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COMPARISON BETWEEN CELLS AND CANCER STEM-LIKE CELLS ISOLATED FROM GLIOBLASTOMA AND ASTROCYTOMA ON EXPRESSION OF ANTI-APOPTOTIC AND MULTIDRUG RESISTANCE–ASSOCIATED PROTEIN GENES

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Abstract—This study is to explore and compare the features of the cells and cancer stem-like cells (CSCs) isolated from both glioblastoma and astrocytoma on expression of anti-apoptotic and multidrug resistance–associated protein (MRP) genes. As a result, the mRNA expression of livin, livinα and MRP1 was up-regulated in human CSCs from 2 times to 85 times, but the gene expression of MRP3 was down-regulated from 0.09 times to 0.5 times. After just differentiation the mRNA expression of livin, livinα and MRP3 was up-regulated from9 times to 64 times, but the mRNA expression of MRP1 was down-regulated from 0.01 times to 0.03 times. It is a rare report that glioma stem-like cells can be induced successfully from a grade 2–3 astrocytoma tissue. The properties of glioblastoma and astrocytoma stem-like cells on anti-apoptotic and MRP genes are: anti-apoptotic gene livin and survivin are elevated in CSCs but are the most increased in just differentiated CSCs; MRP1 gene is significantly increased and MRP3 is decreased in CSCs, but when differentiating the MRP3 gene starts a remarkable increase in CSCs; the expression of anti-apoptotic and MRP genes shows no differences between the CSCs isolated from glioblastoma and astrocytoma tissues.

Key words: glioma, cancer stem-like cell, glioblastoma, astrocytoma, livin, multidrug resistance–associated protein.

Gliomas are the most common type of primary brain tumor. Nearly two-thirds of gliomas are highly malignant lesions that account for a disproportionate share of brain tumor–related morbidity and mortality (Sathornsumetee et al., 2007). Despite recent advances, 2-year survival for glioblastoma with optimal therapy is less than 30%. While surgery, radiation therapy and chemotherapy have roles to play in the treatment of patients with gliomas, these therapies are self-limited because of the intrinsic resistance of glioma cells to therapy, and the diffusely infiltrating nature of the lesions (Nakada et al., 2007). Even among patients with low-grade gliomas that confer a relatively good prognosis, treatment is almost never curative (Norden and Wen, 2006). Though the last decade produced a number of important advances, some of which have translated directly into survival benefits, the prognosis for many patients with gliomas is poor.

It is now known that malignant gliomas arise from a number of well-characterized genetic alterations and activations of oncogenes and inactivation of tumor suppressor genes. Those genetic alterations disrupt critical cell cycle, growth factor activation, apoptotic, cell motility, and invasion pathways that lead to phenotypic changes and neoplastic transformation (Bansal et al., 2006). Recently, the concept of cancer stem cells was focused on increasingly (Schulenburg et al., 2006). The findings of brain cancer stem cells were made by applying the principles for cell culture and analysis of normal neural stem cells (NSCs) to brain cancer cell populations and by identification of cell surface markers that allow for isolation of distinct cancer cell populations that can then be studied *in vitro* and *in vivo*. A population of brain cancer cells can be enriched for brain cancer stem cells by cell sorting of dissociated suspensions of cancer cells for the NSC marker CD133. These CD133 positive cells, which also expressed the NSC marker nestin, but not differentiated neural lineage markers, represent a minority fraction of the entire brain cancer cell population, and exclusively generate clonal cancer spheres in suspension culture and exhibit increased self-renewal capacity (Singh et al., 2004a). Moreover, progressive explorations have discovered CD133 positive cancer stem cells have a capacity for unlimited self-renewal, as well as the ability to initiate and drive tumor progression in an animal model (Singh et al., 2004b). The cell-membrane protein CD133 has been identified as a marker of a subset of NSCs in the adult CNS as well as of glioma stem or stem-like cells (Bao et al., 2006; Singh et al., 2003, 2004b).

In gliomas, WHO (World Health Organization) system assigns a grade from 1 to 4, with one being the least

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Abbreviations: ACC, autologous cancer cell; bFGF, basic fibroblast growth factor; CSC, cancer stem-like cell; Ct, cycle threshold; DMEM/F12, Dulbecco's modified Eagle's medium/nutrient mixture F-12 Ham's; EGF, epidermal growth factor; FBS, fetal bovine serum; GFAP, glial fibrillary acidic protein; IAP, inhibitor of apoptosis protein; LIF, leukemia inhibitory factor; MRP, multidrug resistance–associated protein; NSC, neural stem cell; PBS, phosphate-buffered saline; WHO, World Health Organization.

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aggressive and four being the most aggressive. Glioblastoma, also called grade 4 astrocytoma, glioblastoma multiforme, is a fast-growing type of CNS tumor that forms from glial (supportive) tissue of the brain and spinal cord and has cells that look very different from normal cells. Astrocytoma is a tumor that begins in the brain or spinal cord in small, star-shaped cells called astrocytes and includes WHO grades 1–3. As in the past all glioma stem-like cells were isolated from glioblastoma, astrocytoma stem cells have not been known so far, and the properties of stem-like cells isolated from glioblastoma and astrocytoma on expression of anti-apoptotic and multidrug resistance–associated protein (MRP) genes have not been compared before.

Thereby, we cultured and disassociated cancer stem-like cells (CSCs) from the cell lines and tumor mass of inpatients suffering from both glioblastoma and astrocytoma, and explored the anti-apoptotic and drug resistant gene expression in order to find novel properties of those CSCs.

