# Dysregulation of the PI3K/Akt signaling pathway affects cell cycle and apoptosis of side population cells in nasopharyngeal carcinoma

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**Abstract.** Increasing evidence has suggested that certain types of cancer possess their own stem-like cells, and that one subset of these cells, termed the side population (SP), may have an important role in tumorigenesis and cancer therapy. However, the molecular mechanisms underlying the modulation of SP cells in nasopharyngeal carcinoma (NPC) have remained elusive. In the present study, it was hypothesized that dysregulation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt signaling pathway may influence SP and non-SP (NSP) phenotype. SP cells from the HK-1 NPC cell line were identified, and cancer stem cell markers were found to be highly expressed in SP cells compared with that of NSP cells. Freshly sorted SP cells demonstrated a significant increase in the proportion of cells in G0/G1 phase, while the majority of NSP cells were in the proliferative phase. Following 48 h of culture subsequent to cell sorting, the differences in cell cycle distribution between the SP and NSP cells converged. In addition, the apoptotic ratio of NSP cells was higher than that of SP cells at 24 h following sorting, but had no significant differences 48 h following sorting. To elucidate the potential mechanism mediating the cell cycle and apoptosis in SP cells, the expression levels of key molecules in the PI3K/Akt signaling pathway were evaluated. PI3K and Akt were upregulated, while 14-3-3σ protein was downregulated in SP cells when freshly sorted (0 h). However, there was no significant difference in the expression of these molecules between SP and NSP cells following 48 h of culture. These results suggested that dysregulation of the PI3K/Akt signaling pathway may be associated with the cell cycle and apoptosis of SP cells in NPC. However, further investigation is required to elucidate the detailed mechanisms underlying these effects.

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*Abbreviations:* CSC, cancer stem cell; NPC, nasopharyngeal carcinoma; SP, side population; NSP, non side population; ABCG2, adenosine triphosphate-binding cassette transporter superfamily G member 2; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; FCM, flow cytometry

*Key words:* nasopharyngeal carcinoma, side population cells, cell cycle, apoptosis, signal pathway

## Introduction

Increasing evidence has suggested that specific types of cancer may contain their own stem-like cells, known as cancer stem cells (CSCs), which have key roles in the initiation, maintenance and recurrence of tumors (1-3). In particular, attention has been paid to a subset of CSCs, termed the side population (SP), which was identified by flow cytometry. These SP cells are able to exclude the DNA binding dye, Hoechst 33342, and are highly enriched for stem cells in numerous types of tissue (4-6). SP cells have been isolated from multiple solid tumors, and studies have suggested that they may have significant roles in tumorigenesis and cancer therapy. A SP of cells in nasopharyngeal carcinoma (NPC) were found to exhibit characteristics of stem-like cancer cells (7-12). However, the molecular mechanisms underlying the modulation of these stem-like cell populations in NPC have remained elusive.

Cellular proliferation is a critical process underlying the growth, development and regeneration of eukaryotic organisms, and appropriate control of the cell cycle is required for the proliferation of normal cells (13,14). Deregulation of the cell cycle is responsible for the aberrant cell proliferation characteristic of cancer, and the loss of cell cycle checkpoint control,

which promotes genetic instability. The cell cycle machinery, which functions as an integration point for information transduced via upstream signaling networks, is a target for potential diagnostic and therapeutic interventions (13-15). Apoptosis is a physiological cell death process that has key functions in normal development, as well as in the pathophysiology of various diseases (16,17). A balance between the expression of anti-apoptotic and pro-apoptotic factors underlies apoptosis; and this balance may be altered by certain extracellular signals. Significant alterations to this regulatory pathway may result in the development of various diseases, including autoimmune and neurodegenerative diseases, as well as certain types of cancer (16-19). The phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathway is known to have key roles in cell proliferation, apoptosis and survival in various cell types (20). The PI3K/Akt signaling pathway has been shown to regulate metastasis in multiple cancer cells (21,22).

