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Original Article

Biological characteristics of CD133$^+$ cancer stem cells derived from human laryngeal carcinoma cell line

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Abstract: Objective: To investigate the *in vitro* invasive capability, clone-forming ability, resistance to anti-tumor treatments of CD133$^+$ human laryngeal carcinoma stem cells, and characterize the related signaling pathways in these cells. Methods: Human laryngeal carcinoma Hep-2 cells were subjected to flow cytometry sorting to obtain CD133$^+$ stem cells. Transwell chamber assay and clone-formation forming test were performed to evaluate the invasive capability and the clone-forming ability of CD133$^+$ laryngeal carcinoma tumor stem cells, respectively. MTT assay was used to assess the resistance of CD133$^+$ Hep-2 cells to radiotherapy and chemotherapy, respectively. Western blot and real-time PCR were applied to characterize the signaling pathways in these stem cells. Results: Our results from the transwell chamber assay indicated that the migrating capability of CD133$^+$ Hep-2 cells was significantly higher than CD133$^-$ cells, and the invasive capability of CD133$^+$ Hep-2 cells was also significantly elevated. Moreover, clone-formation forming test showed higher clone-forming ability for CD133$^+$ Hep-2 cells, compared with CD133$^-$ cells. Furthermore, CD133$^+$ Hep-2 cells displayed significant resistance to radiotherapy and chemotherapy. The Bcl-2/Bax ratio was increased, and Hedgehog, Wnt, and Bmi-l signaling pathways were all activated, in CD133$^+$ laryngeal carcinoma stem cells, which might be involved in the self-renewal process of these stem cells. Conclusion: The invasive capability, clone-forming ability, and resistance to anti-tumor treatments are enhanced, and anti-apoptotic and proliferation-related signaling pathways are activated in CD133$^+$ laryngeal carcinoma tumor stem cells. These findings might provide new insights into the prevention and/or treatment of laryngeal carcinoma, especially concerning target-oriented therapies.

Keywords: CD133, laryngeal carcinoma, tumor stem cells, chemotherapy, radiotherapy

Introduction

Cancer stem cells (CSCs) are a group of cells with eternal life or infinite self-renewal ability, which have high migrating, infiltrative, and metastatic abilities [1, 2]. Initially identified in hematopoietic cancers, a number of CSCs have been isolated from various solid human malignancies [3-7]. Studies have shown that tumor-initiating cells are responsible for tumor formation and progression. Tumor clone is heterogeneous with respect to proliferation and differentiation. Interestingly, these tumor-initiating cells share with stem cells the key feature of self-renewal. They can produce other heterogeneous tumor cells in the tumor [8, 9]. Though CSCs only accounted for a small proportion in tumors, the high resistance to traditional therapy exempts them from therapy killing and thus they can reconstruct tumors. Therefore, therapy targeting stem cell has played an important role in the treatments of malignant tumors [10].

Previous studies have found that heterogeneity of cancer cells extensively exists in head and cervical cancers. Prince *et al.* [11] have reported that CD44$^+$ cancer cells are detected in the primary laryngeal carcinoma. Though CD44$^+$ cancer cells only account for less than 10%, they have very high tumor-formation ability *in vitro*. Human CD133 gene is about 152 kb long, which is located on the fourth chromosome, containing at least 37 exons. CD133 protein belongs to the membrane protein superfamily, and it is a glycoprotein composed of 865 amino acids, with a molecular weight of ~120 kD [12, 13]. Singh *et al.* [14] have reported for the first time that CD133 can be used as a characteris-

Table 1. Information of primary antibodies

Antibody	Company	Genus	Dilution ratio
Bax	CST	Rabbit	1:2000
Bcl-2	CST	Rabbit	1:1000
Fas	Abcam	Rabbit	1:1000
c-Myc	CST	Rabbit	1:1000
Survivin	Santa Cruz	Mouse	1:1000
β-catenin	CST	Rabbit	1:500
Bmi-1	Santa Cruz	Rabbit	1:500
PTCH	Abcam	Rabbit	1:2500
Gli-1	Abcam	Rabbit	1:1000
SHH	CST	Rabbit	1:1000
SMO	Abcam	Rabbit	1:1000
β-actin	Sigma	Rabbit	1:3000

tic marker on the surface of brain tumor stem cells. Subsequently, increasing evidence supports that CD133 may be a specific molecule expressed on the surface of CSCs, and CD133 may become a new effective target for tumor therapy. Some experimental results have indicated that CD133 is one of the markers for laryngeal carcinoma stem cells [3].

It has been found in previous studies that CSCs can be detected in laryngeal carcinoma cell line [9, 10]. The present study was carried out to further investigate the *in vitro* invasive capability and clone-forming ability of CD133+ human laryngeal carcinoma cells to investigate its derivation from stem cells, which can further determine the marker and provide evidence for target-oriented therapies.

