Serological Identification of URGCP as a Potential Biomarker for Glioma

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SUMMARY

Aim: Glioblastoma multiforme (GBM) is one of the most frequent human brain tumor and causes dismal outcome. To identify tumor-associated antigens in GBM patients may find potential diagnostic markers and immunotherapeutic targets. In this study, we identified a gene termed *URGCP* using the serological identification of antigens by recombinant A2B5 positive glioma cDNA library. The gene product of URGCP is immunogenic in GBM after tested in allogenic patients serum screening. **Methods and results:** GBM patients with an auto-antibody response against URGCP show longer survival than those without URGCP response. In addition, we show that URGCP was high expression in most GBM tissues and cell lines compared with normal brain tissues and majorly co-expressed with stem cell marker A2B5. **Conclusion:** We identified a potential new biomarker of GBM, URGCP. The findings indicate that URGCP is immunogenic in human GBM and suggest its potential use as diagnostic and immunotherapeutic for GBM patients.

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Introduction

GBM is one of the most malignant brain tumors with a median survival only 14 months. Various strategies for therapy of glioma have been made, including surgery, chemotherapy, and radiotherapy, without obtaining any obvious improvement [1–3]. However, so far few immunological markers for survival have been introduced into clinical application. Thus, novel clinical immune markers and novel therapeutic concepts are urgently needed for this CNS malignancy.

Cancer stem cells are small subsets of cancer cells and have the ability for self-renewal and multipotency. These subsets of cancer cells are responsible for most tumor therapy failure and recurrence [4,5]. In GBM, glioma stem-like cells have been implicated to play one of the important roles in treatment failure. CD133 was well known as a stem cell marker and was universally expressed by most of malignant brain tumors. However, recent studies have defined A2B5+CD133-tumor-initiating cells in human glioma [6,7]. The prevalence of A2B5 reactivity in these cells suggests that human glioma contains multiple subsets of cells with the capacity to form tumors and specifically distinct from CD133+ cells.

Serological analysis of recombinant tumor cDNA expression libraries (SEREX) is a method designed to isolate tumor-associated antigens that have elicited high-titer IgG responses in human hosts [8,9]. SEREX analysis has identified a number of tumor-associated antigens that have associated with the etiology, diagnosis, and therapy of cancer. To date, more than 600 tumor-associated antigens have been identified using by SEREX [9,10]. Most of the SEREX-based studies aimed at the identification of novel tumor antigens that elicit an antibody response in tumor tissue or cell lines [11–16]. Until now, there was no antigen identified by SEREX based on tumor stem cell, which is notorious for GBM recurrence and malignance [5,17,18].

Previously studies have demonstrated that A2B5 was a new marker of glioma stem cell [6,7]. In this study, we firstly present to screen antigen-specific immune responses associated with glioblastoma by screening cDNA libraries of A2B5-positive glioma cells. We identified a candidate new biomarker of GBM, URGCP and suggest its use as an immunotherapeutic target for GBM patients.

Materials and methods

Sera and Glioma Tissue

Sera were obtained from patients with GBM and healthy volunteers after they had provided written informed consent. Glioma patients underwent surgery, and the histology was grade II–IV at Department of Neurosurgery, Huashan Hospital, Fudan University. Each serum was centrifuged at $3\,000 \times g$ for 5 min and then frozen at -80°C until use. Glioma tissue was collected from operations. Control brain tissue was isolated from nonneoplastic CNS tissues, which were collected during a surgery from patients with intractable epilepsy. The Local Ethical Review Board of the Huashan hospital, Fudan University, approved this study.

cDNA Library

The total RNA was extracted from the A2B5-positive glioma cells sorting by MACS MicroBead Technology. Using the TRIZOL reagent (Invitrogen), mRNA was extracted and purified on an oligo (dT) column. The first-strand cDNA synthesis was performed using Hind III Random Primers, MMLV reverse transcriptase. Then the cDNA was ligated to *Eco*RI and Hind III adaptors and digested with *Eco*RI and Hind III. Double-strand cDNA fragments were cloned into T7Select[®]10-3b vector (Merck KGaA, Darmstadt, Germany) and then finished *in vitro* packaging using an *in vitro* packaging kit (Merck).