EXPERIMENTAL PROCEDURES

Chemicals and reagents

Dulbecco's modified Eagle's medium/nutrient mixture F-12 Ham's (DMEM/F12) with high glucose medium was purchased from HyClone (Logan, UT, USA). Fetal bovine serum (FBS), trypsin, B-27 (1×) serum-free supplements were from Gibco (Grand Island, NY, USA). Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), leukemia inhibitory factor (LIF) was obtained from Peprotech (Rocky Hill, NJ, USA). Rabbit anti-human nestin, rabbit anti-human glial fibrillary acidic protein (GFAP) and mouse anti-human β-tubulin antibody were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Goat anti-mouse IgG-FITC antibody and goat anti-rabbit IgG-FITC antibody were from Sigma (St. Louis, MO, USA). CD133 cell isolation kit (MACS method) was purchased from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany).

Tumor specimens and primary cell culture

Glioma specimens were collected from 17 inpatients in China Wuhan Union Hospital and their pathological diagnoses were brain glioma. In those patients 10 subjects were of glioblastoma and the others suffered from astrocytoma. The exsected tissues were washed twice with D-Hanks' solution in aseptic condition. After clearing out of visible blood vessel and necrotic tissue, the glioma tissues were sheared into paste by eye scissors. Then following digestion with 0.25% trypsin for 20 min and blown and stirred by pipette, cell suspension was made prior to terminating digestion by 10% FBS. The suspension was filtered through a 200-mesh sieve and centrifuged at 1000 r.p.m. After disposing of supernatant, the primary glioma cells were washed again with serum-free medium. Then having been centrifuged, the cells were collected and inoculated into a serum-free medium (NSC medium) which contained DMEM/F12 with high glucose medium, 20 ng/mL EGF, 20 ng/mL bFGF, 10 ng/mL LIF and b27 (1×). Last, the cells were placed in incubator on conditions of 37 °C, 5% CO_2 and saturated humidity. Every 3–4 days the medium was renewed. Then the induced glioma stem-like cells were cultured in conditions of DMEM/F12 with high glucose medium with 10% FBS for inducing differentiation. The use of human tissue was authorized by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology.

Glioma cell line culture

Glioblastoma U251 cell line was provided by the China Center for Typical Culture Collection (CCTCC) (Wuhan, China). Astrocytoma GL15 cell line was kindly provided by Institute of Neuroscience, Tongji Medical College, Huazhong University of Science and Technology. The two cell lines were cultured in the same conditions as the primary cell culture.

Isolation of CD133 positive glioma stem-like cells

After the neurospheres grew a large amount, the spheres were collected and the CD133 positive cells were separated by magnetic cell sorting technique (MACS). The sorting process was conducted according to the instruction of the CD133 cell isolation kit.

Immunofluorescence staining on glioma tumor stem cells

The well-grown cell spheres were selected for growing on the slides which were coated by polylysine. After drying at 37 °C, the slides were washed by phosphate-buffered saline (PBS) three times in order to clear up medium. At room temperature, the cells were fixed by paraform for 30 min, and then were washed by PBS again three times. After blocked by 5% goat serum at 37 °C for 30 min, rabbit anti-human nestin (1:200) (1st antibody) was added and the cells were placed in a wet box for one night. The day following PBS washing, goat anti-rabbit IgG-FITC antibody (2nd antibody) was added for incubation for 30 min at 37 °C. Meanwhile a negative control in which PBS was used instead of the 1st antibody was performed. The slides were observed with an Olym-

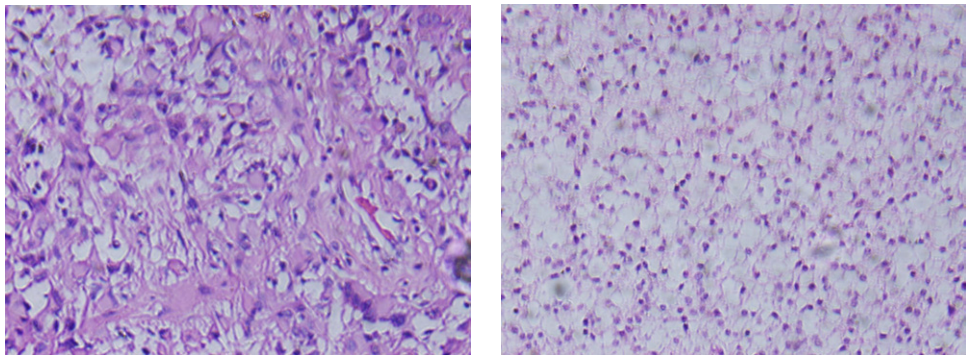

Fig. 1. Glioma tissues with HE staining (20×10). (A) Glioblastoma tissue (No.912110); (B) astrocytoma (WHO 2–3 grade) tissue (No. 916718).

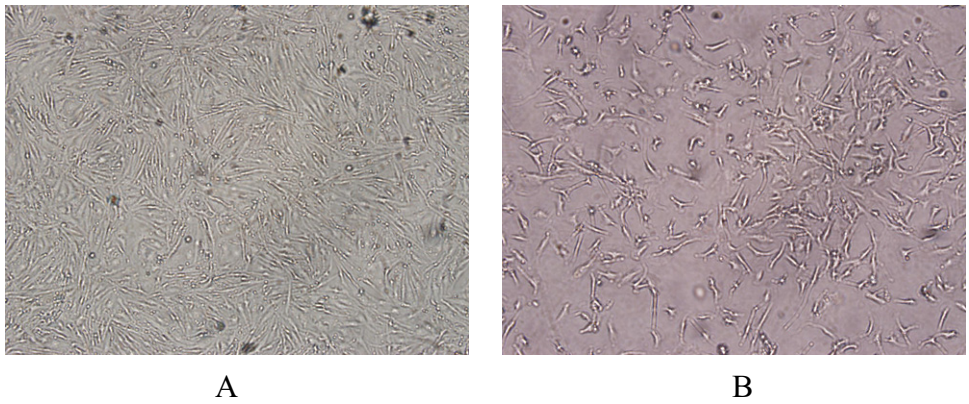

Fig. 2. Normal glioma cells (10×10). (A) Normally cultured GL15 cells. (B) Normally cultured U251 cells.

pus BX51 fluorescence microscope. The procedure of immunofluorescent assay on GFAP and β-tubulin on differentiated glioma stem cells followed the course of nestin except for respective related antibodies.

