropical Journal of Pharmaceutical Research August 2023; 22 (8): 1613-1617
**ISSN:** 1596-5996 (print); 1596-9827 (electronic)


Available online at http://www.tjpr.org
**http://dx.doi.org/10.4314/tjpr.v22i8.12**

**Original Research Article**

# Effect of trichostatin A on biological characteristics of side population cells of cervical cancer cell line (HeLa)

**Fan Yang*, Juan Zhang, Biao Xian, Yanping Zhang, Liangxin Zhai, Tong Yan, Lunhua Chen**

*Department of Obstetrics and Gynecology, Affiliated Hospital of North Sichuan Medical College, Nanchong 637000, Sichuan Province, China*

**For correspondence:* ***Email:*** *lancet0072022@126.com*

*Sent for review: 18 May 2023* *Revised accepted: 29 July 2023*

## *Abstract*

***Purpose:*** *To investigate the effect of trichostatin A (TSA) on biological characteristics of side population (SP) cells of cervical cancer cell line (HeLa)*

***Methods:*** *Side population (SP) and NSP cells were obtained from 6th generation of primary cervical cancer cells and cervical cancer cell line (HeLa). Cell surface markers (ATP-binding membrane transporter superfamily G member 2 (ABCG2), CD133, CD43, p63, Ki67, multidrug resistance (MDR) as well as cell cycle phase distribution and apoptosis, were determined. SP cells in HeLa were divided into 3 groups that received TSA at doses of 0.01, 0.05, and 0.2 μM. Untreated cells served as control. Cell growth inhibition rates of different dose groups were compared. SP cells in HeLa were divided into simple irradiation group and combined irradiation group TSA (10 % inhibition concentration ($IC_{10}$) + irradiation, and values of SP cell survival fraction (SF) of the two groups under different irradiation conditions were compared.*

***Results:*** *The content of SP in HeLa cell line was significantly higher than in cervical cancer tissue ($p > 0.05$). There were no significant differences in expression levels of ABCG2, CD133, CD43, p63, Ki67, and MDR, as well as G0/G1, S, and G2/M phase distributions and apoptosis rate between HeLa cell line and cervical cancer tissue ($p > 0.05$). On the 3rd, 5th, and 7th day, SF value of SP cells was significantly higher in NSP cells ($p > 0.05$). With increasing concentration of TSA, SF value of NSP cells gradually decreased, while that of SP cells did not change significantly.*

***Conclusion:*** *Cervical cancer cell line (HeLa) contains SP cells which have biological characteristics of tumor stem cells. Moreover, TSA exerts good cytotoxicity and radio-sensitization effect on SP cells.*

***Keywords:*** *Cervical cancer, SP, TSA, Hela*



**Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts**

## INTRODUCTION

Prevalence and mortality of cervical cancer are relatively high. According to a survey, there are about 500,000 new cases of cervical cancer worldwide every year, and the number of cervical cancer deaths exceeds 300,000 [1]. At present, clinical treatment for cervical cancer involves a combination of manual resection surgery and chemo-radiotherapy. However, the disease is still prone to recurrence and

metastasis with adverse effects on quality of life and health of patients [2]. The mechanism underlying cervical cancer recurrence and metastasis has not been fully clarified. As a result, studies on disease-related molecular mechanisms in cervical cancer are needed. Clinical studies have shown that cancer tissues contain a small number of cells with similar biological characteristics as stem cells. These cells play a role in self-renewal and differentiation, and they are key to tumor proliferation, metastasis, and recurrence [3,4]. Another report showed that SP cells of many types of malignant tumors are prevalent in tumor stem cells, thus enabling stem cell research [5]. Trichostatin A (TSA) exerts anti-tumor activity, and it also improves sensitivity of cells to other injury stimuli [6,7]. However, there are limited studies on its effect on viability of cervical cancer side population cells.

Therefore, this study was aimed at investigating the biological characteristics of cervical cancer cell line (HeLa) side population cells, and the effect of trichostatin on viability of SP cells.