Materials and methods

Cell line

Human laryngeal carcinoma Hep-2 cell line was obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

Cell culture and sorting

Cells were cultured with RPMI1640 complete medium (GIBCO, Grand Island, NY, USA), in a 5% CO_2, 37°C incubator. The medium was changed every other day. For flow cytometry sorting, cells in the exponential phase were digested with 0.25% trypsin, resuspended with 0.01% PBS, filtered with a strainer of 400-mesh

Table 2. Primer sequences for real-time PCR

Primer sets	Sequences
β-actin F	5'-CCTGGCACCCAGCACAATG-3'
β-actin R	5'-CGCCGATCCACACGGAGTAC-3'
Bax F	5'-CCCGAGAGGTCTTTTTCCGAG-3'
Bax R	5'-CCAGCCCATGATGGTTCTGAT-3'
Bcl-2 F	5'-TTGGATGCACAACATGAATCAGG-3'
Bcl-2 R	5'-TCTTCTGACTGAGAGCTATGGTC-3'
Fas F	5'-TCTGGTTCTTACGTCTGTTGC-3'
Fas R	5'-CTGTGCAGTCCCTAGCTTTCC-3'
c-Myc F	5'-CTTCTCTCCGTCCTCGGATTCT-3'
c-Myc R	5'-GAAGGTGATCCAGACTCTGACCTT-3'
Survivin F	5'-GGCCCAGTGTTTCTTCTGCTT-3'
Survivin R	5'-GCAACCGGACGAATGCTTT-3'
β-catenin F	5'-AGGGATTTTCTCAGTCCTTC-3'
β-catenin R	5'-CATGCCCTCATCTAATGTCT-3'
Bmi-1 F	5'-CTGGTTGCCCATTGACAGC-3'
Bmi-1 R	5'-CAGAAAATGAATGCGAGCCA-3'
SHH F	5'-CTCGCTGCTGGTATGCTCG-3'
SHH R	5'-ATCGCTCGGAGTTTCTGGAGA-3'
PTCH F	5'-CCAGAAAGTATATGCACTGGCA-3'
PTCH R	5'-GTGCTCGTACATTTGCTTGGG-3'
SMO F	5'-TCGAATCGCTACCCTGCTG-3'
SMO R	5'-CAAGCCTCATGGTGCCATCT-3'
Gli-1 F	5'-AGGGAGTGCAGCCAATACAG-3'
Gli-1 R	5'-ATTGGCCGGAGTTGATGTAG-3'

grid, and then centrifuged at 1000 r/min for 5 min. FITC-CD133 antibody (1:200 dilution; eBioscience, California, CA, USA) was added to incubate the cells. After incubated at room temperature in dark for 0.5 h, the cells were rinsed with 0.01% PBS, and then resuspended with FACS buffer. Cell sorting was performed with a flow cytometer (BD Biosciences, Hercules, CA, USA). After sorting, CD133+ and CD133- cells were centrifuged, rinsed, and resuspended with fresh RPMI1640 complete medium. Culture medium was changed every three days, and cell proliferation was observed under an inverted microscope, at 0 h, 24 h, 48 h, and 72 h.

Transwell chamber assay

Laryngeal carcinoma stem cells were rinsed with 0.01% PBS, and resuspended with culture medium containing 1% fetal bovine serum (FBS; HyClone, Logan, Utah, USA). 100 μL cell suspension was added into the transwell chamber (Corning, Corning, NY, USA) at a density of

5×10^5 cells/mL. Then 600 μL medium containing 20% FBS was added into the chamber, below the 24-well plate. 48 h later, the cells were rinsed with 0.01% PBS and fixed in 0.5% methanol for 30 min. The cells were stained with 0.1% gentian violet (GIBCO) for 20 min, and rinsed with 0.01% PBS. Cells penetrating the membrane were counted in five random visual fields (200×), and the mean value was calculated.

Clone-forming test

Cells in the exponential phase of growth were digested, and suspended in RPMI1640 culture medium containing 10% FBS. The cell suspension was serially diluted, and then inoculated in the 24-well plate containing 10 mL medium at a density of 100 cells/well. After 2-3 wk, clone spheres were formed in the dishes. The cells were rinsed with 0.01% PBS, and then fixed with 5 mL 4% paraformaldehyde for 15 min. GIEMSA (Invitrogen, Carlsbad, CA, USA) was added for staining for 15 min, and the samples were rinsed with flow water, and then air-dried. The dishes were kept inverted, covered with a transparent film with grids. The number of clones with more than 10 cells was counted under the microscope. Clone formation rate was calculated according to the following formula:

Clone formation rate = (number of clones/number of inoculated cells) × 100%.

The clone formation rates for three successive cell passages were determined.

Drug administration and irradiation treatment

Paclitaxel was used to assess the resistance of tumor stem cells to chemotherapy. Cells were inoculated in the 96-well plate, at a density of 2×10^4 cells/well, in 100 μL medium. Paclitaxel was added to incubate the cells, with the final concentration of 1×10^{-8} mol/L, for 24 h, 48 h, and 72 h, respectively.

For irradiation treatment, $CDl33^+$ and $CDl33^-$ cells were subjected to serum-free culture, and 4×10^3 cells were planted in the 96-well plate. Medium was used as the blank control. Then the cell cultures were subjected to irradiation with a linear accelerator at 10 Gy for 36 h. Irradiation of 0 Gy was used as control.