Immunoscreening

Sera from the mixed GBM patients were diluted in 1% bovine serum albumin/tris-buffered saline (TBS) and preabsorbed with transformed *E. coli* lysates and *E. coli* infected with T7Select[®]10-3b phage. Recombinant phages at a concentration of 5×10^8 /10 cm plate were amplified for 6 h at 37°C , and then covered with nitrocellulose membranes (Amersham, Buckinghamshire, England) and incubated for an additional 3 h at 37°C to transfer the encoded proteins onto the filter membranes. Membranes were then blocked with 5% (w/v) skim milk/TBS. After washing with TBS containing 0.05% Tween 20 (TBS-T), membranes were incubated in prepared sera for 15 h at 4°C . This was followed by incubation in horseradish peroxidase (HRP)-conjugated mouse anti-human IgG for 1 h at 37°C , and then membranes were washed in TBS-T and TBS and incubated with ECL RPN 2106 (Amersham) for 1 min and exposed to LAS 4000 to detect antibody-reactive phage plaques. Positive recombinant clones were picked up and purified by an additional cycle of plating and screening.

Sequence Analysis of Identified cDNA Clones

Immunoreactive phage clones were amplified by PCR using the Ex Taq kit (Takara Shuzo) and sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit and an ABI Prism automated sequencer (Perkin-Elmer, Branchburg, NJ, USA). The sequenced DNAs were analyzed by a BLAST search of genetic databases at the National Center for Biotechnology Information.

qRT-PCR

For the analysis of URGCP messenger RNA (mRNA) expression, complementary DNA (cDNA) synthesis was performed using random primers under standard conditions. mRNA expression was quantified using the $2^{-\Delta\Delta\text{Ct}}$ method. GAPDH served as the internal control. All reactions were performed in triplicate.

Immunohistochemistry and Immunofluorescence

Immunohistochemistry and Immunofluorescence assay was performed as previously described [19,20]. Briefly, URGCP expression was analyzed using immunocytochemical staining of GBM, low-grade glioma, and normal brain tissues. The tissue section was incubated with URGCP (1:500) for 12 h, and then washed and incubated with biotinylated goat anti-rabbit IgG (1:3000) for 30 min at room temperature. The sections were immersed in a solution with the avidin–biotin complex (Vector Laboratories, Burlingame, CA, USA) for 30 min, developed with diaminobenzidine and counterstained with eosin. The sections were scanned at magnification ($200 \times$) using light microscopy. Two pathologists evaluated the immunoreactivity and staining for each section. For immunofluorescence assay, primary antibodies for cultured tumor cells and clinical samples were anti-URGCP, anti-A2B5, and antinestin. The secondary antibodies were Alexa Fluor 488, 594, or 647-conjugated donkey anti-mouse or rabbit or anti-goat IgG. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Fluorescence signals were detected with a two-photon confocal laser-scanning microscopy.

Elisa

For enzyme-linked immunosorbent assays, 96-well flat plates were coated with purified URGCP protein (150 ng/well) at 4°C overnight. After washing three times with PBST, the plates were blocked with FCS. Then, 100 μL /well of 500-fold diluted sera from normal controls or glioma patients were added to each plate. The plates were washed in PBST after 1.5 h incubation at 37°C and further incubated with 1:1000-diluted horseradish peroxidase (HRP)-conjugated goat-anti-human IgG for 1 h at 37°C . Finally, 100 μL /well of tetramethyl-benzidine substrate solution was added, and the reaction was stopped by adding 50 μL of 1 mol/L sulfuric acid. Optical absorbance was measured at 450 nm.