In the present study, SP cells were identified in the HK-1 NPC cell line, and the SP and NSP cells within this population were sorted for analysis of the cell cycle and apoptosis at differential time-points. In addition, the expression levels of key molecules associated with the PI3K/Akt signaling pathway, including PI3K and Akt, were evaluated by western blotting at the corresponding time-points. The results of the present study may aid the elucidation of the involvement of dysregulation of the PI3K/Akt signaling pathway in cell cycle and apoptosis of SP cells in NPC.

## Materials and methods

*Cell culture*. HK-1 human NPC cells, a highly differentiated NPC cell line, were provided by the Chinese University of Hong Kong (Hong Kong, China), and cultured in RPMI-1640 (Gibco Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco Life Technologies), 100 U/ml penicillin and 100 $\mu$g/ml streptomycin (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) at 37˚C in a 5% $CO_2$ incubator.

*Identifying and sorting of SP cells by flow cytometry (FCM)*. HK-1 cells were cultured in RPMI-1640 with 10% FBS until they reached ~70% confluence. The cells were trypsinized with 0.25% Trypsin (Sigma-Aldrich, St. Louis, MO, USA) at 37˚C in a 5% $CO_2$ cell incubator. Following centrifugation at 500 x g for 5 min at room temperature, the single cell suspension was resuspended in prewarmed RPMI-1640 culture medium containing 2% FBS at a concentration of $1x10^6$ cells/ml. Hoechst 33342 (10 mg/ml; Biotium Inc., Hayward, CA, USA) was added at a final concentration of 5 $\mu$g/ml with or without 50 $\mu$mol/l verapamil (5 mmol/l; Sigma-Aldrich), an adenosine triphosphate binding cassette (ABC) transporter inhibitor, to determine whether the fluorescent efflux effect was altered. The cell suspensions were incubated in a 37˚C circulating water bath for 90 min with gentle shaking every 15 min. Subsequently, the cells were washed twice with pre-cooled phosphate-buffered saline (PBS; Solarbio Science and Technology Co., Ltd., Beijing, China), resuspended in iced PBS with 2% FBS buffer and 1 $\mu$g/ml propidium iodide (PI; Sigma-Aldrich) was added to exclude dead cells. The entire protocol was performed in the dark. A MoFlo™ XDP high-performance cell sorter (Beckman Coulter, Brea, CA, USA) was used for analysis of the SP profile and subsequent cell sorting. In the flow cytometry graphs, SP cells displayed a low Hoechst staining intensity. Finally, SP and NSP cells were sorted from the HK-1 cell line for further experiments. Data and images were acquired using Summit v.5.2 software (Beckman Coulter).

*CSC marker assay in SP and NSP cells*. The total expression and cell surface expression levels of various CSC markers were evaluated in sorted SP and NSP cells by flow cytometric analysis (MoFlo™ XDP). CSC cell surface marker expression was determined by washing freshly sorted SP and NSP cells with PBS, prior to incubation with the following fluorescent conjugated antibodies (5 $\mu$g/$10^5$-$10^7$ cells): ABC superfamily G member 2 (ABCG2)-phycoerythrin (PE) (eBioscience, Inc., San Diego, CA, USA), CD133-PE [Miltenyi Biotec Technology & Trading (Shanghai) Co., Ltd. Shanghai, China], CD34-electron-coupled dye [ECD (PE-Texas Red)] (Beckman Coulter), CD26-fluorescein isothiocyanate (FITC; Beckman Coulter), cytokeratin 14-FITC (Biological, Swampscott, MA, USA) for 1 h at 4˚C. PE mouse immunoglobulin G $(IgG)_{2b}$ isotype control (eBioscience, Inc.), mouse $IgG_{2b}$ isotype control FITC (eBioscience, Inc.) and mouse $IgG_{2b}$ isotype control ECD (eBioscience, Inc.) were used as negative controls for non-specific background signals.

To determine the total expression of these CSC markers, the sorted SP and NSP cells were fixed in 4% paraformaldehyde (Solarbio Science and Technology Co., Ltd.) for 30 min, washed in PBS (3 x 30 sec) and incubated with 0.1% Triton-X 100 (Solarbio Science and Technology Co., Ltd.) for 20 min. Subsequently, the cells were suspended in PBS and the corresponding aforementioned antibodies were added according to the manufacturer's instructions. Mouse $IgG_{2b}$ isotype control antibodies were used as the negative control. The results were analyzed by flow cytometry (MoFlo™ XDP).