MTT analysis

After indicated treatments, 20 μL methyl thiazolyl tetrazolium (MTT) was added into each well for a further incubation for 4 h. The supernatant was removed, and 100 μL dimethyl sulfoxide (DMSO) was added into each well, shaking for 10 min. An automatic microplate reader was used to determine the optical density at 490 nm (A value), and the reference wavelength was set at 630 nm. The relative survival rate and inhibition rate of the cells were calculated according to the following formulas, respectively:

Relative survival rate (%) = $(A_{\text{the treated group}}-A_{\text{the control group}}) \times 100\%$,

Inhibition rate (%) = $(A_{\text{the control group}}-A_{\text{the treated group}})/A_{\text{the control group}} \times 100\%$.

The tests were repeated for three times, and the mean value was calculated.

Western blot

Cells were lysed by ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer, containing 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 μg/mL leupeptin, 1 μg/mL aprotinin, and 1 μg/mL pepstatin, at 4°C for 15 min. After centrifugation, the supernatant was collected and the protein concentration was determined using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). The sample was mixed with Laemmli buffer, and heated at 95°C for 5 min. 50 mg samples were loaded in each lane and separated by SDS-polyacrylamide gel electrophoresis (PAGE). After electrophoresis, proteins were electrophoretically transferred onto a polyvinylidene difluoride (PVDF) filter membrane (0.45 mm; Amersham, Amersham, Buckinghamshire, UK). The membrane was blocked in Tris-buffered saline Tween-20 (TBST) with 5% non-fat milk, and then incubated with indicated primary antibodies (**Table 1**), at 4°C overnight. The membrane was further incubated with secondary antibody (horseradish peroxidase-conjugated anti-rabbit IgG) for 1 h. The signals were visualized by an enhanced chemiluminescence reaction (ECL) system (Millipore, Billerica, MA, USA). The staining was quantified by scanning the films and the band density was determined with Quantity-One software (Bio-Rad, Hercules, CA, USA).

