



# Isolation of Cancer Stem Cells and Astrocytes from Human Glioblastoma: Morphological Characterization of Two Cells Types

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## Abstract

**Background:** Glioblastoma multiforme is the most aggressive astrocytoma in adults. Glioblastoma is a heterogenous tumor formed from various cells including astrocyte and cancer stem cells. Here, we explain the isolation, culture, morphology and specific markers of human glioblastoma astrocyte and stem cells.

**Methods:** We isolated astrocyte and cancer stem cells from human glioblastoma tissue. The obtained glioblastoma tissues were digested and cultured in DMEM12, B27 supplemented with basic fibroblast growth factor and epidermal growth factor. The morphology and specific markers were assessed in astrocyte and cancer stem cell of human glioblastoma through immunocytochemistry.

**Results:** Results indicated that there were two morphology types in cell culture including epithelioid morphology and fibroblastic morphology. The astrocyte confirmed via expression of the Glial fibrillary acidic protein (GFAP) protein. Cancer stem cells were round and floating in the culture medium. Immunocytochemical staining indicated that nestin and SRY-box 2 (SOX2) antigens were positively expressed in primary neurospheres.

**Conclusion:** The expression of glial and stem cell markers shows that both cells are in the human glioblastoma.

**Keywords:** Glioblastoma multiforme; Astrocyte; Cancer stem cell; Morphology.

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## Introduction

astrocytomas are the most common group of primary brain tumors in the brain comprising 30–40% of all brain tumors.<sup>1</sup> Glioblastoma multiforme constitute 52% of primary brain tumors. The most prevalent age of glioblastoma is about 50-55 years and men are more prone to this brain tumor. Prognosis of glioblastoma is poor and the survival rate is 14.6 months after diagnosis.<sup>2</sup> Important risk factors for glioblastoma are high dose radiation, hereditary syndromes, and age increase.<sup>3</sup>

Glioblastoma is formed of a heterogenous tissue containing differentiated astrocytes and cancer stem cells.<sup>4</sup> The two properties of cancer stem cells are unlimited self-renewal and multipotency. There are two stem cell niches in the adult mammalian brain including the dentate gyrus<sup>5</sup> and the subventricular zone.<sup>6</sup> Neural precursor cells seem to play an important role in the initiation and progression of different neurological diseases including Alzheimer's disease, Parkinson's disease, stroke, epilepsy, and schizophrenia.<sup>7</sup> Also, theories were reported that neural precursor cells can be the origin of primary brain tumors.<sup>8</sup> Specific gene alterations and diverse cellular differentiation can lead to primary brain tumors.<sup>5</sup> However, there is strong evidence that neural precursor

cells are the origin of glial tumors.<sup>9</sup> Two models have been suggested to clarify the initiation and progression of brain tumors including the stochastic model and the hierarchical model. The stochastic model explained that the tumor is formed of various or heterogeneous cells and all the cells are able to produce tumor. On the other hand, the hierarchical model refers to malignancies that are composed of cancer stem cells or initiating cells of tumor. Furthermore, cancer stem cells have the potential to produce tumors<sup>10</sup> and through drug resistance and genetic disorders, they can lead to therapy failure in tumors.<sup>11</sup> In this study, we focused on two cells types forming human glioblastoma and evaluated the morphology and antigen of differentiated astrocyte and cancer stem cells.

## Material and Methods

### Isolation of Astrocyte From Human Glioblastoma Tissue

Glioblastoma tissue was confirmed by pathological assessment. Tissue was digested with 0.05% trypsin-EDTA for 10 minutes at 37°C in a water bath. Tissue centrifugation was performed at 1000 rpm for 5 minutes, DMEM/F12 medium containing 1% (100 U/mL) antibiotic/antimycotic and 2% fetal bovine serum (FBS) was added to cells and then, incubated in 5% CO<sub>2</sub> at 37°C

for 14 days. The medium containing FBS was changed every three days in the culture.

#### **Isolation of Cancer Stem Cells From Human Glioblastoma Tissue**

The pieces of glioblastoma tissue were washed three times in calcium and magnesium free Hank's balanced salt solution (HBSS) with 1 % penicillin and streptomycin. Then, the tissue was digested and incubated in trypsin for 20 minutes. Digestion was stopped by trypsin inhibitor (80  $\mu\text{g}/\text{mL}$ ) in HBSS solution. Then, centrifugation was done at 1000 g for 5 minutes, at room temperature. The digested tissues were triturated in DMEM/F12 with a flamed polished Pasteur pipette and filtering was performed using a 70  $\mu\text{m}$  strainer. The cell suspension was cultured in six well-plates with DMEM/F12 containing 20 ng/mL, epidermal growth factor (EGF), 20 ng/mL, basic fibroblast growth factor (bFGF), 1% glutamax 1% B27, 1% penicillin–streptomycin. The cells were incubated at 5%  $\text{CO}_2$ , 37°C. The medium in the supplements was changed every 2-3 days.