*RNA isolation and reverse-transcription-quantitative polymerase chain reaction (RT-qPCR) analysis*. Total RNA was extracted from the SP and NSP cells using an RNeasy® kit (Qiagen, Inc., Valencia, CA, USA) and complemetary (c)DNA synthesis was performed using the RevertAid First Strand cDNA Synthesis kit (CWBio, Beijing, China) according to the manufacturer's instructions. Subsequently, qPCR was conducted using the GoTaq qPCR master mix (Promega Corp., Madison, WI, USA). The primers used for RT-qPCR are presented in Table I. RT-qPCR was performed using the BIO-RAD CFK96TM Real-Time System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The data were analyzed with Bio-Rad CFK Manager 2.0 software (Bio-Rad Laboratories, Inc.). Messenger (m)RNA expression was assessed by evaluating the threshold cycle (CT) values. GAPDH was used as an internal control.

*Flow cytometric analysis of the cell cycle*. The SP and NSP cells of the HK-1 cell line were sorted by flow cytometry as previously described, and divided into two groups, respectively. One group was for analysis of the cell cycle of sorted SP and NSP cells (0 h). The other was for the analysis of the cell cycle of SP and NSP cells following culture in RPMI-1640 supplemented with 10% fetal bovine serum for 24 or 48 h. Cells were harvested at 0, 24 and 48 h and fixed in 70% ethanol at

Table I. Human-specific primer sequences used in the present study. To avoid false positive signals originating from DNA contamination, all human-specific polymerase chain reaction primers were designed with known amplicon size, and where possible flanking a region that contained a minimum of one intron.

| Target gene | Forward primer | Reverse primer |
|---|---|---|
| ABCG2 | AGCTGCAAGGAAAGATCCAA | TGCCCATCACAACATCATCT |
| CD133 | TTGTGGCAAATCACCAGGTA | TCAGATCTGTGAACGCCTTG |
| CD34 | CAAGCCACCAGAGCTATTCC | TCCACCGTTTTCCGTGTAAT |
| CD26 | CAAATTGAAGCAGCCAGACA | CACACTTGAACACGCCACTT |
| CK14 | TTCTGAACGAGATGCGTGAC | GCAGCTCAATCTCCAGGTTC |

4˚C. The cells were then washed with cold PBS and stained with PI in working solution (0.5 mg/ml RNase and 0.1 mg/ml PI in PBS). The cell cycle distribution was determined by flow cytometric analysis using a MoFlo™ XDP High-Performance Cell Sorter (Beckman Coulter) and the data were analyzed using Summit v.5.2 software.

*Flow cytometric analysis of apoptosis*. The SP and NSP cells of the HK-1 cell line were sorted by flow cytometry. The sorted cells were cultured in RPMI-1640 supplemented with 10% FBS for 24 or 48 h, prior to harvest. The cell apoptosis ratio was analyzed using an Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Briefly, $5x10^5$ cells were stained with Annexin V-FITC (5 $\mu$l) and 100 $\mu$g/ml PI (1 $\mu$l) in 100 $\mu$l binding buffer and incubated at room temperature for 15 min in the dark. Subsequently, 400 $\mu$l of binding buffer was added and mixed gently, and the stained cells were analyzed using a MoFlo™ XDP flow cytometer. The data were evaluated using Summit v.5.2 software.