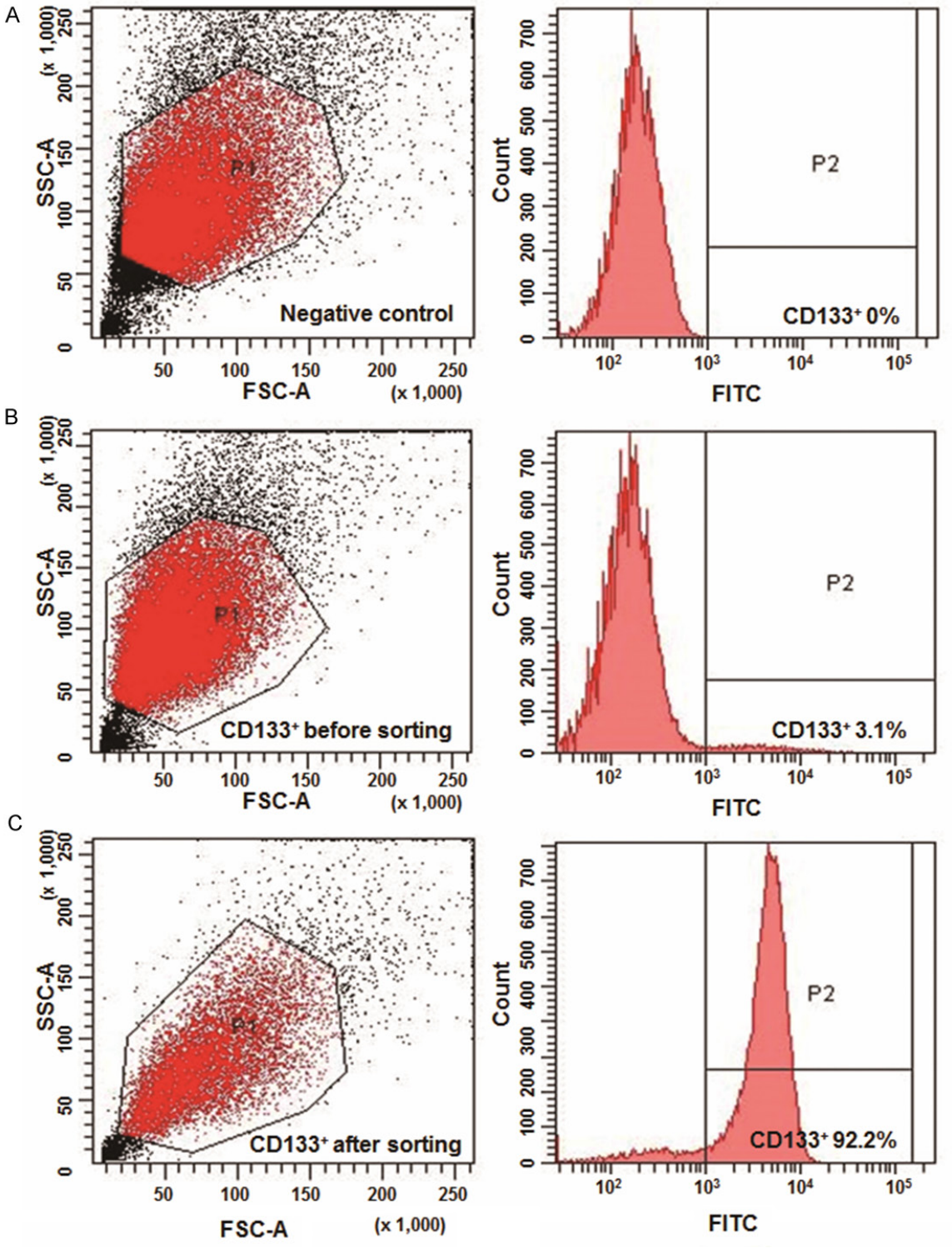

Figure 1. Flow cytometry sorting of CD133+ laryngeal carcinoma stem cells. A. Negative control group. B. Determination of the percentage for CD133+ cells in the Hep-2 cells before sorting. C. Proportion of CD133+ Hep-2 cells after FACS sorting.

Real-time PCR

Total RNA was extracted from the tumor stem cells using an RNeasy Mini Kit (Qiagen, Hilden, Germany). First stand cDNA was synthesized using a Sensiscript RT Kit (Qiagen), according to the manufacturers' instructions. The mRNA expression levels of the genes encoding Bax,

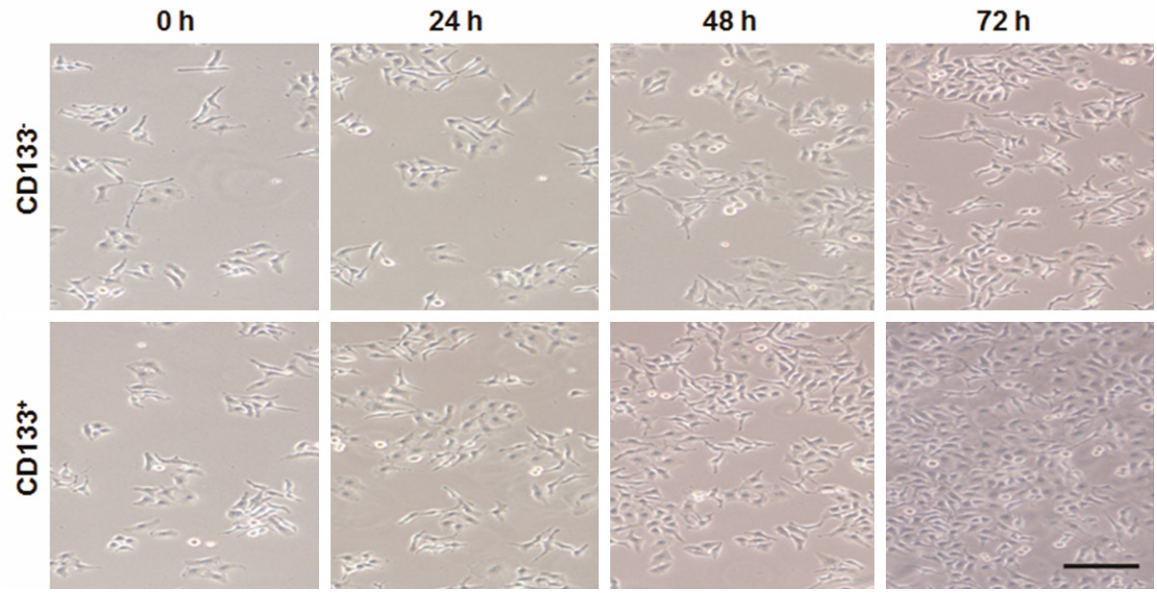

Figure 2. Culture of CD133+ laryngeal carcinoma stem cells. Growing status of CD133- (upper panel) and CD133+ (lower panel) Hep-2 cells at 0 h, 24 h, 48 h, and 72 h after sorting. Scale bar, 100 μm.

Bcl-2, Fas, c-myc, survivin, β-catenin, Bmi-1, SHH, PTCH, SMO, Gli-1, and the housekeeping gene β-actin were determined by real-time PCR using SYBR Green master mix under standard thermocycler conditions (Applied Biosystems, Foster City, CA, USA), with the primer sequences shown in **Table 2**. Data were analyzed using an ABI Prism 7900 sequence detection system (Applied Biosystems).

Statistical analysis

The data were expressed as mean ± SD. SPSS 11.0 software was used for statistical analysis. Paired t-test was carried out for the comparison between groups. $P < 0.05$ was considered as statistically significant.

Results

Flow sorting and culture of CD133+ laryngeal carcinoma stem cells

Human laryngeal carcinoma Hep-2 cells were subjected to flow cytometry sorting. As shown in **Figure 1**, the percentage of Hep-2 cells positive for CD133 was 3.10 ± 0.21% before sorting, and this percentage became 90.20 ± 5.51% after sorting. CD133+ and CD133- tumor cells were cultured separately. On the first day, both the CD133+ and CD133- tumor cells exhibited fusiform and flat morphology, with abundant cytosol and round nuclei, and the cell density and distribution were similar between groups. On the second day, clusters were observed in CD133+ cells, and the cell density was significantly higher than CD133- cells. On the third day, CD133+ cells reached 80-90% confluence, while the density of CD133- cells was dramatically lower (**Figure 2**). In the following experiments, the invasive capability, clone-forming ability, resistance to anti-tumor treatments, and related signaling pathways, in the sorted CD133+ laryngeal carcinoma tumor cells were investigated.

