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Original Article

Alterations of expression profile of cancer-related genes in co-cultured human bone marrow-derived mesenchymal stromal cells with brain glioma cells in vitro: propose concerns about oncological risk before clinical application

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Abstract: Background: Human bone marrow-derived mesenchymal stromal cells (hMSCs) may serve as ideal delivery vehicles for gene therapy for human gliomas. The stability of hMSCs in culture had been concerned and studied, while the stability of these cells in glioma environment had been overlooked and remains unclear. In this study, we investigate the alterations of expression profile of cancer-related genes in hMSCs under the influence of glioma cells in vitro. Methods: HMSCs were obtained from normal adult persons and identified by analysis of distinct surface markers by flow cytometry and tests of their stemness, and hMSCs were co-cultured with U251 glioma cells and the expression profile of cancer-related genes were investigated by microarray assay, and the results of microarray were verified by Real-time quantitative RT-PCR. Results: The obtained hMSCs express distinct surface markers of mesenchymal stromal cells and could be induced to differentiate into neural lineage cells in vitro. Of the 440 cancer-related genes covered by Oligo GEArray Human Cancer Microarray OHS-802, 16 were found to be significantly up-regulated (>3-fold) but none down-regulated in hMSCs co-cultured with U251 glioma cells. The up-regulations of some of those genes were confirmed by Real-time quantitative RT-PCR. The up-regulated genes include some important oncogenes. Conclusions: The present study is the first to show that the co-culture of hMSCs with human glioma cells lead to up-regulated expressions of some important oncogenes in hMSCs, the over-expression of which has been demonstrated to contribute to tumorigenesis. We propose concerns about oncological risk of hMSCs under the influence of glioma cells, and highlight the need for further studies before their clinical application as therapeutic vectors to treat human gliomas.

Keywords: Mesenchymal stromal cells, glioma, gene therapy, tumorigenicity, cancer-related gene

Introduction

Mesenchymal stromal cells (MSCs) were demonstrated to be effective for delivering transgenes to treat experimental gliomas based on their unique migratory properties within the central nervous system (CNS) and their good tropism to glioma cells [1]. The potential of MSCs for targeted gene delivery in the context of cancer is an exciting area of research that has gained considerable momentum in recent years, with studies reporting engineered MSCs specifically targeting multiple tumor types. However, concerns about the biosafety of MSCs must be addressed fully to allow MSC-mediated therapy for cancer to realize its full potential.

The transformation potential of MSCs in culture had been concerned. Expansion of MSCs in vitro will be required for therapeutic application

and so their stability in culture is paramount. The majority of studies have shown that human MSCs are stable, while murine MSCs are more prone to genetic transformation during in vitro culture, and may be capable of forming sarcomas in vivo [2, 3]. Although transformation of human MSCs appears unlikely, and very rare, these studies certainly emphasize the importance of stringent monitoring of MSCs before application in the clinical setting.

However, there is still another important aspect of this problem, that is the transformation risk of human MSCs (hMSCs) in tumor environment, had not attracted much attentions. When applied to treat tumors hMSCs would leave the original living place and enter into the targeted tumor environment and the milieu of growth factors and inflammatory cytokines present in the tumor environment is very different from that in the original living place of hMSCs. It was reported that co-culture of human adipose tissue derived stem cells with human squamous cell carcinoma cells could cause significant changes of gene expressions and protein synthesis in human adipose tissue derived stem cells, which arouse worries about oncological risk [4]. hMSCs could serve as ideal genes vector to treat human gliomas. For that application hMSCs would leave the bone marrow then reach and stay in the glioma environment, and interact with glioma cells. Whether malignant transformation of hMSCs would be induced in glioma environment is what we concern about here. To make a primary probe into this question we design this experiment. Since tumorigenesis could be attributed to genetic characteristics, we co-culture hMSCs with U251 glioma cells and investigated the alterations of expression profile of cancer-related genes in hMSCs, in order to find out whether there were any characteristics that could help in the evaluation of the risk of malignant transformation of hMSCs in human glioma environment.