*Western blot analysis*. SP and NSP cells at 0 and 48 h following sorting, were lysed in radioimmunoprecipitation buffer (CWBio, Beijing, China) and total protein concentration was determined using a Pierce® BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Extracts containing 50 $\mu$g protein were separated with 10% SDS-PAGE and electroblotted onto nitrocellulose membranes (HyClone; GE Healthcare Life Sciences). The membranes were inhibited using Tris-buffered saline/Tween-20 (25 mM Tris-HCl, 150 mM NaCl and 0.05% Tween-20; pH 7.5; Solarbio Science and Technology Co., Ltd.) containing 5% non-fat milk followed by overnight incubation at 4˚C with the following primary antibodies: rabbit anti-PI3K polyclonal antibody (catalog no. 4292; Cell Signaling Technology, Inc., Danvers, MA, USA; dilution, 1:500); rabbit anti-Akt polyclonal antibody, (catalog no. 9272; Cell Signaling Technology, Inc.; dilution, 1:300) and mouse anti-14-3-3σ monoclonal antibody (E-11; catalog no. sc-166473; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Following three washes, the membranes were incubated with horseradish peroxidase-conjugated mouse anti-rabbit (catalog no. sc-2491; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; dilution, 1:5,000) and goat anti-mouse (catalog no. sc-2039; Santa Cruz Biotechnology, Inc.; dilution, 1:5,000) IgG secondary antibodies for 1 h at room temperature and the signals were visualized using an enhanced chemiluminescence detection system (Universal Hood II; Bio-Rad Laboratories, Inc.) with Image Lab™ software. Anti-GAPDH antibody (Santa Cruz Biotechnology, Inc.; 1:3,000) was used as a loading control.

*Statistical analysis*. Results were statistically analyzed by Student's unpaired t-test using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± standard error of the mean. $P<0.05$ was considered to indicate a statistically significant difference between values.

## Results

*Putative CSC markers are differentially expressed in SP and NSP cells of the HK-1 cell line*. The SP fraction of the NPC cell line, HK-1, was determined using a flow cytometric SP discrimination assay. The percentage of SP cells was found to be 3.65±1.51%. The proportion of SP cells was significantly blocked by verapamil (Fig. 1A and B). Subsequently, the MoFlo™ XDP High-Performance Cell sorter was used to isolate the SP and NSP cells from the HK-1 cells and the cell surface expression levels of CSC markers were evaluated. The expression levels of putative CSC markers, including ABCG2, CD133, CD34 and CD26, were higher in SP cells compared with those of NSP cells ($P<0.05$). ABCG2 expression was higher in SP cells (4.27%) than NSP cells (0.41%). CD133 was 3.28 and 1.90% in SP and NSP cells, respectively. CD34 and CD26 exhibited analogous expression patterns. However, no differential expression of CK14 was detected between SP and NSP cells (Fig. 1C).

*Protein and mRNA expression levels of putative CSC markers differ between SP and NSP cells in NPC*. Subsequently, the total protein expression levels of the putative CSC markers were examined in sorted SP and NSP cells by flow cytometry. The results demonstrated that CD133, CD34, and CK14 expression was higher in SP cells compared with that of NSP cells. Expression levels were 28.09, 28.17 and 11.89% in SP cells, and 4.82, 24.90 and 5.23% in NSP cells, respectively. The fraction of ABCG2 and CD26 expression was high in SP and NSP cells (Fig. 1D). In addition, the expression levels of these markers in SP and NSP cells were evaluated by RT-qPCR. It was demonstrated that there were significant differences in the mRNA expression levels of ABCG2, CD26, and CK14 between SP and NSP cells of the HK-1 cell line ($P<0.05$). However, there was no significant difference in the expression of CD133 and CD34 (Fig. 1E). In SP and NSP cells, the mRNA and protein expression levels of CSC markers, including CD133 and CD34, is not consistent.