GBM mRNA data and Statistical Analysis

The TCGA and Rembrandt mRNA expression microarray data were downloaded from the following portal: <http://tcga-data.nci.nih.gov/tcga/homepage.htm> and <https://cainegrator.nci.nih.gov/rembrandt/home.do>. Data were presented as mean values and standard deviation (SD). Statistical differences between two groups were evaluated by the unpaired Student's t-test by SPSS 14.0. Overall survival analysis was performed according to the Kaplan–Meier method. Tests with $P < 0.05$ are considered statistically significant.

Result

Serological Screening of A2B5 Positive cDNA Library

The phage expression library was constructed using mRNA derived from the A2B5-positive glioma cells (Figure 1). To identify glioma-associated tumor antigens, cDNA expression libraries were screened using mixed sera from 10 GBM patients and 23 reacting clones were recognized by serum IgG

Figure 1 A2B5+ glioma cells sorting and characteristics. (A) A2B5+ glioma cells were sorted by A2B5 MicroBead and proliferated in culture as nonadherent spheres, whereas GBM tumor cells adhered to culture dishes did not form spheres. (B) GBM tumor cells and A2B5+ glioma cells were immunostained for A2B5 and subjected to flow cytometry for quantification of A2B5 expression. (C) A2B5+ primary tumor spheres from GBMs are immunostained for characteristic neural stem cell marker nestin and A2B5.

antibodies. DNA sequence was analyzed by comparing the homologous sequences from NCBI BLAST database. Finally, we found that these 23 isolated clones comprised 16 independent genes, and the rest of 7 clones were overlapped (Table 1).

Allogenic Serum Screening on URGCP Antigen

Among the collected 16 gene fragments by SEREX, we firstly focus on URGCP, which was mostly associated with tumor phenotype and involved in cell cycle regulation of cyclin D1 [21]. To confirm the specificity and relevance of the auto-antibody to URGCP, the allogenic GBM patient serum screening was performed. We plated the *E. coli* cells transfected with the URGCP clone and GBM cDNA library containing cells separately on an agar plate. After 12 hours, plaques were transferred to nitrocellulose membranes, reacted with GBM sera, and then scored positive by visual inspection when compared to cDNA library plaques (Figure 2A). The specific immune reaction was detected in 14 of 40 GBM sera (Table 2). To confirm the specificity of antibody reaction in GBM, we applied sera from healthy volunteers. None of the 14 control sera contained antibodies against cDNA library plaques and URGCP (Figure 2A).

URGCP Elicited Specific Humoral Responses in GBM Patients

Next, we assessed the frequency of anti-URGCP immune responses *in vivo*. Using an ELISA assay, we observed immune reaction of URGCP in 14 healthy donors and 40 GBM patients. The mean OD₄₅₀ value of anti-URGCP IgG was higher in GBM patients than those in healthy donors (Figure 2B; $P < 0.001$). After setting the cut-off value by mean OD₄₅₀ plus 2 SD ($OD_{mean} + 2SD$) from healthy donors, we found that all serum samples from healthy donors tested were negative. URGCP-specific immune reaction was detectable in 14 of 40 samples from GBM patients (Figure 2B). It was consistent with results obtained from allogenic serum screening *in vitro*.

Prognostic Value of URGCP Immune Response

To evaluate the role of the URGCP antibody response in GBM survival, we compared the OS of the patients with different immune response of URGCP antibodies. The URGCP-positive patients showed a better survival than URGCP-negative patients (Figure 2C). The median survival of URGCP-positive patients was 19 months, while the URGCP-negative patients was only 11 months ($P = 0.041$).

URGCP Expression in Normal and Tumor Tissues and in Tumor Cell Lines

To demonstrate the expression pattern of URGCP transcripts in glioma cell lines and control brain tissues, real-time PCR assay was performed. Our results suggested that URGCP was expressed highly in most of glioma cell lines (LN229, U87, LN308, A172, T98, U251, and SNB19), but low expression in normal brain tissue

Table 1 List of gene fragments obtained by serological analysis of recombinant cDNA libraries from an A2B5-positive glioma cDNA library