Invasive capability assay of CD133+ laryngeal carcinoma stem cells

To investigate the invasive capability of CD133+ laryngeal carcinoma tumor cells, the transwell chamber assay was performed. Transwell chambers were used to mimic tumor environment, and polycarbonate membrane (pore diameter, 12 μm) was applied to separate the upper and lower chambers to mimic the extracellular matrix. The results showed that there were 526 ± 39 CD133+ Hep-2 cells in each visual field (200×), which was significantly higher than CD133- Hep-2 cells under the same experimental conditions (220 ± 20) ($P < 0.01$) (**Figure 3**). These results indicate that the migrating capability of CD133+ Hep-2 cells is significantly higher than that of CD133- cells,

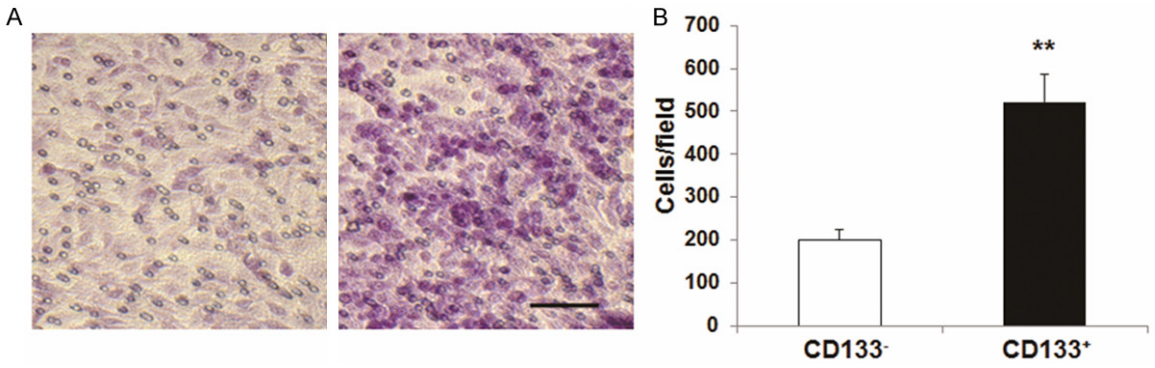

Figure 3. Invasive capability assay of CD133+ laryngeal carcinoma tumor stem cells. Transwell chamber assay was performed to assess the invasive capability of CD133+ Hep-2 cells. A. CD133- (left) and CD133+ (right) Hep-2 cells penetrating the membrane. Scale bar, 100 μm. B. Statistical analysis of the numbers of CD133- and CD133+ Hep-2 cells penetrating the membrane. Compared with the CD133- control cells, $^{**}P < 0.01$.

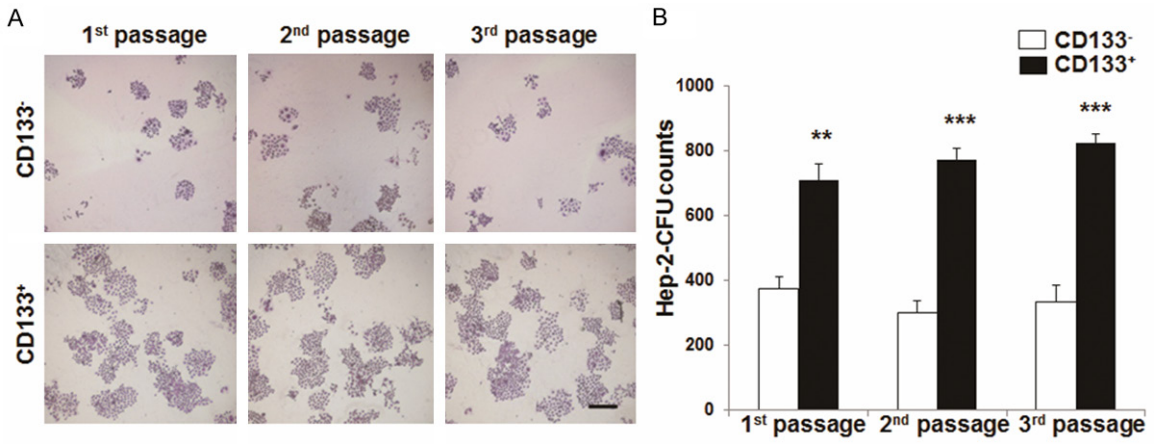

Figure 4. Assessment of clone-forming ability for CD133+ laryngeal carcinoma stem cells. Clone-forming ability was assessed for three consecutive passages of CD133- and CD133+ Hep-2 cells. A. Pictures form clone-forming tests for three passages of CD133- (upper panel) and CD133+ (lower panel) Hep-2 cells. Scale bar, 100 μm. B. Statistical analysis of the numbers of clone clusters for CD133- and CD133+ laryngeal carcinoma stem cells. Compared with the CD133- control cells, $^{**}P < 0.01$, $^{***}P < 0.001$.

and the invasive capability of CD133+ Hep-2 cells is significantly elevated.