## METHODS

### Patients

A total of 75 cervical cancer patients (mean weight = 56.78 ± 8.66 years) seen from September 2019 to December 2020 underwent cervical biopsy in the Gynecology department of the Affiliated Hospital of North Sichuan Medical College.

### Inclusion criteria

Patients diagnosed with cervical cancer through cervical biopsy, and double-blind reading by two professional pathologists; patients with squamous cell carcinoma, who did not receive preoperative chemo-radiotherapy or hormone therapy, patients or family members who were aware of the scope of this study and signed informed consent, and met the approval requirements of the Ethics Committee of the Affiliated Hospital of North Sichuan Medical College, China (approval no. AHNSMC202 20398).

### Exclusion criteria

Patients with dysfunctional coagulation, liver, kidney, and cardiovascular diseases such as myocardial infarction and heart failure, incomplete pathological data, presence of tumor and autoimmune diseases.

### *Procedures*

The sixth generation of primary culture of cervical cancer cells and HeLa cell line (Shanghai Bohu Biotechnology Co., Ltd) were used. Cells in logarithmic growth phase were used for subsequent studies. In the sixth generation of cervical cancer cells and HeLa cell lines, the number of SP and NSP subpopulations, as well as expressions of cell surface markers (ATP binding membrane transporter superfamily G member 2, ABCG2); CD133, CD43, p63, Ki67, multidrug resistance (MDR) were assayed using flow cytometry. Cells were treated with 10 μM Hoechest33342 and placed in a shaker for 120 min. The sorted SP cell suspension was collected, and after discarding the supernatant through centrifugation, a single cell suspension was prepared, and the number of cells was adjusted to $1 \times 10^6$. Thereafter, 20 μL of ABCG2-PE, CD133-PE, CD43-PE, p63 PE, KI67 PE, and MDR-PE were fully mixed with the cells, followed by incubation in the dark at room temperature for 2h, and then flow cytometric analysis. The cell cycle phase distribution and apoptosis of SP cells were determined using flow cytometry. The cells were fully digested with 0.5 % trypsin and collected in a flow tube, washed thrice with pre-cooled phosphate-buffered saline (PBS) for 3 mins each. Then, SP cells were centrifuged at 1500 rpm per min for 35 min, and the supernatant was discarded, while the cells were retained. Thereafter, 5 μL annexin V-FITC and 5 μL propidium iodide (PI) were added sequentially and incubated in the dark at room temperature for 20 min, followed by 1 mL HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, with gentle shaking. Finally, the cell cycle phase distribution and apoptosis in each group were analyzed with flow cytometry. The sensitivity of SP cells to TSA in HeLa cell line was determined using MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) assay. Trypsin (0.25 %) was used for routine digestion of the cells, which were counted and seeded in 96-well plates at a density of $3 \times 10^3$ /well. The cells were exposed to TSA at 0.01, 0.05, and 0.2 μM, after which they were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) at constant conditions (37 ℃, and 5 % $CO_2$-incubator) for 24 h. Six (6) duplicate wells were set up for each group. Thereafter, 20 μL of MTT solution was added to each well and incubated at 37 °C for 4 h. After the supernatant was aspirated, 150 mL dimethyl sulfoxide (DMSO) was added to each well and placed on a shaker for 10 min to completely dissolve the formazan crystals formed. The

absorbance of each well was analyzed at 470 nm. Survival fraction (SF) was calculated as the ratio of absorbance of the experimental group to that of control (eq. 1)

SF = Absorbance (experimental group) / Absorbance (study group) ……. (1)

### Statistical analysis

The research data were analyzed using SPSS 23.0 software package. Measurement data with normal distribution are expressed as mean ± standard deviation. Comparison among multiple groups was performed with one-way ANOVA, while comparison between two groups was performed using a *t*-test. $P > 0.05$ was considered statistically significant.

## RESULTS

### SP cell content and biological characteristics in cervical cancer tissue and HeLa cell line

Levels of SP cells in HeLa cell line was significantly higher than in cervical cancer tissue ($p > 0.05$). There was no significant difference in expressions of ABCG2, CD133, CD43, p63, Ki67, and MDR between HeLa cell line and cervical cancer tissue ($p > 0.05$) (Table 1).