Materials and methods

Isolation of hMSCs

To isolate hMSCs, bone marrow aspirates were taken from the iliac crest of normal adult donors after informed consent and under a protocol approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical University. Nucleated cells were isolated by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient and resuspended in DMEM-F12 (Gibco BRL, Gaithersburg, MD, USA) plus 10% FBS, and incubated at 37°C with 5% CO_2. After 24 hours, nonadherent cells were discarded, and adherent cells were thoroughly washed twice with PBS and continued to incubate with the new medium. After that medium was changed every 3 days and cells were passaged once a week. Human glioma U251 cells were obtained from China Center for Type Culture Collection (Shanghai, People's Republic of China) and maintained in DMEM-F12 plus 10% FBS at 37°C with 5% CO_2.

Epitope analysis of hMSCs

Obtained hMSCs were analyzed by flow cytometry. Detached cells were washed and resuspended in PBS. Approximately 10^5 cells were incubated on ice for 30 min with conjugated, monoclonal antibodies against CD34, and CD45 (Becton Dickinson, San Jose, CA, USA), CD73 (PharMingen, Uppsala, Sweden), and CD 166 (Ansell, Bayport, MN, USA). Nonspecific fluorescence was determined, using equal aliquots of cell preparation incubated with an-ti-mouse monoclonal antibodies (Becton Dickinson). Finally, the cells were assayed in a flow cytometer (FACSort, Beckton Dickinson), and the data analyzed with Cellquest software (Becton Dickinson).

Determination of hMSCs' stemmness

After passages 2-5, the conversion of hMSCs into neurosphere-like structures was initiated. Specifically, cells were dissociated with 0.25% trypsin/0.01% EDTA and plated on uncoated flasks at a concentration of 1×10^5 cells/cm^2 in serum-free DMEM/F12 medium containing B27/N2 (all from Gibco BRL, Gaithersburg, MD) plus 20 ng/mL of both epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), both from Sigma (St. Louis, MO) and incubated at 37°C with 5% CO_2. The medium was changed once a week and growth factors were added twice a week. After 10-15 days, neurosphere-like structures could be observed. To induce differentiation in vitro, cell aggregates were plated on fibronectin-coated plates in B27 media containing 10% FBS.

Immunocytochemistry

Cells were fixed in 4% paraformaldehyde in PBS. Immunocytochemistry was carried out using standard protocols. Cell nuclei were

counterstained with 4,6-diamidino-2-phenylindole (DAPI; Roche Molecular Biochemicals, Mannheim, Germany). The antibodies used and their dilutions were: nestin, 1:500 (Chemicon International, Temecula, CA); GFAP, 1:100 (Chemicon International); NG2, 1:100 (Sigma); MAP2ab, 1:100 (Chemicon International); anti-rabbit IgG Cy3 conjugate, 1:100 (Chemicon International); and antimouse IgG FITC conjugate, 1:100 (Sigma).

Co-culture of hMSCs and U251 glioma cells

Co-culture of hMSCs and U251 glioma cells was performed in a transwell system. For that 2×10^4 hMSCs were seeded onto a polyester membrane transwell-clear insert (Corning, pore size 0.4 μm) while 2×10^4 U251 cells were seeded onto the bottom of a six-well cell culture plate. Cells were cultured up to five days in 4 ml DMEM-F12 plus 10% FBS per well and the medium was changed every 48 h. 2×10^4 hMSCs cultured alone in transwell inserts served as the control and were treated the same as the co-culture.

Preparation of RNA samples

After co-cultured with U251 glioma cells or cultured alone (as control) for five days as described above, isolation of RNA samples in hMSCs were initiated. Total RNAs were isolated by using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA).

Microarray expression analysis

In order to investigate the influence of glioma cells on the expression of cancer-related genes in hMSCs, we co-cultured hMSCs with U251 cells for five days, and the expression profile of cancer-related genes were investigated by microarray test, and the hMSCs cultured alone were treated as the control group. Using the TrueLabeling-AMP Linear RNA amplification kit (SuperArray Bioscience, Frederick, MD, USA), the mRNA was reversely transcribed to obtain cDNA and converted into biotin-labeled cRNA using biotin-16-UTP (Roche, Mannheim, Germany) by *in vitro* transcription. Prior to hybridization, the cRNA probes were purified with the ArrayGrade cRNA cleanup kit (SuperArray Bioscience). The purified cRNA probes were then hybridized to the pretreated Oligo GEArray Human Cancer Microarrays OHS-802 (SuperArray Bioscience), which cover 440 cancer-related genes. Following several washing steps, array spots binding cRNA were detected using alkaline phosphatase-conjugated streptavidin and CDP-Star as chemiluminescent substrate. Chemiluminescence was detected by exposing the Array membranes to x-ray film. The image data were transformed into numerical data using the software GEArray Expression Analysis Suite (SuperArray Bioscience). The numerical data were then further evaluated with Microsoft Excel 2003. Data evaluation included background correction (subtraction of minimum value) and median normalization. Data filtering criteria were as follows: at least one of the spot intensities to be compared had to be more than twice the background intensity, and the spot intensity ratios had to be higher than 3.0 (for overexpressed) or lower than -3.0 (for down-expressed).