Real-time quantitative RT-PCR

The samples were collected and each 50–100 mg tissue samples or 10^6 cell samples were collected and added 1 mL TRIzol (Jin et al., 2008). The total RNA from glioma cells and glioma stem cells was prepared by adding TRIzol Reagent (Gibco BRL) according to manufacturer's protocol. The RNA solution was stored at −80 °C until used. All reactions were performed in duplicate with a negative control (no template) and the mean value of the threshold cycle (the start of exponential amplification) of each sample was normalized with the threshold cycle of glyceraldehyde-3-phosphate dehydrogenase (GAPD), obtaining the Δ cycle threshold (Ct) value. Quantitative PCR was performed in ABI-7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA). The reverse transcription was performed with M-MLV reverse transcriptase. The reverse transcriptional reaction system included: 5.5 μL H_2O, 1.0 μL Oligo(dT18) (50 μg/mL), total RNA 6.0 μL, 70 °C 5 min to ice for unfolding the secondary structure of mRNA; 0.5 μL RNasin (40 U/μL), 4.0 μL 5× buffer, 2.0 μL dNTP (10 mM), 1.0 μL RTase (200 U/μL), 42 °C 60 min to 95 °C 5 min to 4 °C.

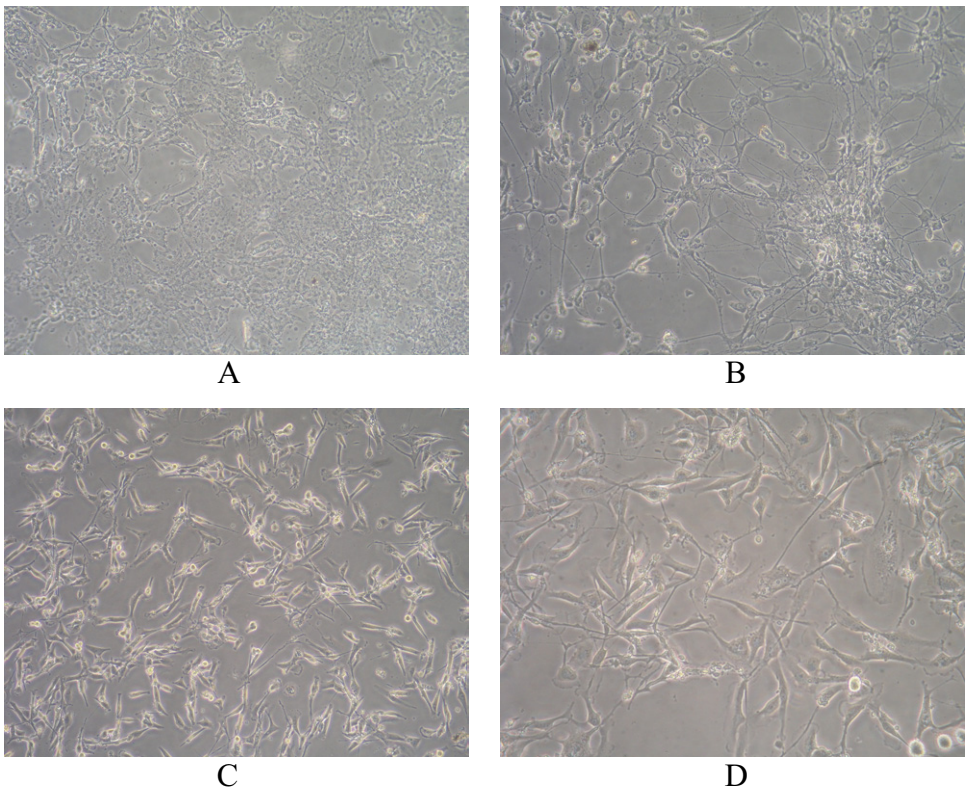

Fig. 3. Glioma cells cultured from tissues and cell lines (20×10). (A) Primary cells derived from glioblastoma tissue (No. 912110) at the 5th culturing day in NSC medium. (B) Primary cells derived from astrocytoma tissue (No. 916718) at the 5th culturing day in NSC medium. (C) GL15 cells at the 3rd culturing day in NSC medium. (D) U251 cells at the 3rd culturing day in NSC medium.

Real time PCR reaction was performed with SYBR GreenIfluorochrome. The standard curve was obtained and Ct value was calculated. Each 50 μL PCR system contained 1/50 of the original cDNA, 7 μL (25 mM) $MgCl_2$, 0.8 μL (20 pmol/μL) of each primer, 1 μL (10 mM) dNTP, 1 μL SYBR GreenI, 0.5 μL (5 U/μL) *Taq*DNA polymerase (Promega, Madison, WI, USA) and 5 μL 10$\times$ buffer. Fifty cycles of amplification were performed: after 94 °C 3 min, reaction cycle with 94 °C, 30 s, to 57 °C, 30 s, then to 72 °C, 30 s was carried out. The fluorescence signal was detected at the end of each cycle. Melting curve analysis was used to confirm the specificity of the products. The $2^{-\Delta\Delta CT}$ method was performed to analyze the results (Livak and Schmittgen, 2001). The primer was as below:

Homo-livin
Forward: 5′-ACAGAGGAGGAAGAGGAGGAGG-3′
Reverse: 5′-GCAGTCAGCGGCCAGTCATAG-3′
Homo-livinα
Forward: 5′-CTGTCAGTTCCTGCTCCGGTC-3′
Reverse: 5′-GGGCTCAAGAACCCACCAC-3′
Homo-survivin
Forward: 5′-ACTGCCCCACTGAGAACGAGC-3′
Reverse: 5′-AAGGAAAGCGCAACCGGACGAAT-3′
Homo-MRP1
Forward: 5′-CACCACTGGAGCATTGACTACC-3′
Reverse: 5′-GTAATTACAGCAAGCCTGGAACC-3′
Homo-MRP3
Forward: 5′-CCTGTATGTGGGTCAAAGTGCG-3′
Reverse: 5′-CCCAGCCTCAGGGAAGTGTTG-3′
Homo-β-actin
Forward: 5′-CTTAGTTGCGTTACACCCTTTCTTG-3′
Reverse: 5′-CTGCTGTCACCTTCACCGTTCC-3′

Statistical analysis

Each test was perform and then repeated two times. The data were normalized as ratio of CSC/ACC (autologous cancer cells). The data were presented as mean$\pm$S.D. Comparisons of the data were performed with Student *t*-test. Statistical significance was considered significant when $P<0.05$. The statistical process was performed with SPSS 12.0 software.

RESULTS

Induction of glioma stem-like cells

We found that the primary cancer cells from two tissues, U251 and GL15 cell line (Figs. 1, 2 and 3) could become floating neurospheres by NSC-medium cultivation. When cultured in NSC medium at the 7th–10th culturing day, the floating neurospheres emerged from primary glioma cells, while at the 5th–7th culturing day the GL15 cells produced neurospheres and at the 15th–20th culturing day the U251 cell generated neurospheres (Fig. 4). By CD133 isolation, glioma stem-like cells were induced successfully from two glioma specimens from one glioblastoma (No. 912110) and one grade 2–3 astrocytoma subject (No. 916718), and from U251 and GL15 cell lines (Fig. 5). The CSCs which were cultured in NSC medium grew in suspension and presented the features of stem cells: sphere-like shape, self-renewal and ability to differentiate. Nestin immunoflu-

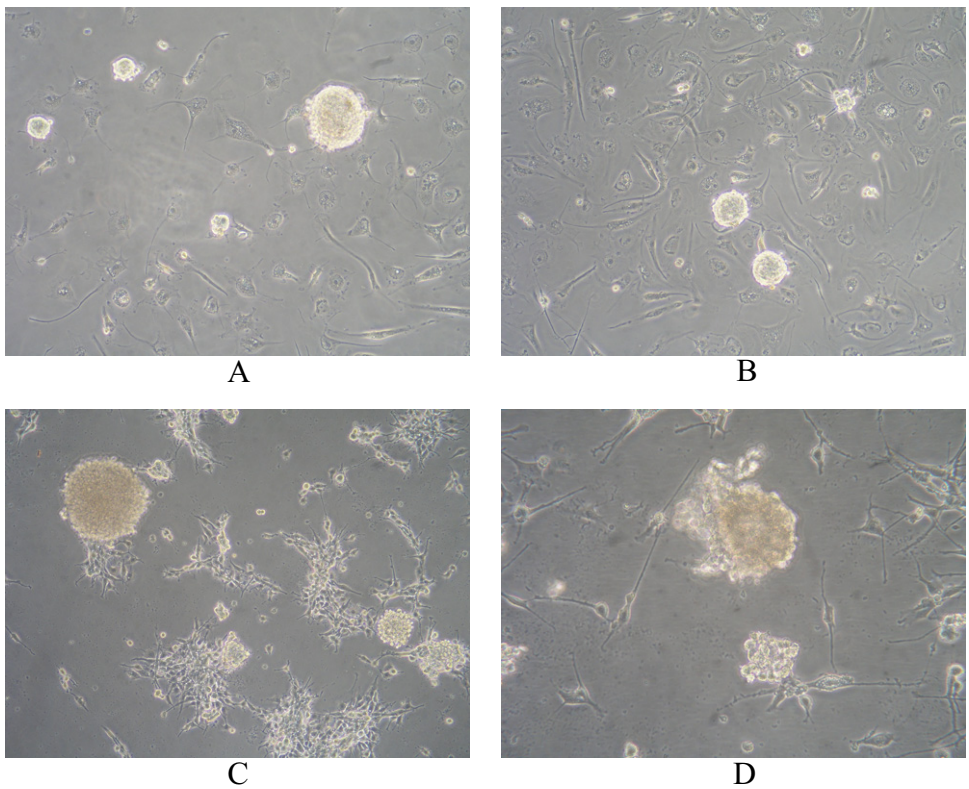

Fig. 4. Neurospheres emerged from primary cells and cell lines (20$\times$10). (A) Neurospheres emerged from primary glioblastoma cells (No. 912110) at the 7th culturing day in NSC medium. (B) Neurospheres emerged from astrocytoma cells (No. 916718) at the 7th culturing day in NSC medium. (C) Neurospheres emerged from GL15 cells at the 5th culturing day in NSC medium. (D) Neurospheres emerged from U251 cells at the 15th culturing day in NSC medium.