NO.	Gene name	EntrezGene	Definition
MY-A2B5-01	NUDC	10726	Homo sapiens nuclear distribution C homolog (<i>A. nidulans</i>) (NUDC), mRNA
MY-A2B5-02	GATC	283459	Homo sapiens glutamyl-tRNA(Gln) amidotransferase, subunit C (GATC), transcript variant 1, mRNA
MY-A2B5-03	LOC283459	283459	Homo sapiens hypothetical protein LOC283459, mRNA
MY-A2B5-04	SRSF9	8683	Homo sapiens serine/arginine-rich splicing factor 9 (SRSF9), mRNA
MY-A2B5-05	EEF1A1	1915	Homo sapiens elongation factor 1-alpha 1 (EEF1A1L14) mRNA
MY-A2B5-06	EEF1A1	1915	Homo sapiens elongation factor 1-alpha 1 (EEF1A1L14) mRNA
MY-A2B5-07	EEF1A1	1915	Homo sapiens elongation factor 1-alpha 1 (EEF1A1L14) mRNA
MY-A2B5-08	URGCP	55665	Homo sapiens Upregulator Of Cell Proliferation, mRNA
MY-A2B5-09	MRPS24	64951	Mitochondrial ribosomal protein S24
MY-A2B5-10	MORF4L1	10934	Homo sapiens mortality factor 4 like 1, mRNA
MY-A2B5-11	URGCP	55665	Homo sapiens upregulator Of cell proliferation, mRNA
MY-A2B5-12	RAB3GAP2	10933	Homo sapiens RAB3 GTPase activating protein subunit 2
MY-A2B5-13	MORF4L1P1	25782	Homo sapiens MORF4L1 mRNA
MY-A2B5-14	MORF4L1P1	25782	Homo sapiens MORF4L1P1 mRNA
MY-A2B5-15	URGCP	55665	Homo sapiens upregulator Of cell proliferation, mRNA
MY-A2B5-16	USP15	9958	Homo sapiens ubiquitin-specific peptidase 15
MY-A2B5-17	MCLD	9958	MCLD 16S ribosomal RNA, complete sequence
MY-A2B5-18	pG1 protein		Homo sapiens pG1 protein mRNA
MY-A2B5-19	NKAIN2	154215	Homo sapiens Na ⁺ /K ⁺ transporting ATPase interacting 2 (NKAIN2), mRNA
MY-A2B5-20	RP1-157N22	154215	Human DNA sequence from clone RP1-157N22 on chromosome 6q22.32-23.2
MY-A2B5-21	WB20		WB20 16S ribosomal RNA gene
MY-A2B5-22	WB18 16S		WB18 16S ribosomal RNA gene
MY-A2B5-23	NUDC	10726	Homo sapiens nuclear distribution C homolog (<i>A. nidulans</i>) (NUDC), mRNA

Figure 2 UGRCP-specific immunal responses. **(A)** GBM patient sera indicated at the top of the figure were incubated with clones expressing UGRCP antigen and with the A2B5-positive GBM cDNA library. Positive plaques indicating an antibody reaction show more intensive staining than cDNA library background. Normal control sera contained no antibodies response against cDNA library and UGRCP. **(B)** Serum samples from healthy donors and GBM patients were diluted at 1:100 and were analyzed by enzyme-linked immunosorbent assay (ELISA) to detect antibodies reactive to recombinant UGRCP protein. The dashed line indicates a cut-off value (ODmean $\pm$ 2 SD) as determined by ELISA assays using sera from healthy individuals. **(C)** Kaplan–Meier survival curves indicating cumulative survival that patients with UGRCP-positive response experienced longer survival.