Real-time quantitative RT-PCR

To confirm the up-regulation of some of those oncogenes indicated by microarray, Real-time quantitative RT-PCR was performed. Primers were designed using the primer software (version 5.0, Whitehead Institute for Biomedical Research, Cambridge, MA, USA) under consideration of the special design criteria for real-time PCR primers. Primer sequences (forward, reverse) and lengths of the amplified products were as follows: β-actin (5'-CCTGTACGCCAACACAGTGC-3', 5'-ATACTCCTGCTTGCTGATCC-3', 211 bp); KIT (5'-TTGTCATCAGCCACCATCCT-3', 5'-CCAGTCCATACCTCCCTCTCTT-3', 121 bp); CAPNS1 (5'-GATAGCGACACCACAGGCAAG-3', 5'-GAGATTTGAAGGCACGGAACAT-3', 283 bp); TK1 (5'-GTGATTCTCGGGCCGATGTT-3', 5'-GCGAGTGTCTTTGGCATACTTGAT-3', 114 bp); MMP-1 (5'-CGCACAAATCCCTTCTACC-3', 5'-CTCTGTCGGCAAATTCGTAAG-3', 103 bp); CCND1 (5'-TGGCGTTTCCCAGAGTCATC-3', 5'-CCACCTCCCTTCAACACTTCC-3', 195 bp); CDC20 (5'-TCCCTGGCAGTCCAATGTCC-3', 5'-GGAGACCAGAGGATGGAGCAC-3', 135 bp); RELA (5'-CGCTGCATCCACAGTTTCCA-3', 5'-GCACAGCATTCAGGTCGTAGTCC-3', 151 bp); STC1 (5'-GAAGCCTGCTGGAATGTGAT-3', 5'-GGGATGTGCGTTTGATGTG-3', 237 bp). The RNA samples were reversely transcribed using the MMLV-reverse transcriptase (Promega). An oligo-dT primer was used for priming the reverse transcription. Beta-actin was chosen as the reference gene for normalization of the results. The quantitative real-time PCR was performed using a