#### **Immunocytochemistry**

Cells were fixed in 4% paraformaldehyde in 0.2M phosphate-buffered saline (PBS) for 20 minutes, and then washed with PBS three times. Then, cells were permeabilized in 0.2% Triton X-100 in PBS for 5 minutes at room temperature. Then, cells were blocked in 10% albumin bovine serum for 1 hour at room temperature and incubated with primary antibodies of rabbit anti-nestin (1:250; Abcam), rabbit anti-SOX2 (1:500; Abcam), and rabbit anti-GFAP (1:300, Abcam) overnight at 4°C. After being washed with PBS, the cells were incubated with secondary antibody of 1:200 fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit (Abcam, USA) for 2 hours at room temperature. Finally, protein expression in the cells were observed using an inverted fluorescence microscopy.

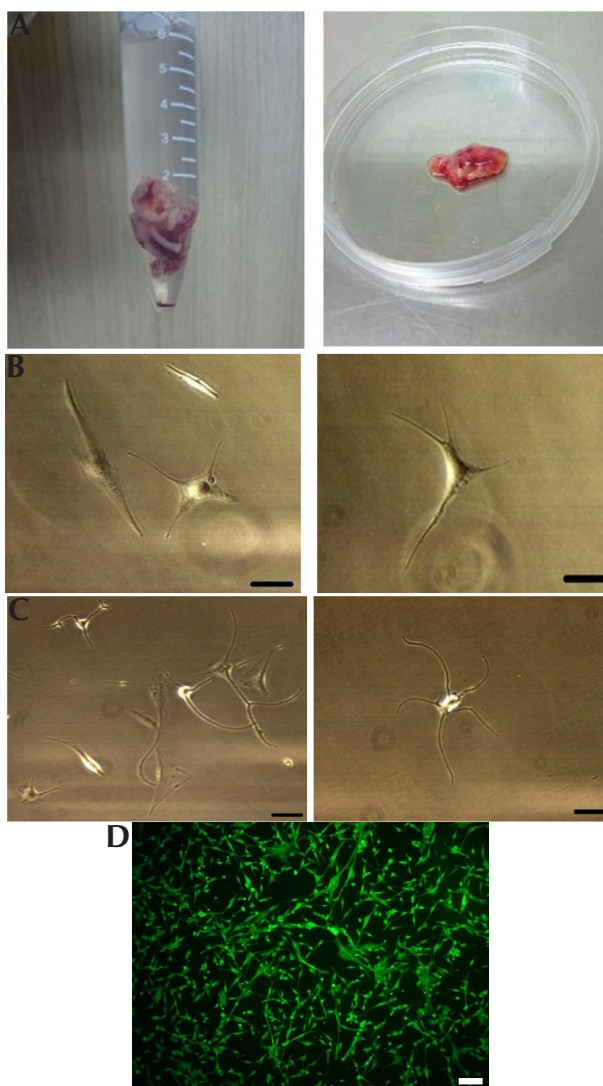
#### **Statistical Analysis**

All tests were repeated in the three independent experiments. The data were qualitative and were reported as images.

### **Results**

#### **Astrocytes Morphology of Human Glioblastoma Multiforme**

Astrocytes were derived from human glioblastoma tissue (Figure 1A). Results indicated that there were two morphology types in cell culture. 1) Epithelioid morphology was observed to be large, abundance cytoplasm and interconnecting processes (Figure 1B). 2) Fibroblastic morphology was slender, elongated, and spindle-shaped (Figure 1C). The immunocytochemistry test indicated the expression of GFAP protein in the cells, confirming the presence of the astrocyte in the culture



**Figure 1.** Morphology of the Astrocyte Types. (A) tissue of the human glioblastoma. (B) Astrocytes with epithelioid morphology, scale bar: 100  $\mu\text{m}$ . (C) Astrocytes with fibroblastic morphology, scale bar: 100  $\mu\text{m}$ . (D) Confirmation of cultured astrocytes through expression of GFAP protein in the cells, scale bar: 50  $\mu\text{m}$ .