Table 2 Summary of SEREX results with allogenic GBM patients' sera

Sera	Sex	Age	Pathology	SEREX
G1	Female	56	GBM WHO IV	—
G2	Female	62	GBM WHO IV	—
G3	Male	55	GBM WHO IV	+
G4	Male	56	GBM WHO IV	+
G5	Female	72	GBM WHO IV	—
G6	Female	60	GBM WHO IV	+
G7	Male	58	GBM WHO IV	—
G8	Female	62	GBM WHO IV	—
G9	Male	43	GBM WHO IV	—
G10	Male	58	GBM WHO IV	+
G11	Female	54	GBM WHO IV	—
G12	Male	60	GBM WHO IV	—
G13	Female	27	GBM WHO IV	—
G14	Female	38	GBM WHO IV	—
G15	Female	66	GBM WHO IV	+
G16	Male	66	GBM WHO IV	—
G17	Female	43	GBM WHO IV	—
G18	Male	55	GBM WHO IV	+
G19	Male	32	GBM WHO IV	—
G20	Female	58	GBM WHO IV	—
G21	Female	52	GBM WHO IV	—
G22	Male	57	GBM WHO IV	—
G23	Male	43	GBM WHO IV	—
G24	Male	52	GBM WHO IV	—
G25	Female	55	GBM WHO IV	—
G26	Female	45	GBM WHO IV	+
G27	Male	67	GBM WHO IV	+
G28	Female	55	GBM WHO IV	+
G29	Male	44	GBM WHO IV	—
G30	Male	52	GBM WHO IV	—
G31	Female	56	GBM WHO IV	+
G32	Female	53	GBM WHO IV	+
G33	Male	66	GBM WHO IV	—
G34	Male	48	GBM WHO IV	—
G35	Female	53	GBM WHO IV	—
G36	Male	58	GBM WHO IV	+
G37	Female	37	GBM WHO IV	+
G38	Female	65	GBM WHO IV	+
G39	Female	54	GBM WHO IV	—
G40	Male	42	GBM WHO IV	+

(Figure 3A). Consistent with the cell lines' data, we found that the protein expression of URGCP in GBM tissues was also significantly higher than normal brain tissues in an independent set of 227 human GBM tumors from Rembrandt database (Figure 3B).

To further analyze URGCP protein expression, IHC assay for URGCP was performed in normal brain and different grade gliomas. In glioma, strong positive expression of URGCP was observed in both cytoplasm and nucleus (Figure 3C). The levels of URGCP expression were increased according to the pathology grade of tumors. That is to say high-grade tumor was heavily stained, while low-grade and normal brain tissue were barely stained. In addition, URGCP was also majorly co-expressed with A2B5 in neurosphere cells and clinical

samples (Figure 4). These results suggest that URGCP could serve as a new tumor-associated biomarker that is expressed frequently in GBMs.

Discussion

Using a combined immunological and molecular approach based on the auto-antibody response against tumor-associated antigens, we isolated URGCP, a new biomarker in GBM. Then we reported that URGCP was immunogenic in 14/40 of GBM patients, but not in every healthy control. The obviously high expression in GBM patients compared with the normal brain tissue and co-localization with A2B5 supports the hypothesis that URGCP may play a key role in GBM.

SEREX was first developed by Michael Pfreundschuh at Homburg's Saarland University [9]. By screened diluted serum of the patient with cDNA library from fresh tumor cells, tumor-associated antigen can be identified. So far, more than 600 tumor-associated antigens have been identified by SEREX method. Several SEREX studies have focused on glioma specimens, cell lines, and sera from glioma patients. In the initial analysis, Struss *et al.* [22] screened a human GBM cDNA library with autologous patient serum and identified a gene termed PHF3. GBM patients with auto-antibody response against PHF3 show significant longer survival than those without PHF3 response [23]. To extend the number of cDNA library, 5 cDNA libraries from astrocytomas and other grade gliomas were screened by Rudolf *et al.* [24]. This resulted in the isolation of 10 gene products, including the GFAP, Bax-inhibitor 1, 3 markers involved in gene regulation and proliferation, SP40, TCP-1, calnexin, and 2 new gene products. The other studies also applied SEREX to identify glioma antigens, including SH3GL1, GLEA2, and SOX6 [23–25]. Our present study was firstly designed to identify cancer antigens for human glioma by SEREX, using a cDNA library from A2B5-positive glioma cells, which were crucial for the initiation and maintenance of GBM malignant [6,7]. In this screening, we isolated a new biomarker URGCP and confirmed its expression and immunoreactivity in human GBM.