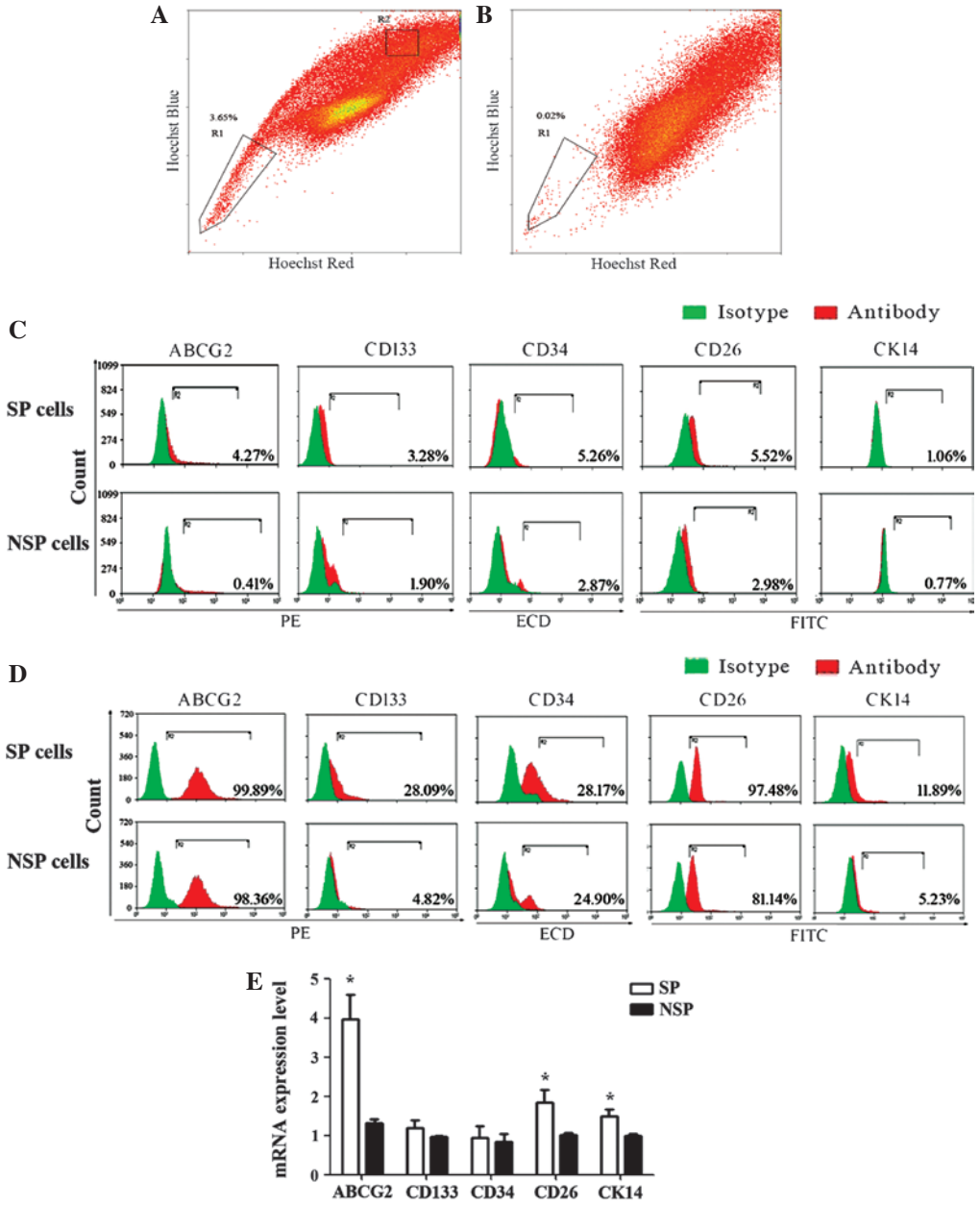


Figure 1. Human nasopharyngeal carcinoma cell lines contain a fraction of SP cells and CSC-associated markers are differentially expressed in SP and NSP cells. (A) Scatter-blot analysis of HK-1 cells stained with Hoechst 33342. (B) Scatter-blot analysis of HK-1 cells stained with Hoechst 33342 plus verapamil treatment. SP and NSP cells are indicated in boxes R1 and R2, respectively. (C) CSC marker, ABCG2, CD133, CD34, CD26 and CK14, expression on the cell surface of sorted SP and NSP cells was evaluated by flow cytometry. (D) Total expression levels of CSC markers, ABCG2, CD133, CD34, CD26 and CK14, in SP and NSP cells. Flow cytometric analysis revealed that CSCs markers were highly expressed in SP cells. (E) Relative mRNA expression levels of ABCG2, CD133, CD34, CD26 and CK14 in SP and NSP cells were determined by reverse transcription-quantitative polymerase chain reaction. Three independent experiments were performed. The results are shown as the mean ± standard error and refer to freshly sorted cells (0 h). *P<0.05 vs. NSP cells. SP, side population; CSC, cancer stem cell; NSP, non-side population; mRNA, messenger RNA; PE, phycoerythrin; ECD, electron-coupled dye (PE-Texas Red); FITC, fluorescein isothiocyanate; ABCG2, adenosine triphosphate-binding cassette transporter superfamily G member 2.