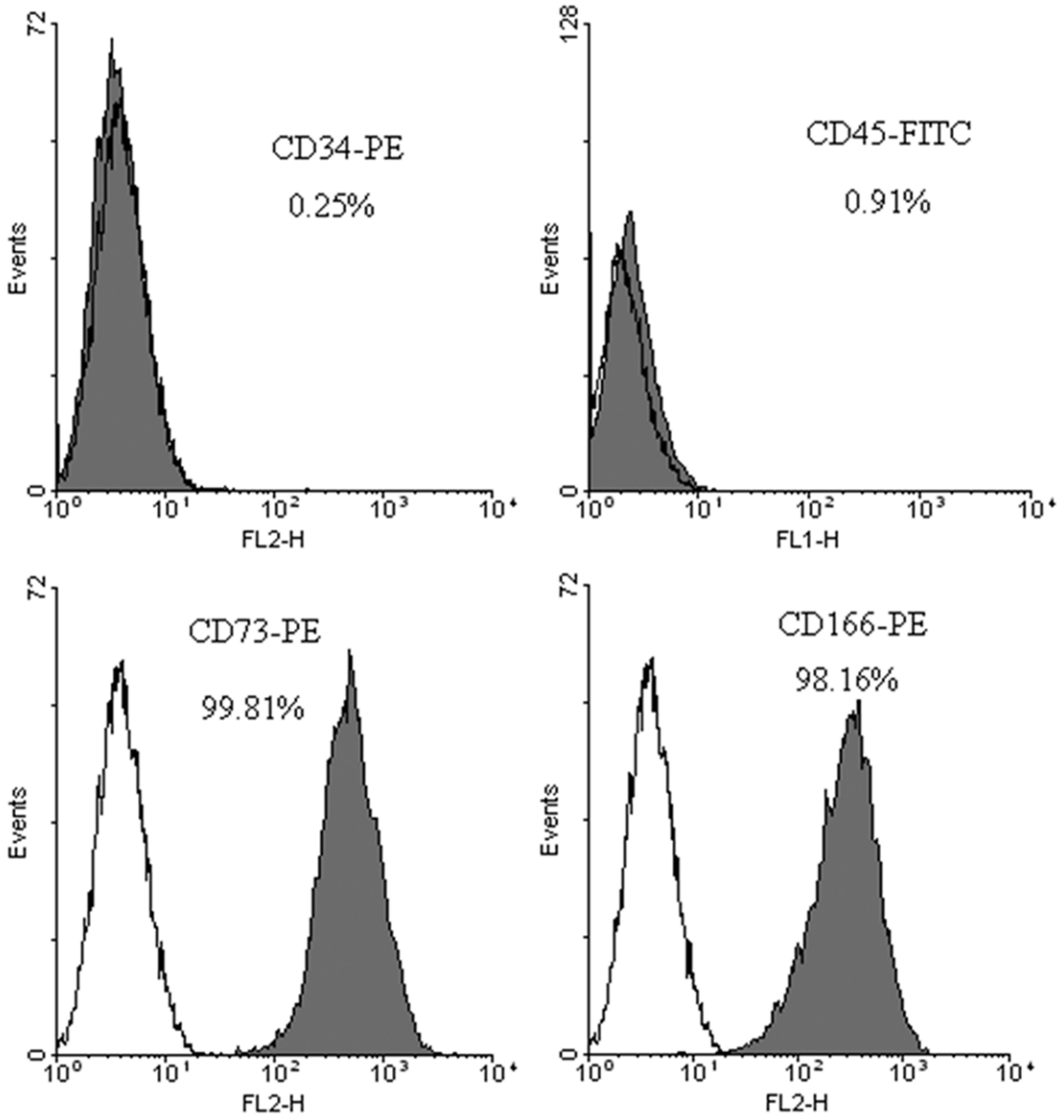

Figure 1. Epitope analysis of human bone marrow-derived mesenchymal stromal cells (hMSCs). Flow cytometric analysis of cultured hMSCs with monoclonal antibodies against CD34, CD45, CD73, and CD166. hMSCs were negative for CD34 and CD45 and positive for CD73 and CD166.

Rotor-Gene 3000 thermal cycling system (Corbett Research, Sidney, Australia) with Syber green (Molecular Probes, Eugene, OR, USA) as the detection system. The results were analyzed with the Rotor-Gene 6.0 software (Corbett Research) and Microsoft Excel 2003.

Results

Identification of hMSCs

Applied hMSCs were identified by analysis of distinct surface markers in flow cytometry and tests of their stemness. Flow cytometric analysis of hMSCs with monoclonal antibodies against CD34, CD45, CD73, and CD166. hMSCs were negative for CD34 and CD45 and positive for CD73 and CD166 (**Figure 1**).

Applied hMSCs could be converted into cells with characteristics of NSCs in vitro, which formed cell aggregates that were morphologically similar to NSC-derived neurospheres (**Figure 2A**) and highly expressed the neural stem cell marker nestin (**Figure 2B**). And the