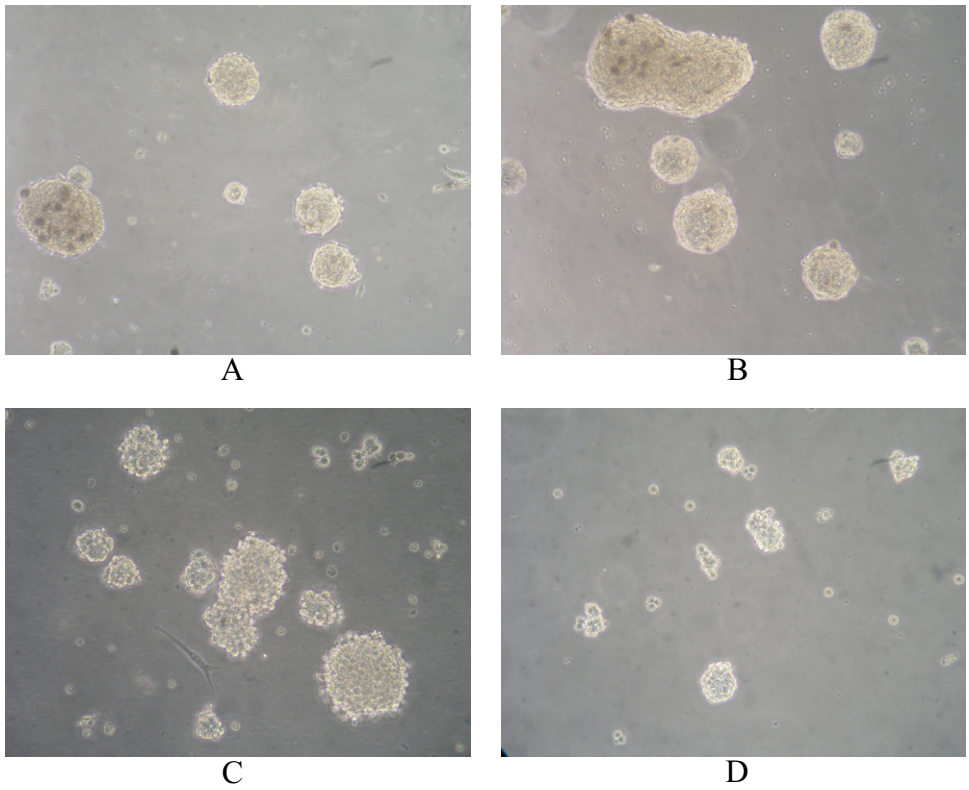

Fig. 5. Stem-like cell sphere derived from primary cells and cell lines (20×10). (A) Stem-like cell sphere derived from glioblastoma tissue (No. 912110). (B) Stem-like cell sphere derived from astrocytoma tissue (No. 916718). (C) Stem-like cell sphere derived from GL15 cell line. (D) Stem-like cell sphere derived from U251 cell line.

orescence staining of the stem-like cells presented positive but the glioma cells did not show this feature (Fig. 6).

Differentiation of glioma stem-like cells

The induced glioma stem-like cells started to differentiate after culture conditions of DMEM/F12 medium with 10% FBS. At the 4th hour after culture, the stem-like spheres derived from glioma tissues adhered to the wall and dendrite- or axon-like pseudopodia emerged at the surface of spheres. At the 3rd–5th day, thick dendrite-like pseudopodia grew from all the spheres. As a contrast, the stem-like cells cultured by NSC medium did not show the similar changes. The differentiated cells presented positive β-tubulin and GFAP stain but the glioma stem-like spheres did not show this feature (Fig. 7).

Comparison of anti-apoptotic and MRP gene expression between glioma CSC and ACC

As shown in Table 1, the mRNA expression of livin, livinα and MRP-1 was up-regulated in CSCs in both glioma primary cells and cell lines from 2 times to 62 times in CSC derived from cell line and 2 times to 85 times CSC from primary tissues, but the gene expression of MRP-3 was down-regulated from 0.09 times to 0.5 times. The trends of livinβ were also coincident with livinα according to the changes of livin and livinα. The increasing extent from GL15 cell line was significantly more than that from U251 cell line ($P<0.01$) but the difference did not exist between the CSC from two tissues.

Comparison of anti-apoptotic and MRP gene expression between CSC before and after differentiation

As shown in Table 2, the mRNA expression of livin, livinα and MRP-3 was up-regulated after differentiation in CSC deriving from both glioma specimens from9 times to 64 times. The trends of livinβ were also coincident with livinα according to the changes of livin and livinα. However, the mRNA expression of MRP-1 was down-regulated from 0.01 times to 0.03 times.

DISCUSSION

Stem cells are defined by their ability to self-renew, proliferate, and generate progeny that differentiate into the multiple cell types that make up the tissue from which they are derived. The hypothesis that certain cancers may be the result of unregulated stem cells is founded on the suggested similarities between cancer stem cells and somatic stem cells (Ghods et al., 2007). Recently, it has been confirmed by identification of brain cancer stem cells by CD133 (Bao et al., 2006; Singh et al., 2003, 2004b). Identification of brain cancer stem cells provides a powerful tool for the investigation of the tumorigenic process in the CNS,