*Cell cycle distribution differs between freshly sorted SP and NSP cells of the HK-1 cell line*. Cell cycle progression was compared between SP and NSP cells 0, 24 and 48 h following sorting by flow cytometric analysis. When cells were freshly sorted, SP cells revealed a significant increase in the proportion of cells in G0/G1 phase and a reduction in the percentage of cells in S phase (Fig. 2A). The percentages of G0/G1, S and G2/M phases were 64, 32.22 and 3.78%, respectively. By contrast, the majority of NSP cells were in the proliferative phase, and the percentages of cells in G0/G1, S and G2/M phases were 40.76, 54.83 and 4.41%, respectively. Immediately following sorting, SP and NSP cells demonstrated significant differences in cell cycle distribution. However, following 24 h of culture, the differences in cell cycle distribution between SP and NSP cells were abrogated. The percentages of cells in G0/G1, S and G2/M phases in SP and NSP cells at 24 h were 44.09, 19.55 and 36.37 vs. 40.5%, 37.27% and 22.23%, respectively (Fig. 2B). In accordance, the cell cycle distribution of SP and NSP cells 48 h following sorting also coincided. The percentages of cells in G0/G1, S and G2/M phase in SP and NSP cells at 48 h were 39.5%, 41.05% and 19.45% vs. 47.17%, 35.3% and

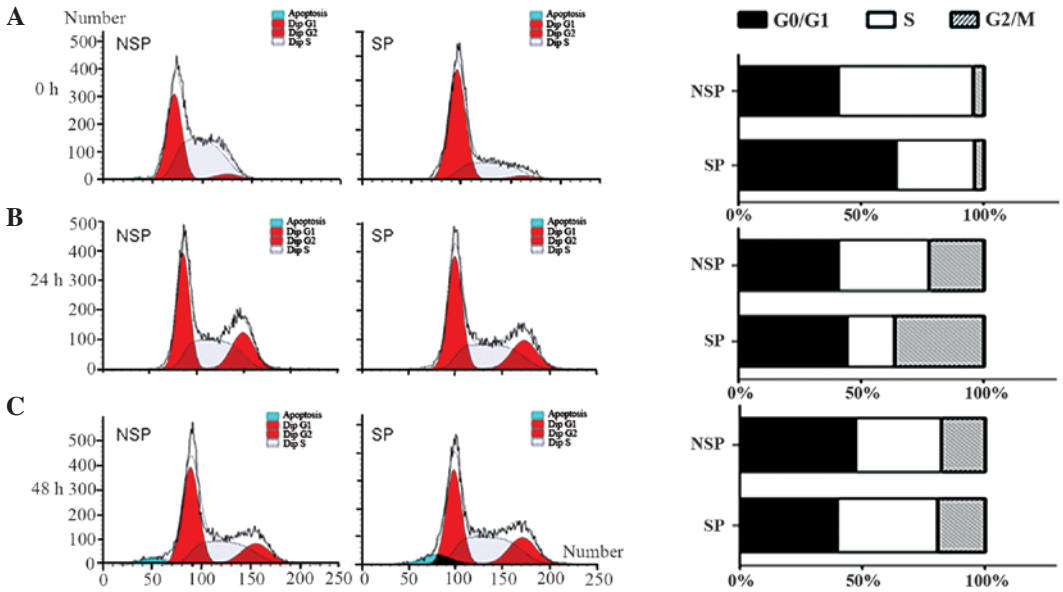


Figure 2. SP and NSP cells exhibited altered cell cycle profiles. Cell cycle distribution of SP and NSP cells was evaluated by flow cytometry (A) 0, (B) 24 and (C) 48 h following cell sorting. SP, side population; NSP, non-side population.