Assessment of clone-forming ability of CD133+ laryngeal carcinoma stem cells

The clone-forming ability of CD133+ Hep-2 cells was then assessed. Our results showed that the clone formation rates for three passages of CD133+ Hep-2 cells were 30.00 ± 4.69%, 32.25 ± 3.59%, and 32.75 ± 3.40%, while the clone formation rates for CD133- Hep-2 cell passages were 15.25 ± 2.21%, 12.00 ± 2.49%, and 13.75 ± 3.30%, respectively. Statistical analysis showed that the differences between the same passages were statistically significant ($P < 0.01$ for all the three passages) (**Figure 4**). These results indicate that the clone-forming ability of CD133+ Hep-2 cells is significantly higher than the CD133- Hep-2 cells.

Resistance of CD133+ laryngeal carcinoma stem cells to anti-tumor treatments

To determine the resistance to anti-tumor treatments of CD133+ and CD133- Hep-2 cells, they were subjected to the treatments of a linear accelerator (10 Gy) and a common chemotherapeutic agent for head and neck neoplasms, paclitaxel, respectively. The experiments showed that the growth inhibition rate for CD133+ Hep-2 cells after irradiation treatment was 30 ± 7.12%, which was significantly lower than CDl33- cells (55 ± 6.33%) (**Figure 5A**; $P < 0.01$).

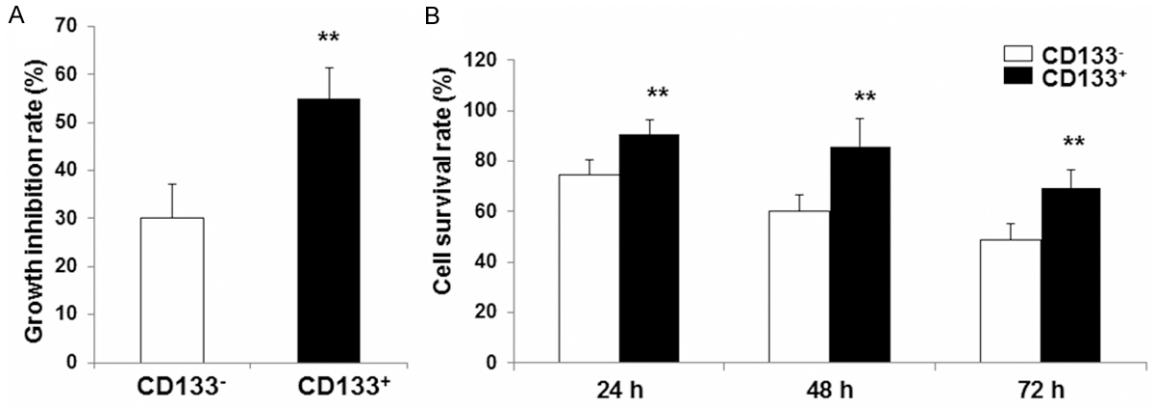

Figure 5. Resistance of CD133+ laryngeal carcinoma stem cells to anti-tumor treatments. A. The growth inhibition rate for CD133- and CD133+ Hep-2 cells after irradiation treatments of 10 Gy for 36 h. B. Relative survival rates of CD133- and CD133+ Hep-2 cells after treated with paclitaxel for 24 h, 48 h, and 72 h, respectively. Compared with the CD133- control cells, $^{**}P < 0.01$, $^{***}P < 0.001$.

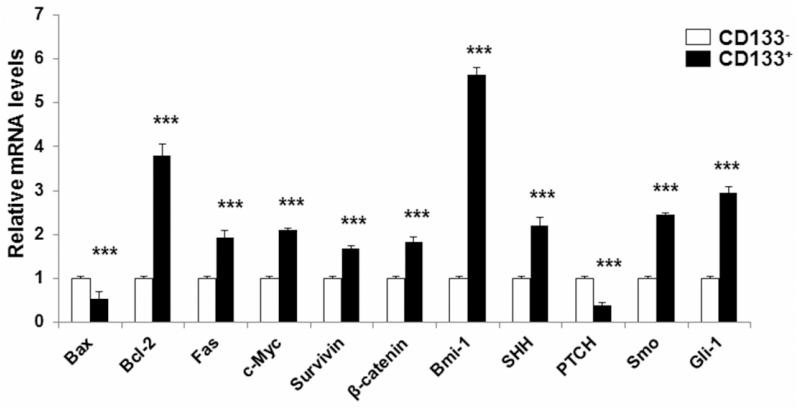

Figure 6. Characterization of signaling pathways in CD133+ Hep-2 cells by real-time PCR. The relative mRNA expression levels of proteins in apoptosis- and/or proliferation-related signaling pathways in CD133+ Hep-2 cells were assessed by real-time PCR.