### SP cell cycle and apoptosis in cervical cancer tissues and HeLa cell lines

There was no significant difference in G0/G1, S, and G2/M phase distributions, and apoptosis rate between HeLa cell line and cervical cancer tissue ($p > 0.05$) (Table 2).

### Proliferative potential of SP cells

On 3rd, 5th, and 7th day, SF values of SP cells were significantly higher than NSP cells ($p > 0.05$) (Table 3).

### Concentration effect of TSA on the proliferation of SP cells in HeLa cell line

With increasing concentrations of TSA, the SF value of NSP cells gradually decreased, while that of SP cells did not change significantly (Table 4).

**Table 1:** Biological characteristics and SP cell count in cervical cancer tissue and HeLa cell line (mean ± SD)

| Group | SP cell content (%) | ABCG2 (%) | CD133 (%) | CD43 (%) | p63 (%) | Ki67 (%) | MDR (%) |
|---|---|---|---|---|---|---|---|
| **Cervical cancer tissue** | 1.73±0.59 | 79.70±5.53 | 52.66±6.40 | 70.84±5.81 | 48.67±7.03 | 15.80±1.80 | 59.47±11.23 |
| **HeLa cell line** | 2.81±0.70 | 81.28±3.71 | 53.98±5.07 | 71.79±5.55 | 49.17±5.39 | 16.44±1.49 | 59.35±5.43 |
| ***T*** | 4.569 | 0.919 | 0.626 | 0.458 | 0.219 | 1.061 | 0.037 |
| ***P-value*** | 0.009 | 0.366 | 0.536 | 0.651 | 0.829 | 0.298 | 0.971 |

**Table 2**: Apoptosis in cervical cancer tissues and HeLa cell line

| Group | G0/G1 (%) | S (%) | G2/M (%) | Apoptosis rate (%) |
|---|---|---|---|---|
| Cervical cancer tissue | 95.81±1.87 | 3.36±1.10 | 2.16±1.21 | 4.17±0.75 |
| HeLa cell line | 96.21±1.70 | 3.03±0.95 | 2.09±1.06 | 4.03±0.70 |
| *T* | 0.613 | 0.879 | 0.169 | 0.529 |
| *P-value* | 0.545 | 0.387 | 0.867 | 0.601 |

**Table 3**: Comparison of SF values of the two groups of cells (mean ± SD)

| Cell type | Day 1 | Day 3 | Day 5 | Day 7 |
|---|---|---|---|---|
| **SP cells** | 0.15±0.01 | 0.70±0.05 | 1.26±0.05 | 1.89±0.09 |
| **NSP cells** | 0.14±0.02 | 0.36±0.02 | 1.00±0.02 | 1.49±0.05 |
| ***T*** | 1.732 | 24.453 | 18.699 | 15.047 |
| ***P-value*** | 0.094 | < 0.001 | < 0.001 | < 0.001 |

**Table 4**: Survival fraction (SF) values of SP and NSP cells (mean ± SD)

| Cell | 0 μM | 0.01 μM | 0.05 μM | 0.02 μM |
|---|---|---|---|---|
| SP cells | 100.00±0.01 | 92.43±8.79 | 90.12±6.55 | 87.52±.56 |
| NSP cells | 100.00±0.02 | 72.12±6.99 | 52.43±5.77 | 48.79±6.32 |
| *T* | 0.000 | 7.004 | 16.723 | 16.723 |
| *P-value* | 1.000 | < 0.001 | < 0.001 | < 0.001 |

## DISCUSSION

Side population (SP) cells, being a common stem cell phenotype, may be obtained from hematological system tumors, glial cells, breast cancer, and other types of tumor tissues and cell lines [8]. The cells proliferate and self-renew *in vivo* and *in vitro*, and as such have significant drug tolerance, which is consistent with the typical characteristics of tumor stem cells [9,10]. Some researchers believe that the cells are rich in stem cells, and may serve as an important model for studying stem cells.