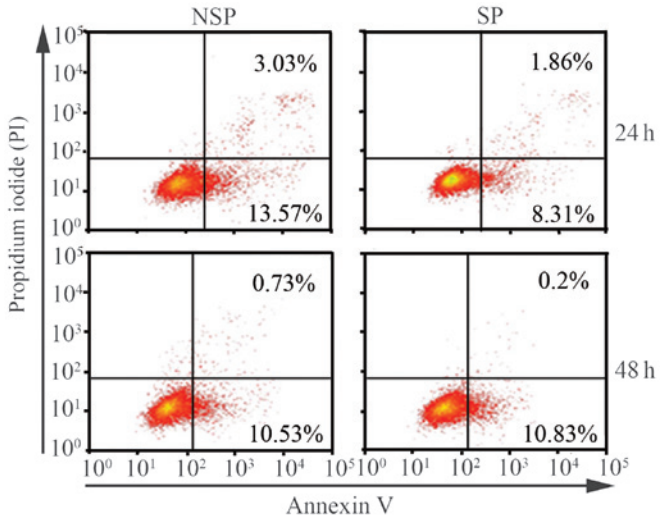


Figure 3. SP and NSP cells exhibited altered cell apoptosis profiles. Cell apoptotic analysis of SP and NSP cells by flow cytometry at 24 and 48 h. Annexin V-positive/PI-negative cells indicated early apoptotic cells. Annexin V-positive/PI-positive cells indicated late apoptotic cells. The rate of cell apoptosis was calculated by early plus late apoptotic cells. SP, side population; NSP, non-side population; PI, propidium iodide.

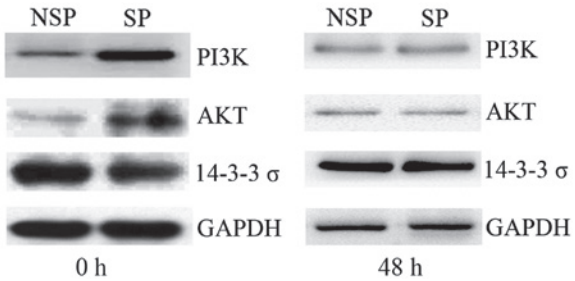


Figure 4. Western blot analysis of SP and NSP cells from HK-1 cell line for the indicated proteins at 0 and 48 h after cell sorting. GAPDH was used as the loading control. Expression levels of PI3K, AKT and 14-3-3σ differed significantly at 0 h between SP and NSP cells. At 48 h, no significant difference in expression was observed. SP, side population; NSP, non-side population; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase.

17.53%, respectively (Fig. 2C). These results suggested that the differences in cell cycle distribution between SP and NSP cells converged with time.

*The apoptotic ratio of NSP cells is higher than that of SP cells 24 h following sorting.* Given that SP cells were found to be rich in CSCs markers compared with NSP cells, whether SP cells had a lower apoptotic ratio than NSP cells was determined. Annexin V-FITC and PI staining were used to analyze the percentage of apoptotic cells in SP and NSP cells at various time-points by flow cytometry. The apoptotic ratio of NSP cells was higher than that of SP cells 24 h following sorting, without any external stimuli to the cells. The apoptotic ratios of SP and NSP cells were 10.17 and 16.6%, respectively. As observed in cell cycle distribution, no significant differences in the apoptotic proportion were detected between SP and NSP cells 48 h following sorting. The apoptotic ratios of SP and NSP cells at 48 h were 11.03 and 11.36%, respectively (Fig. 3).

*Dysregulation of The PI3K/Akt signaling pathway is dysregulated in SP cells of HK-1 cells immediately following sorting.* To elucidate the potential mechanism underlying the mediation of the cell cycle and apoptosis in SP cells, the expression levels of key molecules associated with the PI3K/Akt signaling pathway were detected by western blot analysis. Immediately following cell sorting by flow cytometry (0 h), PI3K and Akt expression levels were upregulated in SP cells compared with those of NSP cells of the HK-1 cell line, whereas 14-3-3σ protein expression was downregulated in SP cells. Once the sorted cells had been cultured for 48 h, no significant difference in PI3K, Akt or 14-3-3σ protein was detected between SP and NSP cells of HK-1 cells (Fig. 4). Combined with the

aforementioned results of cell cycle and apoptosis analysis, it was hypothesized that dysregulation of the PI3K/Akt signaling pathway was associated with the alterations in the cell cycle and apoptosis of SP cells in NPC.