On the other hand, these cells were treated with 1×10^{-8} mol/L paclitaxel for 24 h, 48 h, and 72 h, respectively. Our results showed that the survival rates were decreased along with the time for both CD133+ and CD133- Hep-2 cells. At each time point, the survival rates of CD133+ Hep-2 cells were significantly higher than the CD133- controls (**Figure 5B**; $P < 0.01$ for 24 h, 48 h, and 72 h, respectively). These results indicate that the CD133+ Hep-2 cells display significant resistance to radiotherapy and chemotherapy.

Characterization of signaling pathways in CD133+ laryngeal carcinoma stem cells

To further characterize the signaling pathways in CD133+ laryngeal carcinoma stem cells, the mRNA and protein expression levels of several apoptosis- and/or cell proliferation-related proteins were determined by real-time PCR and Western blot, respectively. As shown in **Figure 6**, results from real-time PCR indicated that, the mRNA expression levels of Bcl-2, Fas, c-Myc, and survivin were significantly up-regulated, while the Bax mRNA expression was significantly down-regulated, in CD133+ Hep-2 cells, compared with the CD133- controls. For the gene encoding proteins, the mRNAs of Bmi-1, SHH, SMO, and Gli-1 were expressed at significantly higher levels in CD133+ Hep-2 cells than CD133- controls; in the meantime, the mRNA expression level of PTCH was relatively decreased in CD133+ Hep-2 cells. On the other hand, Western blot analysis showed that, in CD133+ Hep-2 cells, the protein expression levels of Fas, c-Myc, and survivin were elevated. Up-regulated Bcl-2 expression and down-regulated Bax expression lead to increased Bcl-2/Bax ratio in CD133+ Hep-2 cells. Moreover, the protein expression levels of β-catenin, Bmi-1, SHH, SMO, and Gli-1 were also significantly increased, while the PTCH protein expression level was declined, in the CD133+ Hep-2 cells. These results indicate that the anti-apoptotic capability is enhanced, and Hedgehog, Wnt,

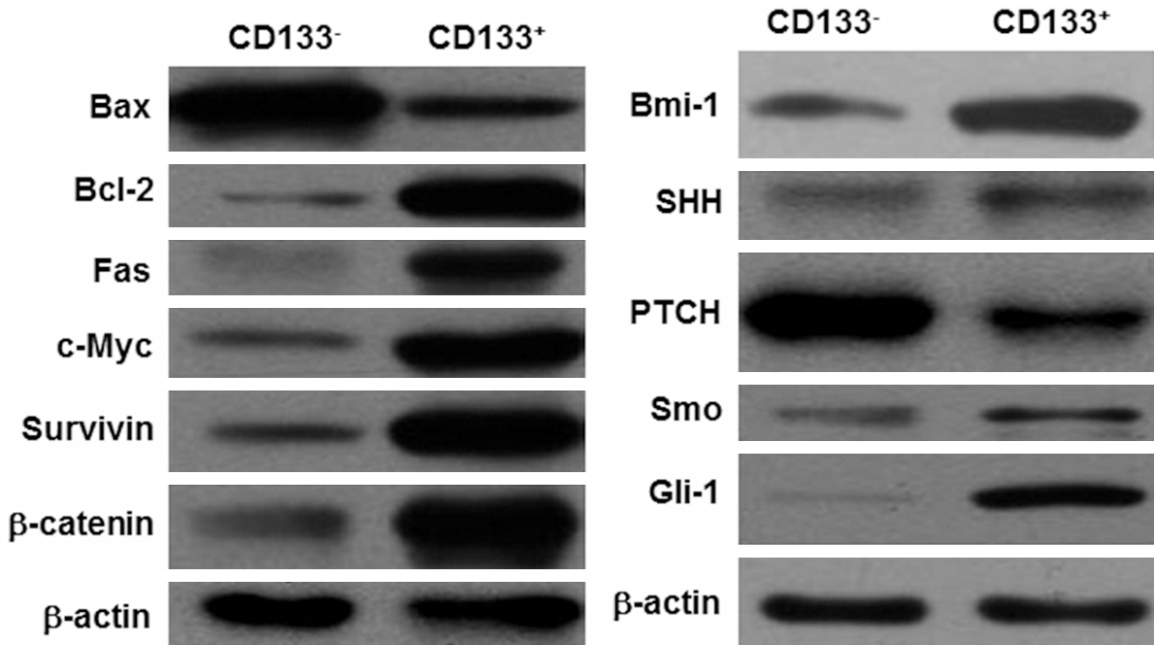

Figure 7. Characterization of signaling pathways in CD133+ Hep-2 cells by Western blot. The protein expression levels molecules involved in apoptosis- and/or proliferation-related signaling pathways in CD133+ laryngeal carcinoma stem cells were evaluated by Western blot analysis.

and Bmi-I signaling pathways are all activated in CD133+ laryngeal carcinoma stem cells, which might be involved in the self-renewal process of these stem cells.