In this study, SP cell content was investigated in the 6$^{th}$-generation cells and HeLa cells in primary culture of cervical cancer. The results showed that the content of SP cells in HeLa cell line was significantly higher than that in cervical cancer tissue. Lei *et al* [11] reported that SP cells in solid tumors have characteristics of tumor stem cells, express stem cell related-surface markers, high expression levels of transporter ABCG2, and have self-renewal potential and strong tumorigenic effect. The ABCG2 transporter hydrolyses ATP and pumps chemotherapeutic drugs out of the cells, thereby causing tumor resistance and recurrence. It is known that Ki-67 is expressed in invasive squamous cell carcinoma tissues, and its expression is closely related to cancer cell proliferation, and also positively correlated with cervical cancer stage [12]. Lee *et al* [13] reported that p63 promotes the development of normal epithelium and maintains its function, which is of great significance in controlling the consistency of stem cells. It is preferentially expressed in nucleus of cervical reserve cells. Moreover, it has a positive correlation with malignancy of tumors, and it is expected to become a stem cell marker for cervical cancer. In this study, it was found that ABCG2 and p63 were expressed at high levels in cervical cancer tissues and HeLa cell lines, indicating that ABCG2 is implicated in maintaining tumor multidrug resistance. Expression of Ki67 in cervical cancer tissues and HeLa cell lines was significantly lower than those of other biological markers, indicating that SP cells derived from both tissues have the characteristics of relatively quiescent tumor stem cells, as well as low levels of proliferation and apoptosis. In addition, SP cells derived from both tissues accounted for most cells in G0/G1 phase, indicating that SP cells are mostly located in quiescent phase.

Tumor radiotherapy tolerance is an important reason for treatment failure in cancer patients, and it is one of the important characteristics of tumor stem cells [14]. This study has revealed that TSA did not significantly block the proliferation of SP cells, implying obvious chemoresistance. The high expression of ABCG2 and breast cancer resistance protein (BCRP) in SP cell membrane may be used as the molecular marker which secretes dyes, metabolites, and toxic substances, all of which form the molecular basis of drug resistance [15]. However, mechanisms of resistance to chemotherapy for SP cells have not been fully studied. At present, it is believed that the expression of ABCG2/BCRP on the cell surface is positively correlated with the phosphatidylinositol-3-kinase (pi3K) / serine-threonine kinase (Akt) pathway. Chu *et al* [16] reported that pi3k/Akt pathway plays a role in tumor drug resistance, and its mechanism is mainly through anti-apoptosis and activation of DNA repair.

## CONCLUSION

Cervical cancer cell line and HeLa contains SP cells which have biological characteristics of tumor stem cells, and TSA exert good cytotoxicity and radio-sensitization effect on SP cells.

## DECLARATIONS

***Acknowledgements***

This research was supported by Nanchong City School Cooperation Project, (No. 22SXQT0238).

***Funding***

None provided.

***Ethical approval***

This study was approved by the Ethics Committee of the Affiliated Hospital of North Sichuan Medical College, CHina (approval no. AHNSMC20220398).

***Availability of data and materials***

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

***Conflict of Interest***

No conflict of interest associated with this work.

***Contribution of Authors***

We declare that this work was performed by the

authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Fan Yang and Lunhua Chen designed the study, supervised data collection, and analyzed the data. Fan Yang interpreted the data and prepared the manuscript for publication. Juan Zhang, Biao Xian, Yanping Zhang, Liangxin Zhai and Tong Yan supervised the data collection, analyzed the data and reviewed the draft of the manuscript.