## Discussion

NPC is an endemic disease with an incidence rate of 15-50/100,000 individuals in southern China and Southeast Asia, and represents one of the most significant public health issues in these regions (23). Although studies regarding the tumorigenesis of NPC have previously been published (24-29), the molecular basis for NPC is not fully understood. Recent studies have demonstrated that CSCs have a significant role in the pathophisiology of head and neck squamous cell carcinomas (30-32). In particular, research has focused on a specific subset of CSCs, termed SP cells, which were identified by FCM. Therefore, elucidation of the molecular mechanisms of SP in NPC is urgently required for the improvement of clinical diagnosis and therapy.

In the present study, the fraction of SP cells in the HK-1 NPC cell line, was found to be 3.65±1.51%. The proportion of SP cells in this cell line was significantly decreased following verapamil treatment. SP cells expressed high levels of CSC markers compared with those of NSP cells. For example, ABCG2 expression was higher in SP cells (4.27%) than in NSP cells (0.41%), and CD133 expression was 3.28 and 1.90% in SP and NSP cells, respectively. Wang *et al* (7) revealed that SP cells represented ~2.6% of the total cells in the NPC cell line, CNE-2. Another four human NPC cell lines, C-666-1, SUNE-1, HONE-1 and CNE-1, were also found to contain small subpopulations of SP cells and their proportions were 0.1, 6.8, 1.8 and 0.7%, respectively. Certain putative CSC markers are highly expressed in SP cells (7-9), and the results of these studies corroborate the results presented in the present study.

In order to reveal the characteristics of the cell cycle and apoptosis in SP cells, the cells were evaluated at differential time-points following sorting (0, 24 or 48 h). The results of the present study revealed that freshly sorted SP cells demonstrated a significant increase in the number of cells in G0/G1 phase. However, following 48 h of culture, differences in cell cycle distribution between SP and NSP cells were abrogated. In addition, the apoptotic ratio of NSP cells was higher than that of SP cells 24 h following sorting, whereas no significant differences were detected following 48 h of culture. We hypothesize that culturing the SP and NSP cells in complete medium after sorting may have caused the SP cells to differentiate, subsequently losing their stem cell properties. Previous studies have revealed that normal and neoplastic stem cells obtained from neural and epithelial organs only exhibit initial tumor-specific properties when cultured in serum-free medium containing epidermal growth factor (EGF) and fibroblast growth factor (FGF)-2 (33-35). In addition, adherent cells expanded in Laminin-coated culture plates in serum free medium containing N2-supplement, EGF and basic FGF maintain initial tumor-specific properties (36). However, when the cells were cultured in traditional complete medium, stem cells differentiated and lost their stem cell phenotype (37,38). In contrast to embryonic stem cells, a characteristic feature of adult stem cells is their proliferative quiescence. It is widely accepted that this quiescent state is a functionally significant feature of adult stem cells (39-41).

To reveal the potential mechanisms underlying the cell cycle and apoptosis in SP cells, the expression levels of key molecules associated with the PI3K/Akt signaling pathway were detected. PI3K and Akt expression was upregulated, while 14-3-3σ protein expression was downregulated in freshly sorted SP cells (0 h). However, there was no significant difference in the expression of these molecules in SP and NSP cells following 48 h of culture. 14-3-3σ, a potential tumor suppressor protein, is able to negatively regulate cell cycle progression by inducing G2-M phase arrest (42,43). It has previously been demonstrated that 14-3-3σ is transactivated by p53 in response to DNA damage and, in turn, interacts with p53 and positively regulates p53 activity (44). p53 is known to be involved in mediating the complex response to ionizing radiation, inducing irreversible growth arrest and apoptosis (45). The results of the present study are in accordance with those of previous reports.

In conclusion, the results of the present study suggested that dysregulation of the PI3K/Akt signaling pathway may be associated with mediation of the cell cycle and apoptosis of SP cells in NPC. However, elucidation of the detailed mechanisms underlying this process requires further study.

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