Discussion

In the present study, we investigated the *in vitro* biological characteristics of CD133+ Hep-2 tumor cells. Our results indicate that there are significant differences in cell proliferation, clone formation, and *in vitro* invasive capabilities between CD133+ and CD133- Hep-2 cells. These findings provide new insights into the metastasis and recurrence of laryngeal carcinoma, and the disease treatment.

CD133+ Hep-2 cells herein were sorted using flow cytometry. In order to find out whether the *in vitro* growth of CD133+ Hep-2 cells exhibited characteristics of stem cells, the sorted CD133+ cells and CD133- cells were incubated under the same conditions. The results showed that the proliferative capability of CD133+ cells was significantly higher than CD133- cells.

Invasion and metastasis are hallmarks for malignant tumors. Tumor cells can detach from the primary tumor, penetrate the basal membrane, and grow in the interstitial space. It is a complex pathophysiological process, consisting of hydrolysis of extracellular matrix components, cell migration, and tumor angiogenesis. In head and neck, blood vessels are abundant, and nerves are also concentrated. Peripheral infiltration and lymphatic metastasis of laryngeal carcinoma are still the major reasons for death. Our results indicate that CD133+ laryngeal carcinoma cells show high invasive capability, just like CSCs.

Another important characteristic of CSCs is their self-renewal capability, and the daughter cells are identical to the parental cells at genetic and epigenetic levels. Tumor dormancy in cancer therapy may end up with recurrence. In the present study, the clone-forming abilities were confirmed for three generations of CD133+ Hep-2 cells. Actually, the clone-forming ability of CD133+ Hep-2 cells were gradually increased along with the passages, in consistent with the characteristics for malignant tumors. In addition, CSCs have been well accepted to be inherently resistant to radiotherapy and chemotherapy, contributing to tumor recurrence and/or metastasis.

Our results herein showed that CD133+ Hep-2 cells displayed high resistance to paclitaxel, and exhibited low growth inhibition after radio-

therapy, indicating the resistance of CD133+ Hep-2 cells to chemotherapy and radiotherapy.

Tumor stem cells and adult stem cells have similar signaling transduction pathways. However, the gene expression pattern in tumor stem cells was significantly different from well-differentiated tumor cells, which is the biological basis for their specific characteristics, such as anti-apoptotic activities [15, 16]. Our experiments showed that the mRNA level and protein expression levels of the anti-apoptosis genes (survivin and c-Myc), and the ratio of Bcl-2/Bax, were all significantly increased in CD133+ cells, indicating elevated anti-apoptotic capability in these cells. However, the mRNA and protein expression levels of Fas were also up-regulated in CD133+ cells, which might be attributed to the mutations, ectopic expression, or immunological escape of the gene [10, 11, 17, 18]. Recent studies have shown that Hedgehog, Wnt, and Bmi-l signaling pathways are involved in the self-renewal process of tumor stem cells [19-21]. The present study investigated the involvement of these signaling pathways in the self-renewal of CD133+ tumor stem cells. The mRNA and protein levels of SHH, SMO, and Gli-1 were up-regulated, and the expression of PTCH was down-regulated, in CD133+ tumor stem cells. The Hedgehog signaling pathway was also activated in these stem cells. In the Wnt signaling pathway, β-catenin is a crucial mediator, which determines the activation of the Wnt signaling pathway [22, 23]. Our results showed that the mRNA and protein expression levels of β-catenin were at high levels, indicating that the Wnt signaling pathway was activated, in CD133+ tumor stem cells. Recent studies have showed that high mRNA and protein expression levels of Bmi-1 can be detected in CD44+ stem cells from human head and neck squamous cell carcinoma, which were located in specific microdomains in plasma membrane [11]. Our results indicated that, in CD133+ Hep-2 stem cells, the mRNA and protein expression levels of Bmi-l were both increased, indicating the activation of the signaling pathway in the laryngeal CSCs. Currently, tumor stem cells have attracted more and more attention for the treatment of tumors [24]. The illustration of the self-renewal mechanisms of laryngeal CSCs may be greatly helpful in preventing and treating laryngeal cancer in clinical trials. It can be predicted that targeted therapies on CD133+ laryngeal carcinoma cells, such as the blockages of the signaling pathways, the interference of the transcription machinery, and immunological therapies [25, 26], may effectively inhibit these tumor stem cells.

In conclusion, our results showed that the migrating capability of CD133+ Hep-2 cells was significantly higher than that of CD133- cells, and the invasive capability of CD133+ Hep-2 cells was also significantly elevated. Moreover, the clone-forming ability of the CD133+ Hep-2 cells was significantly higher than the CD133- Hep-2 cells, and CD133+ Hep-2 cells displayed significant resistance to radiotherapy and chemotherapy. Furthermore, the anti-apoptotic capability was enhanced, and Hedgehog, Wnt, and Bmi-l signaling pathways were all activated in CD133+ laryngeal carcinoma stem cells, which might be involved in the self-renewal process of these stem cells. Our findings provide new insights into the prevention and/or treatment of laryngeal carcinoma, especially concerning target-oriented therapies.

Acknowledgements

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Disclosure of conflict of interest

None.

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