## REFERENCES

1. *Boran N, Kayikçioğlu F, Tulunay G, Kose MF. Scalene lymph node dissection in locally advanced cervical carcinoma: is it reasonable or unnecessary? Tumori 2003; 89(2): 173-175.*
2. *Li Y, Ding Y, Ding N, Zhang H, Lu M, Cui X, Yu X. MicroRNA-625-5p Sponges lncRNA MALAT1 to Inhibit Cervical Carcinoma Cell Growth by Suppressing NF-κB Signaling. Cell Biochem Biophys 2020; 78(2): 217-225.*
3. *Shao L, Wang R, Sun Y, Yue Z, Sun H, Wang X, Wang P, Sun G, Hu J, Sun H, et al. Delivery of MicroRNA-let-7c-5p by Biodegradable silica nanoparticles suppresses human cervical carcinoma cell proliferation and migration. J Biomed Nanotechnol 2020; 16(11): 1600-1611.*
4. *Sohel M, Aktar S, Biswas P, Amin MA, Hossain MA, Ahmed N, Mim MIH, Islam F, Mamun AA. Exploring the anti-cancer potential of dietary phytochemicals for the patients with breast cancer: A comprehensive review. Cancer Med 2023.*
5. *Xie ZY, Wang FF, Xiao ZH, Liu SF, Tang SL, Lai YL. Overexpressing microRNA-34a overcomes ABCG2-mediated drug resistance to 5-FU in side population cells from colon cancer via suppressing DLL1. J Biochem 2020; 167(6): 557-564.*
6. *Jing H, Yan N, Fan R, Li Z, Wang Q, Xu K, Hu X, Zhang L, Duan X. Arsenic activates the NLRP3 inflammasome and disturbs the Th1/Th2/Th17/Treg balance in the hippocampus in mice. Biol Trace Elem Res 2023; 201(7): 3395-3403.*
7. *Minnelli C, Piva F, Cecati M, Armeni T, Mobbili G, Galeazzi R, Melecchi A, Cristaldi M, Corsaro R, Rusciano D. Meldonium inhibits cell motility and wound-healing in trabecular meshwork cells and scleral fibroblasts: possible applications in glaucoma. Pharmaceuticals 2023; 16(4): 594.*
8. *Jeon D, Kim N, Um SJ. BET inhibitors induce p53-independent growth arrest in HCT116 cells via epigenetic control of the E2F1/c-MYC axis. Biol Pharm Bull 2023; 46(1): 12-18.*
9. *Weng YJ, Zhang XX, Wu X, Guo LL, Wang CY. Distinct side population cell subtypes have different stemness levels in human ovarian cancer cells. Curr Med Sci 2021; 41(1): 127-132.*
10. *Merrien M, Wasik AM, Melén CM, Morsy MHA, Sonnevi K, Junlén HR, Christensson B, Wahlin BE, Sander B. 2-Arachidonoylglycerol modulates CXCL12-mediated chemotaxis in mantle cell lymphoma and chronic lymphocytic leukemia. Cancers 2023; 15(5): 1585.*
11. *Mondal SK, Basak B, Bhattacharya S, Panda UK. Role of WT1, B-cell lymphoma 2, Ki-67 (Mib1), and Her2/Neu as diagnostic and prognostic immunomarkers in ovarian serous and endometroid carcinoma. J Cancer Res Ther 2021; 17(1): 164-169.*
12. *Alhaddad L, Chuprov-Netochin R, Pustovalova M, Osipov AN, Leonov S. Polyploid/multinucleated giant and slow-cycling cancer cell enrichment in response to x-ray irradiation of human glioblastoma multiforme cells differing in radio resistance and TP53/PTEN status. Int J Mol Sci 2023; 24(2): 1228.*
13. *Chai F, Zhang J, Fu T, Jiang P, Huang Y, Wang L, Yan S, Yan X, Yu L, Xu Z, et al. Identification of SLC2A3 as a prognostic indicator correlated with the NF-κB/EMT axis and immune response in head and neck squamous cell carcinoma. Channels 2023; 17(1): 2208928.*
14. *Agaverdiev M, Shamsov B, Mirzoev S, Vardikyan A, Ramirez ME, Nurmukhametov R, Beilerli A, Zhang B, Gareev I, Pavlov V. MiRNA regulated therapeutic potential of the stromal vascular fraction: Current clinical applications - A systematic review. Noncoding RNA Res 2022; 8(2): 146-154.*
15. *Ye GJ, Cai CY, Dong XD, Wu ZX, Teng QX, Wang JQ, Chen ZS, Wang B. Design, synthesis, and biological evaluation of phenyl urea indole derivatives as ABCG2 inhibitors. Bioorg Chem 2023; 135: 106481.*