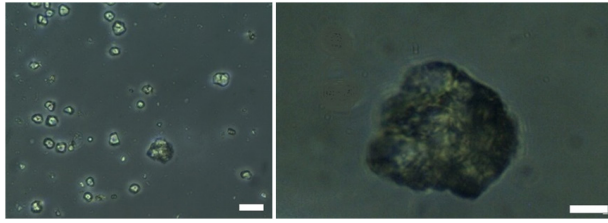
medium (Figure 1D).

#### **Isolation and Culture of Adult Human Glioblastoma Cancer Stem Cells**

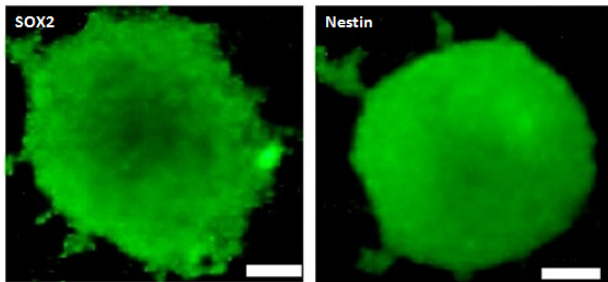
Cancer stem cells were isolated and cultured from human adult glioblastoma tissue. The floating single cells started to grow, divide and form small clusters for 6-8 weeks. Neurosphere morphology was observed to be round and floating in the culture medium (Figure 2).

#### **Antigen Evaluation of Cancer Stem Cells of Human Glioblastoma**

The cancer stem cells of glioblastoma were assessed using immunochemistry test for expression of the nestin and SOX2 antigens. Immunocytochemical staining indicated that nestin and SOX2 antigens were positively expressed



**Figure 2.** Morphology of Cancer Stem Cell Derived From Human Glioblastoma Tissue. Scale bar of the left image: 50 µm, Scale bar of the right image: 100 µm.



**Figure 3.** Expression of the Nestin and SOX2 Antigens in the Cancer Stem Cells Derived From Human Glioblastoma Tissue. Scale bar of the right image: 100 µm.

in primary neurospheres (Figure 3).

### Discussion

In our study, we separated cancer stem cells and astrocyte from glioblastoma tissue, and found that the isolated cells have a morphology similar to cancer stem cells: sphere-like form, self-renewal, potential of differentiation. We further evaluated the other properties of cancer stem cells on our isolated cells. Nestin and SOX2 were selected to assess the property of cancer stem cells. Many studies select nestin as a marker to evaluate the features of brain cancer stem cells.<sup>12</sup> New results also reported that nestin was over-expressed in glioblastoma stem cells.<sup>13</sup> In our study, the isolated cells were nestin positive, indicating cancer stem cells. We cultured the cancer stem cells in the medium similar to neural stem cells and noticed this method could lead to growth of the cancer stem cells. Astrocytes are a differentiated cell in the glioblastoma. This cell is generally located in the periphery part of glioblastoma. It seems that astrocytes of glioblastoma are differentiated from glioblastoma stem cells.<sup>9</sup> Astrocytes were GFAP positive. GFAP is a specific marker of astrocyte.<sup>14</sup>

Cancer stem cells play different roles in glioblastoma including the ability to proliferate and differentiate, tropism and migration potential to other regions, resistance to drugs and toxins, and resistance to apoptosis.<sup>15</sup> Studies were reported that cancer stem cells are dangerous and lead to poor diagnosis, therapy resistance and increase of morbidity and mortality in patients with glioblastoma.<sup>16</sup> Information about cancer stem cells features could be

helpful in how glioblastoma is formed and involved in tumorigenesis. Also, we can assess cellular and molecular mechanism of cancer stem cell tumorigenesis in order to find the suitable therapy approach. Unfortunately, it remains ambiguous whether cancer stem cells are derived from the transformation of normal neural stem cells or whether the differentiation of a mature brain cell is responsible.<sup>17</sup> These theories may lead to new pathways to introduce therapeutic and pharmacological approaches for glioblastoma.

### Conclusion

In this study, we successfully isolated and cultured astrocyte and cancer stem cells from glioblastoma tissue. Furthermore, we reported the features and morphology of cancer stem cells and astrocytes in the human glioblastoma tissue. However, glioblastoma is a heterogenous and complex brain tumor.

### Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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### Ethical Statement

The study was performed according to the principles of the Ethics Committee of Shahid Beheshti University of Medical Sciences with ethical code: IR.SBMU.RETECH.REC.015 for molecular and Cellular study.

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