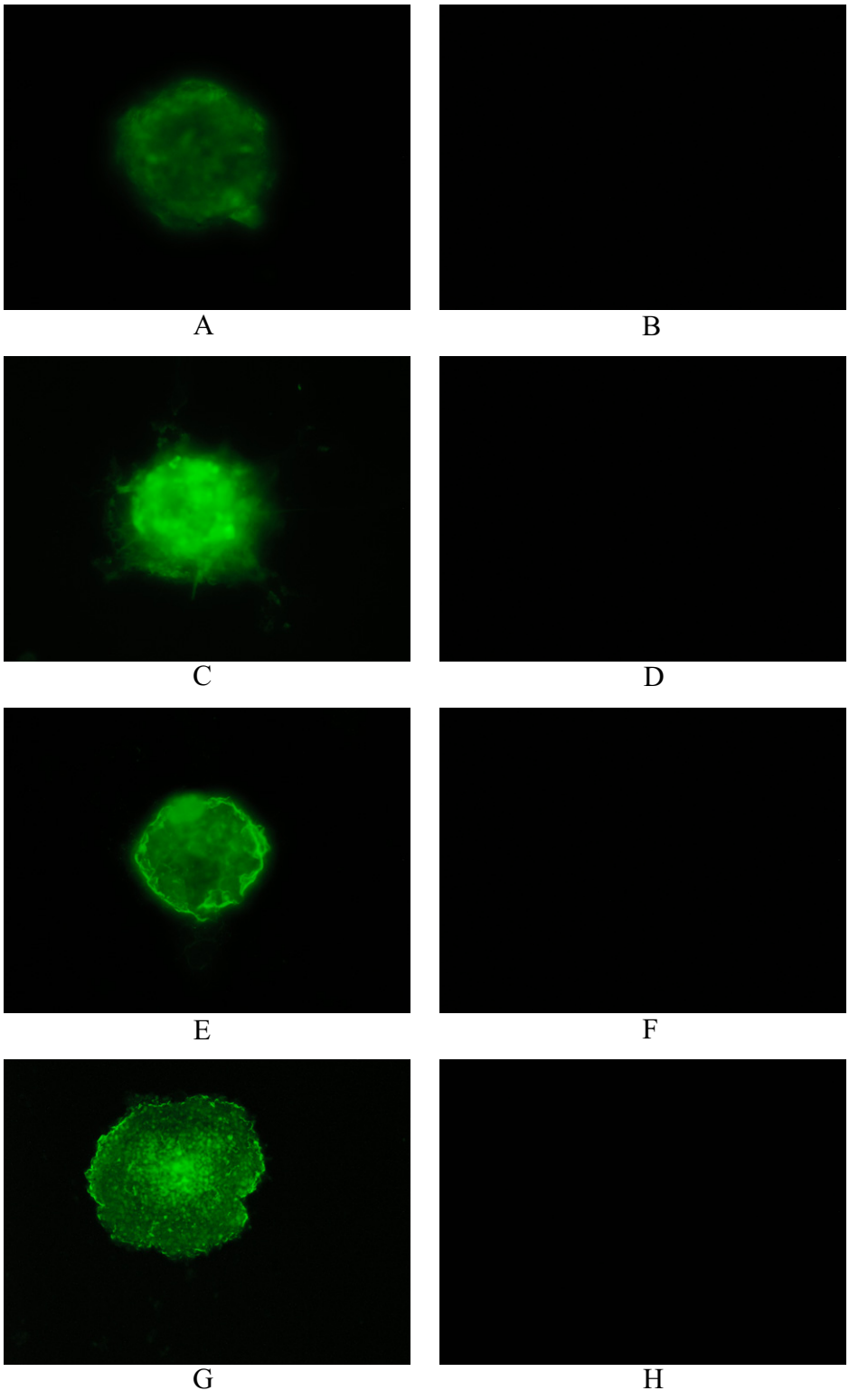

Fig. 6. Nestin immunofluorescence staining (green fluorescence was positive) on glioma stem-like cell spheres and glioma cells (40×10). (A) On spheres derived from glioblastoma tissue (No. 912110). (B) On cells from glioblastoma tissue (No. 912110). (C) On spheres derived from astrocytoma tissue (No. 916718). (D) On cells from astrocytoma tissue (No. 916718). (E) On spheres derived from GL15 cells. (F) On GL15 cells. (G) On spheres derived from U251 cells. (H) On U251 cells.