URGCP was a novel gene located on chromosome 7, firstly identified by PCR select cDNA subtraction [26]. Patients with high expression of URGCP in epithelial ovary cancer and osteosarcoma had shorter survival time, correlated with tumor recurrence and metastasis [27,28]. In addition, overexpression of URGCP in plenty types of cancers promoted cell growth and survival, suggesting that URGCP may be associated with the development of carcinogenesis [27,29–33]. However, there were no studies about URGCP in glioma. In this study, real-time PCR analysis showed that the mRNA level of URGCP was high expressed in human glioma cells compared with normal brain. IHC analyses demonstrated that URGCP was high expressed in both low- and high-grade gliomas. Moreover, URGCP was majorly co-expressed with stem cell marker A2B5 and confirmed in A2B5 neurosphere cells and in GBM samples.

Taken together, we identified candidate new biomarker of GBM, URGCP. These findings show that URGCP is immunogenic in humans' GBM and indicate its potential use as diagnostic and immunotherapeutic target for GBM.

Figure 3 Expression of URGCP in normal brain and gliomas. **(A)** Real-time PCR analyzed the mRNA expression of URGCP in normal brain and glioma cell lines. **(B)** The mRNA expression of URGCP in control brain and GBM in Rembrandt database. **(C)** Immunohistochemistry with URGCP antibody on formalin-fixed paraffin-embedded brain tissue and different grade glioma samples.

Figure 4 URGCP was major co-expressed with A2B5. **(A)** A2B5+ primary tumor spheres from GBM are immunostained for characteristic neural stem cell marker A2B5 and for URGCP. **(B)** Immunofluorescence with URGCP and A2B5 antibody on formalin-fixed paraffin-embedded GBM samples.

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Conflict of Interest

The authors declare no conflict of interest.