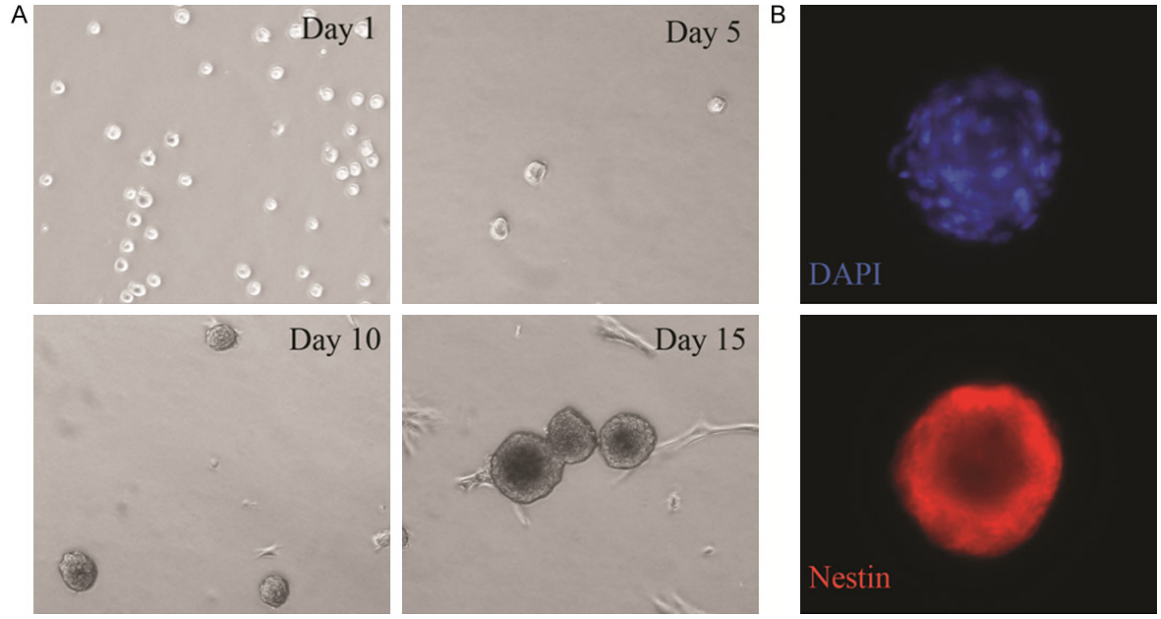

Figure 2. Characteristics of human bone marrow-derived mesenchymal stromal cells (hMSCs) -derived neural stem-like cells. A. Representative sequence of phase-contrast photomicrographs of neural stemlike cells 1, 5, 10, and 15 days after conversion from hMSCs. B. hMSCs-derived neural stemlike cells form neurosphere-like structures, and show high nestin expression. The nuclei were counterstained with DAPI (blue).

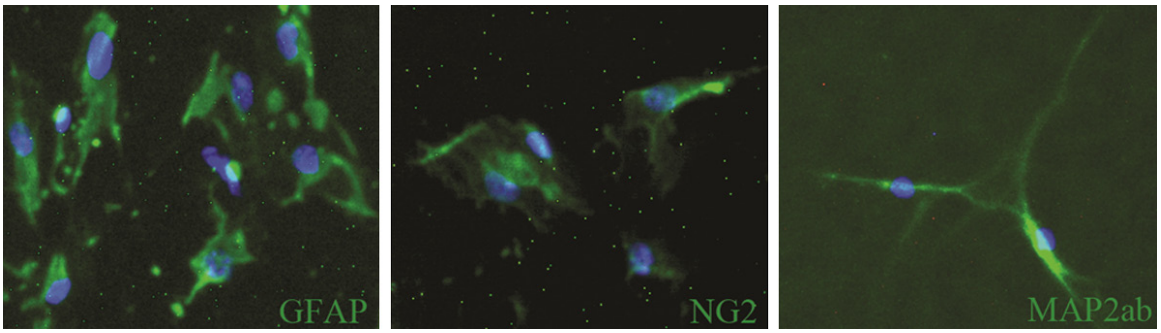

Figure 3. In vitro differentiation of human bone marrow-derived mesenchymal stromal cells (hMSCs)-derived neural stemlike cells into astroglial, oligodendroglial, and neuronal cell types. After 7-10 days in differentiation conditions, the cells were stained for markers for astrocytes (GFAP), oligodendrocytes (NG2), or neurons (MAP2ab). Nuclei were counterstained with DAPI (blue).

cells forming the neurospherelike structures could be induced to differentiate into neural lineage cells in vitro (**Figure 3**).

Microarray expression analysis

hMSCs were co-cultured with U251 glioma cells for five days, and the expression profile of cancer-related genes were investigated by microarray assay. Of the 440 cancer-related genes covered by Oligo GEArray Human Cancer Microarray OHS-802, 16 were found to be significantly up-regulated (>3 fold) but none down-regulated in hMSCs co-cultured with U251 glioma cells compared to that in hMSCs cultured alone (**Figure 4**; **Table 1**). The up-regulated genes in hMSCs co-cultured with U251 cells as indicated by microarray assay include some important oncogenes, the over-expressions of which have been demonstrated to have a positive role in tumorigenicity.