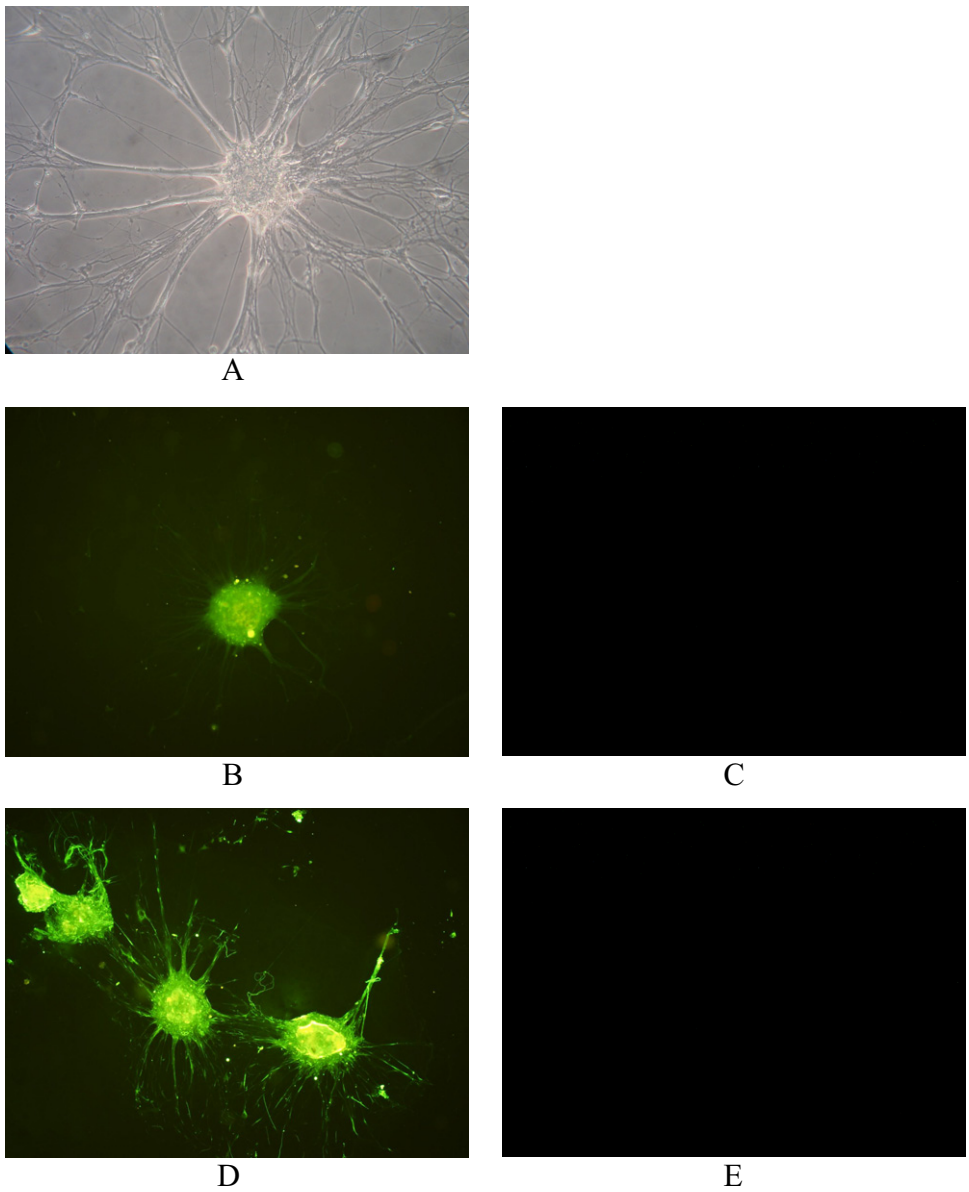

Fig. 7. GFAP and β-tubulin immunofluorescence staining (green fluorescence was positive) on differentiated and undifferentiated glioma stem-like cells (20×10). (A) Differentiated glioma stem-like cell. (B) GFAP immunofluorescence staining on differentiated glioma stem-like cell. (C) GFAP immunofluorescence staining on undifferentiated glioma stem-like cell. (D) β-Tubulin immunofluorescence staining on differentiated glioma stem-like cell. (E) β-Tubulin immunofluorescence staining on undifferentiated glioma stem-like cell.

and will be crucial in developing therapies that use brain cancer stem cells as a target (Singh et al., 2004a).

In our research, we successfully separated CD133 positive cells from glioblastoma, astrocytoma tissues and U251, GL15 cell line, and found the separated cells presented properties of cancer stem cells: sphere-like form, self-renewal, ability to differentiate. Especially, we induced primary glioma stem-like cells from two samples of glioma subjects. One was diagnosed as WHO grade 2–3 astrocytoma. So far almost all glioma stem-like cells have been cultured from glioblastoma tissue. It is a rare report that glioma stem-like cells can be induced from astrocytoma. The finding suggests a mildly malignant tumor still has potential abilities to develop into highly malignant cancer and a clear eradication for cancer tissue is necessary in clinic.

As discovered now, cancer stem cells are a very small group of cells in the tumor bulk. Thereby inducing cancer stem cells from cancer tissue is not easy work and a successful induction is limited by operational technology, such as the successful rate of primary cell culture, cell survival condition and so on. Our induction rate is similar to the literature (Liu et al., 2006). More effective methods will be developed in the future.

We further checked the other properties of glioma stem-like cells on our separated cells. Nestin was selected

Table 1. Anti-apoptotic and MRP gene expression on glioma CSC compared to that on ACC

Gene name	Glioblastoma No. 912110		Astrocytoma No. 916718		GL15		U251	
	ACC	CSC	ACC	CSC	ACC	CSC	ACC	CSC
Livin	1	52.543±4.281	1	57.084±5.362	1	6.695±0.437*	1	3.912±0.231
Livinα	1	11.574±0.852	1	34.279±2.935	1	62.336±5.017*	1	8.699±0.779
Survivin	1	2.457±0.205	1	1.943±0.138	1	4.867±0.403*	1	2.972±0.275
MRP-1	1	64.287±7.803	1	85.736±8.754	1	54.042±4.772*	1	2.015±0.183
MRP-3	1	0.094±0.004	1	0.275±0.017	1	0.533±0.038	1	0.453±0.047

The value of ACC was normalized as 1 and the value of CSC was expressed as relative value.
* $P<0.01$ compared to the value of CSC in U251 group.

to examine the feature of our separated glioma stem-like cells. Nestin is a marker of progenitor cells or precursor cells and now is recognized as a protein marker of NSCs (Hoffman, 2007; Hwang et al., 2007). Many researches chose nestin as a marker to check the properties of brain CSCs. Recent findings also showed nestin has an up-regulated expression in glioma stem cells. In our research, the separated cells were nestin positive, which could support that our induced CD133 positive cells were CSCs. We further cultured the tumor stem cells by NSC medium and found this method could maintain growth of the CSCs. After being induced to differentiation by culture with DMEM/F12 medium, the CSCs from both cancer tissue and glioma cell lines presented GFAP and β-tubulin positive. As GFAP is the astrocyte lineage marker and β-tubulin is considered as the neuronal lineage marker (Ghods et al., 2007), it could be implied that our induced CD133 positive cells had the potential to differentiate into astrocytes and neurons, which shows the cells possess the property to differentiate into different ACCs.

The inhibitor of apoptosis protein (IAP) family encodes a group of baculovirus IAP repeat domain (BIR) –containing proteins that suppress apoptosis (Huang et al., 2006). Survivin is an important member of IAP. This protein is highly expressed in most cancers and associated with chemotherapy resistance, increased tumor recurrence, and shorter patient survival, but rarely is expressed in terminally differentiated adult tissues (Fukuda and Pelus, 2006). Current documents show that biological functions of survivin include inhibition of apoptosis, promotion of mitosis, angiogenesis, which play a causative role in cancer formation and/or progression (Duffy et al., 2007). In our research, it was observed that gene expression of the member of IAP was up-regulated in CSCs, which implies the glioma stem-like cells are a main factor to resist cancer apoptosis and maintain cancer growth and angiogenesis. Furthermore, it was also investigated that survivin continued rising in differentiated glioma stem-like cells. It has been reported that survivin expresses more highly in glioma CSCs (Ghods et al., 2007). Inferred by our finding, the anti-apoptotic gene is the most highly expressed in just differentiated glioma CSCs, which presents a more detailed profile of survivin expression in glioma stem-like cells.