Reference

- Van Meir EG, Hadjipanayis CG, Norden AD, et al. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. *CA Cancer J Clin* 2010;**60**:166–193.
- Stupp R, Mason WP, Van Den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;**352**:987–996.
- Butowski NA, Sneed PK, Chang SM. Diagnosis and treatment of recurrent high-grade astrocytoma. *J Clin Oncol* 2006;**24**:1273–1280.
- Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. *Nature* 2001;**414**:105–111.
- Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;**444**:756–760.
- Ogden AT, Waziri AE, Lochhead RA, et al. Identification of A2B5+CD133+ tumor-initiating cells in adult human gliomas. *Neurosurgery* 2008;**62**:505–514; discussion 14–5.
- Tchoghandjian A, Baeza N, Colin C, et al. A2B5 cells from human glioblastoma have cancer stem cell properties. *Brain Pathol* 2010;**20**:211–221.
- Sahin U, Tureci O, Schmitt H, et al. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc Natl Acad Sci U S A* 1995;**92**:11810–11813.
- Sahin U, Tureci O, Pfreundschuh M. Serological identification of human tumor antigens. *Curr Opin Immunol* 1997;**9**:709–716.
- Old LJ, Chen YT. New paths in human cancer serology. *J Exp Med* 1998;**187**:1163–1167.
- Cooper CD, Lawrie CH, Liggins AP, et al. Identification and characterization of peripheral T-cell lymphoma-associated SEREX antigens. *PLoS ONE* 2011;**6**:e23916.
- Hanafusa T, Mohamed AE, Domae S, et al. Serological identification of Tektin5 as a cancer/testis antigen and its immunogenicity. *BMC Cancer* 2012;**12**:520.
- Hanafusa T, Mohamed AE, Kitaoka K, et al. Isolation and characterization of human lung cancer antigens by serological screening with autologous antibodies. *Cancer Lett* 2011;**301**:57–62.
- Heller A, Zornig I, Muller T, et al. Immunogenicity of SEREX-identified antigens and disease outcome in pancreatic cancer. *Cancer Immunol Immunother* 2010;**59**:1389–1400.
- Shimada H, Shiratori T, Yasuraoka M, et al. Identification of Makorin 1 as a novel SEREX antigen of esophageal squamous cell carcinoma. *BMC Cancer* 2009;**9**:232.
- Wang K, Xu X, Nie Y, et al. Identification of tumor-associated antigens by using SEREX in hepatocellular carcinoma. *Cancer Lett* 2009;**281**:144–150.
- Chen J, Li Y, Yu TS, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* 2012;**488**:522–526.
- Ahmed AU, Auffinger B, Lesniak MS. Understanding glioma stem cells: rationale, clinical relevance and therapeutic strategies. *Expert Rev Neurother* 2013;**13**:545–555.
- Chen L, Huang K, Han L, et al. Beta-catenin/Tcf-4 complex transcriptionally regulates AKT1 in glioma. *Int J Oncol* 2011;**39**:883–890.
- Zhou X, Ren Y, Moore L, et al. Downregulation of miR-21 inhibits EGFR pathway and suppresses the growth of human glioblastoma cells independent of PTEN status. *Lab Invest* 2010;**90**:144–155.
- Xie C, Song LB, Wu JH, et al. Upregulator of cell proliferation predicts poor prognosis in hepatocellular carcinoma and contributes to hepatocarcinogenesis by downregulating FOXO3a. *PLoS ONE* 2012;**7**:e40607.
- Fischer U, Struss AK, Hemmer D, et al. PHF3 expression is frequently reduced in glioma. *Cytogenet Cell Genet* 2001;**94**:131–136.
- Pallasch CP, Struss AK, Munnia A, et al. Autoantibodies against GLEA2 and PHF3 in glioblastoma: tumor-associated autoantibodies correlated with prolonged survival. *Int J Cancer* 2005;**117**:456–459.
- Schmits R, Cochlovius B, Treitz G, et al. Analysis of the antibody repertoire of astrocytoma patients against antigens expressed by gliomas. *Int J Cancer* 2002;**98**:73–77.
- Matsutani T, Hiwasa T, Takiguchi M, et al. Autologous antibody to src-homology 3-domain GRB2-like 1 specifically increases in the sera of patients with low-grade gliomas. *J Exp Clin Cancer Res* 2012;**31**:85.
- Tufan NL, Lian Z, Liu J, et al. Hepatitis Bx antigen stimulates expression of a novel cellular gene, URG4, that promotes hepatocellular growth and survival. *Neoplasia* 2002;**4**:355–368.
- Li W, Zhou N. URG4 upregulation is associated with tumor growth and poor survival in epithelial ovarian cancer. *Arch Gynecol Obstet* 2012;**286**:209–215.
- Huang J, Zhu B, Lu L, et al. The expression of novel gene URG4 in osteosarcoma: correlation with patients' prognosis. *Pathology* 2009;**41**:149–154.
- Song J, Xie H, Lian Z, et al. Enhanced cell survival of gastric cancer cells by a novel gene URG4. *Neoplasia* 2006;**8**:995–1002.
- Satioglu-Tufan NL, Dodurga Y, Gok D, et al. RNA interference-mediated URG4 gene silencing diminishes cyclin D1 mRNA expression in HepG2 cells. *Genet Mol Res* 2010;**9**:1557–1567.
- Dodurga Y, Yonguc GN, Avci CB, et al. Investigation of microRNA expression changes in HepG2 cell line in presence of URG4/URGCP and in absence of URG4/URGCP suppressed by RNA interference. *Mol Biol Rep* 2012;**39**:11119–11124.
- Avci CB, Dodurga Y, Gundogdu G, et al. Regulation of URG4/URGCP and PPARalpha gene expressions after retinoic acid treatment in neuroblastoma cells. *Tumour Biol* 2013 [Epub ahead of print].
- Dodurga Y, Oymak Y, Gunduz C, et al. Leukemogenesis as a new approach to investigate the correlation between up regulated gene 4/upregulator of cell proliferation (URG4/URGCP) and signal transduction genes in leukemia. *Mol Biol Rep* 2013;**40**:3043–3048.