Real-time quantitative RT-PCR

To confirm the results of microarray assay, we chose 8 important oncogenes among those up-

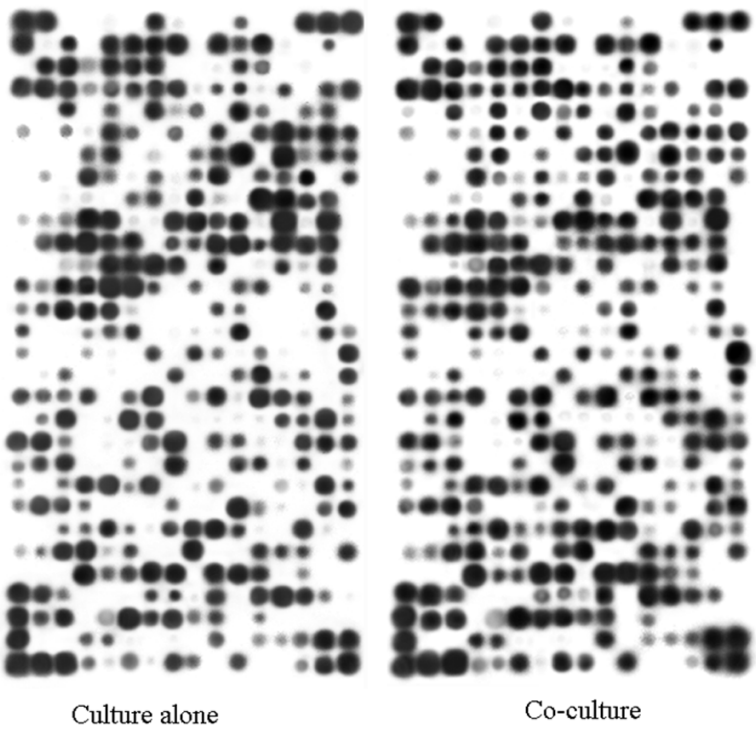

Figure 4. Biotin-labeled cRNA probes were synthesized from total RNA of hMSCs cultured alone or total RNA of hMSCs co-cultured with U251 cells and hybridized to the membrane of Oligo GEArray Human Cancer Microarray OHS-802 spotted with 440 gene-specific oligonucleotide fragments. After hybridization, the membrane was incubated with phosphatase-conjugated streptavidin, and the signal was visualized with CDP-Star and exposed to x-ray film.

regulated genes revealed by microarray assay to be further tested by Real-time quantitative RT-PCR. Real-time quantitative RT-PCR revealed that expression of KIT, CAPNS1, TK1, MMP1, CCND1, CDC20, RELA, and STC1 mRNA were all up-regulated in hMSCs co-cultured with U251 glioma cells compared to that in hMSCs cultured alone, and the magnitude of up-regulation were: KIT, 4.84; CAPNS1, 4.21; TK1, 2.69; MMP1, 8.34; CCND1, 8.94; CDC20, 4.88; RELA, 5.17; STC1, 1.82.

Discussion

Huaman MSCs (hMSCs) represent an optimal cellular vector for gene therapy to treat human gliomas. Nevertheless, research about the influence of human glioma cells on hMSCs is still not in the focus. When server as delivery vector to treat glioma hMSCs would leave the original living place and enter into the targeted tumor environment. What kind of changes would happen to hMSCs under the influence of glioma cells is hardly know. What we concern about here is the bio-safety of hMSCs in aspect of tumorigenic risk in glioma environment. The present study is the first to show that the co-culture of hMSCs with human glioma cells lead to a significant change in the cancer-related gene expression profile of hMSCs.

The microarray analysis revealed that 16 cancer-related genes were found to be significantly up-regulated in hMSCs co-cultured with U251 cells compared to that in hMSCs cultured alone. In particular, the up-regulated genes include 8 important oncogenes: KIT, CAPNS1, TK1, MMP1, CCND1, CDC20, RELA, and STC1, which are involved in cell grow/proliferation, cell invasion/metastasis, cell cycle, and signal transduction pathways. The up-regulation of all these 8 oncogenes mRNA was confirmed by Real-time quantitative RT-PCR.