Livin is also a member of the IAP family of caspase inhibitors that selectively binds the endogenous IAP antagonist (second mitochondria-derived activator of caspases) SMAC and caspase-3, caspase-7, and caspase-9, which encode negative regulatory proteins that prevent cell apoptosis (Chang and Schimmer, 2007). Livin is selectively expressed in the most common human neoplasms and appears to be involved in tumor cell resistance to chemotherapeutic agents (Liu et al., 2007; Kempkensteffen et al., 2007). In our research, it was observed that gene expression of livin and livinα was up-regulated in CSCs. As livin contains two isoforms: livinα and livinβ, it could be deduced that livinβ had a similar elevating expression to livinα in the CSCs and differentiated CSCs. Like survivin the rising expression of livin in glioma stem-like cells also shows its causative role for anti-apoptosis and anti-chemotherapy by inhibiting the function of SMAC and caspase molecules. Furthermore, the fact the highest expression of livin in differentiated CSCs also demonstrates the just differentiating or having differentiated glioma stem-like cells are the most tolerant stage to promote growth of cancer cells and to resistant apoptosis and chemotherapeutic drugs.

The findings of survivin and livin expression in CSC and just differentiated CSC suggest that the group of CSC may be the highest malignant cell population in cancer bulk. This can be interpreted this way: a relapsing cancer is more malignant after treatments because the relapsing cancer tissue may be developed from uneliminated cancer cells, cancer stem cells.

MRP is a 180- to 195-kDa membrane protein associated with resistance of human tumor cells to cytotoxic drugs (Zaman et al., 1994). Numerous members of the

Table 2. Anti-apoptotic and MRP gene expression on CSC compared to that on differentiated stem-like cells (DSC)

Gene name	Glioblastoma No. 912110		Astrocytoma No. 916718	
	CSC	DSC	CSC	DSC
Livin	1	24.783±2.038	1	17.695±1.894
Livinα	1	57.784±5.832	1	82.196±7.539
Survivin	1	12.853±1.439	1	9.361±1.474
MRP-1	1	0.037±0.002	1	0.014±0.003
MRP-3	1	64.739±5.961	1	52.673±6.197

The value of CSC was standardized as 1 and the value of DSC was expressed as relative value.

multidrug resistance associated protein family serve as export pumps that prevent the accumulation of anionic conjugates and glutathione disulfide (GSSG) in the cytoplasm, and play, therefore, an essential role in detoxification and defense against oxidative stress (Homolya et al., 2003). The 190 kDa multidrug resistance protein MRP1 is involved in the multidrug resistance phenotype of human gliomas (Benyahia et al., 2004). In gliomas, mainly MRP1 has been studied thus far and was found to be localized to the tumor vasculature and partially also to the tumor cells (Mohri et al., 2000; Aronica et al., 2003; Andersson et al., 2004; Benyahia et al., 2004). In glioma cell lines, MRP1 is shown to confer resistance to various anticancer drugs (Benyahia et al., 2004; Bronger et al., 2005). Apart from MRP1, some research showed gene MRP3 is hyperexpressed in astrocytomas for the primary resistance to chemotherapy (Calatozzolo et al., 2005) and MRP3 can modulate drug sensitivity to certain anticancer agents in human gliomas (Haga et al., 2001). We detected MRP1 and MRP3 gene expression in both glioma stem-like cells and autologous cancer cells and found MRP1 was the most highly expressed in glioma stem-like cells and MRP3 was much less expressed in CSCs, which was different from the past reports (Salmaggi et al., 2006). That is to say, MRP1 plays a more significant role as multidrug resistance transporter than MRP3 in glioma stem-like cells. However, after differentiation, the expression of MRP3 notably rose, which showed when just differentiating or having differentiated, the cells perform resisting chemotherapeutics via MRP3 function. As the MRP family has been considered as a target for reducing chemotherapeutic drug resistance, the variation of MRPs in CSCs may be important in the future therapeutic strategies.

GL15 cell line is derived from glioblastoma (Bocchini et al., 1991), while the U251 cell line is cultured from astrocytoma (Akhyani et al., 2006). We compared the gene expression between those two cell lines on livin, survivin and MRP1 and found more up-regulations of those genes existed in CSCs derived from the GL15 cell line, which is coincident with malignant degree of glioblastoma. However, the same trends did not occur in glioma stem-like cells derived from tissues, which showed a more complicated influence in human body and the malignant difference between glioblastoma and astrocytoma is not due to the tested anti-apoptotic and MRP genes.

Cancer is among the leading causes of morbidity and mortality in the world (Jin et al., 2008). Despite recent advances, most therapeutic approaches fail to eradicate the entire neoplastic clone. The remaining cells often develop metastasis and/or recurrences and therefore may represent attractive targets of therapy. In this research, we successfully induced and cultured CSCs from both glioma tissues and cell lines. Especially, we induced tumor stem cells from astrocytoma tissue. Moreover, we found the properties of human brain glioma stem-like cells: anti-apoptotic gene is elevated in glioma stem-like cells but is the most increased in just differentiated glioma stem-like cells; MRP1 gene is significantly increased but MRP3 is decreased in glioma stem-like cells, and when differentiating the MRP3 gene starts a remarkable increase in glioma stem-like cells; but the difference of malignant grade between glioblastoma and astrocytoma is not due to the tested anti-apoptotic and MRP genes.

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