One of the most important characteristics of malignant cells is uncontrolled cell growth. The KIT, CAPNS1, and TK1 genes were found to be up-regulated in hMSCs co-cultured with U251 cells. The up-regulation of all these genes was demonstrated to be a positive factor in cell proliferation and to contribute to carcinogenesis. KIT functions as the receptor for stem cell factor (SCF) and this interaction is essential for regulation of proliferation and survival. Up-regulation of KIT signaling has been associated with oncogenic transformation in cells expressing the molecule [5]. Capns1 is a regulatory subunit of Calpain, functions in anti apoptosis and its depletion is coupled to increased sensitivity to apoptosis triggered by a number of autophagy-inducing stimuli in mammalian cells. Calpain System is elevated during transformation; it is required for autophagy and survival of cancer cells and plays a key role in metastatic cell migration and angiogenesis [6]. Thymidine kinase 1 (TK1) is a key enzyme involved in the synthesis of DNA precursors and therefore cell

Table 1. Of the 440 cancer-related genes covered by Oligo GEArray Human Cancer Microarray OHS-802, 16 were found to be significantly up-regulated (>3 fold) but none downregulated in human bone marrow-derived mesenchymal stromal cells (hMSCs) co-cultured with U251 cells compared to that in hMSCs cultured alone

Gene name	Gene symbol	GenBank accession no.	Magnitude of change
Serine peptidase inhibitor, Kunitz type, 2	SPINT2	NM_021102	1.7266E+1
Thymidine kinase 1, soluble	TK1	NM_003258	1.4556E+1
Stanniocalcin 1	STC1	NM_003155	1.0710E+1
Matrix metallopeptidase 1 (interstitial collagenase)	MMP1	NM_002421	9.8170E+0
Cyclin D1	CCND1	NM_053056	9.7077E+0
Sortilin 1	SORT1	NM_002959	7.8167E+0
Septin 6	SEPT6	NM_015129	5.3948E+0
Cell division cycle 20 homolog (S. cerevisiae)	CDC20	NM_001255	5.1840E+0
Src homology 2 domain containing adaptor protein B	SHB	NM_003028	5.1160E+0
Cyclin-dependent kinase 5	CDK5	NM_004935	4.8247E+0
V-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)	RELA	NM_021975	4.3586E+0
X-ray repair complementing defective repair in Chinese hamster cells 4	XRCC4	NM_003401	3.4971E+0
V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	KIT	NM_000222	3.3633E+0
CTP synthase	CTPS	NM_001905	3.2726E+0
Calpain, small subunit 1	CAPNS1	NM_001749	3.2369E+0
Ets variant gene 6 (TEL oncogene)	ETV6	NM_001987	3.2207E+0

proliferation-dependent Thymidine kinase, a cell cycle-dependent marker, can be detected in the serum of patients diagnosed with many different types of cancer. Serum levels of thymidine kinase have also been shown to reflect the progression of cancer as well as an indication of the efficacy of chemotherapeutic intervention [7].

Invasive and metastatic behavior is another important characteristic feature of malignant cells. During tracing glioma cells, hMSCs present good migration ability in the CNS. This property, however, would contribute to the malignancy of this cell population once they obtained uncontrolled cell growth ability, which should be concerned about. Matrix metalloproteinases (MMPs) have long been associated with cell invasion and metastasis, MMPs are up-regulated in almost every type of human cancer, and their expression is often associated with poor survival [8]. MMP1 was found to be up-regulated in hMSCs co-cultured with U251 cells, which may imply enhanced invasion and metastasis abilities in these cells under the influence of glioma cells.

The connection between the cell cycle and cancer is obvious: cell cycle machinery controls cell proliferation, and cancer is a disease of inappropriate cell proliferation. The genes CCND1 and CDC20, which are closely involved in cell cycle process, were found to be up-regulated in hMSCs co-cultured with U251 cells. CCND1 is an important regulator of cell cycle progression and can function as a transcriptional co-regulator; the overexpression of CCND1 has been linked to the development and progression of cancer [9]. CDC20 is an essential cell-cycle regulator required for the completion of mitosis and serves as an integrator of multiple intracellular signaling cascades that regulate progression through mitosis [10]. Overexpression of CDC20 has been observed in cancers, which is associated with impairment of the spindle assembly checkpoint, resulting in chromosomal instability in cancer cells [11, 12].

NF-kappaB signaling controls a number of genes involved in immuno-inflammatory responses, cell cycle progression, inhibition of apoptosis and cell adhesion, thus promoting carcinogenesis and cancer progression [13]. RelA, the p65 subunit of NF-kappaB, was found to be up-regulated in hMSCs co-cultured with U251 cells. RelA has diverse functions but it is often associated with conferring resistance to programmed cell death [14]. Aberrant activation of RELA is associated with many human cancers and the anti apoptotic function of RELA has been found to reduce the efficacy of many common cancer therapies [15].

The genes STC1 involved in cell surface receptor linked signal transduction was found to be up-regulated in hMSCs co-cultured with U251 cells. There is growing evidence that altered STC1 expression patterns may have a role in human cancer and enhanced STC1 gene expression has been found in a number of cancers [16].

Taken together, we found up-regulation of a number of cancer-related genes in hMSCs co-cultured with U251 cells and the up-regulated genes include some important oncogenes, the over-expression of which had been demonstrated to contribute to tumorigenesis. Additional studies will be needed to understand the possible biological significance of these alterations of gene expression. Though no final conclusion could be made yet that tumorigenic potential in hMSCs would be induced under the influence of glioma cells, our findings still propose concerns about that, and highlight the need for further studies on this problem before their clinical application as therapeutic vectors to treat human gliomas.

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Disclosure of conflict of interest

None.

Abbreviations

hMSCs, Human bone marrow-derived mesenchymal stromal cells; FBS, Fetal bovine serum;

DMEM, Dulbecco's modified Eagle's medium; PBS, Phosphate buffer saline.

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References

[1] Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, Chen J, Hentschel S, Vecil G, Dembinski J, Andreeff M, Lang FF. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res 2005; 65: 3307-3318.

[2] Bernardo ME, Zaff aroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Cancer Res 2007; 67: 9142-9149.

[3] Zhou YF, Bosch-Marce M, Okuyama H, Krishnamachary B, Kimura H, Zhang L, Huso DL, Semenza GL. Spontaneous transformation of cultured mouse bone marrow-derived stromal cells. Cancer Res 2006; 66: 10849-10854.

[4] Koellensperger E, Gramley F, Preisner F, Leimer U, Germann G, Dexheimer V. Alterations of gene expression and protein synthesis in co-cultured adipose tissue-derived stem cells and squamous cell-carcinoma cells: consequences for clinical applications. Stem Cell Res Ther 2014; 5: 65.

[5] Nikolaou M, Valavanis C, Aravantinos G, Fountzilas G, Tamvakis N, Lekka I, Arapantoni-Dadioti P, Zizi A, Ghiconti I, Economopoulos T, Pectasides D. Kit expression in male germ cell tumors. Anticancer Res 2007; 27: 1685-1688.

[6] Demarchi F, Schneider C. The calpain system as a modulator of stress/damage response. Cell Cycle 2007; 6: 136-138.

[7] O'Neill KL, Buckwalter MR, Murray BK. Thymidine kinase: diagnostic and prognostic potential. Expert Rev Mol Diagn 2001; 1: 428-433.

[8] Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2: 161-174.

[9] Alao JP. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Mol Cancer 2007; 6: 24.

[10] Yu H. Cdc20: a WD40 activator for a cell cycle degradation machine. Mol Cell 2007; 27: 3-16.

[11] Yuan B, Xu Y, Woo JH, Wang Y, Bae YK, Yoon DS, Wersto RP, Tully E, Wilsbach K, Gabrielson E. Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability. Clin Cancer Res 2006; 12: 405-410.

[12] Mondal G, Sengupta S, Panda CK, Gollin SM, Saunders WS, Roychoudhury S. Overexpression of Cdc20 leads to impairment of the spindle assembly checkpoint and aneuploidization in oral cancer. Carcinogenesis 2007; 28: 81-92.

[13] Okamoto T, Sanda T, Asamitsu K. NF-kappa B signaling and carcinogenesis. Curr Pharm 2007; 13: 447-462.

[14] Kucharczak J, Simmons MJ, Fan Y, Gelinas C. To be, or not to be: NF-kappaB is the answer--role of Rel/NF-kappaB in the regulation of apoptosis. Oncogene 2003; 22: 8961-8982.

[15] Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 2001; 107: 241-246.

[16] Chang AC, Jellinek DA, Reddel RR. Mammalian stanniocalcins and cancer. Endocr Relat Cancer 2003; 10: 359-373.