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Clinical Study

Downregulation of chromatin remodeling factor CHD5 is associated with a poor prognosis in human glioma

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ABSTRACT

Chromodomain helicase DNA-binding protein 5 (CHD5), a member of the CHD family, is involved in key cellular processes including chromatin remodeling, cell cycle regulation, and cellular adhesion. Recent studies have demonstrated that CHD5 is the product of a novel tumor suppressor gene and is implicated in certain tumor types. However, the clinicopathological significance of CHD5 expression in human malignant gliomas remains unclear. To address this problem, CHD5 expression in human gliomas and non-neoplastic brain tissues was measured using real-time quantitative polymerase chain reaction (RT–PCR) assay, Western blot, and immunohistochemistry. The association of CHD5 immunostaining with clinicopathological factors or prognosis of glioma patients was statistically analyzed. Genetic and protein expression of CHD5 were downregulated in glioma tissues compared to corresponding non-neoplastic brain tissues (both $p < 0.001$). Additionally, decreased expression of CHD5 in glioma was significantly associated with pathological grade ($p = 0.007$); high pathological grade was associated with low CHD5 expression. Loss of CHD5 protein expression was also significantly correlated with a low Karnofsky performance scale score ($p = 0.01$). Moreover, overall survival of patients with low CHD5 protein expression was dramatically shorter than those of patients with high CHD5 protein expression ($p = 0.003$). Multivariate Cox regression analysis indicated that CHD5 expression was an independent prognostic factor for patients with gliomas ($p = 0.01$). In conclusion, these data offer convincing evidence for the first time that CHD5 might act as a tumor suppressor in glioma, may act as a regulator of aggressive development, and is a candidate prognostic marker for this malignancy.

1. Introduction

Gliomas are the most common malignancy in the central nervous system for both children and adults. The glioma family includes well-differentiated low grade astrocytomas [World Health Organization (WHO) grade I–II], anaplastic astrocytomas (WHO grade III) and glioblastoma multiforme (GBM, WHO grade IV).[1] Despite great progress in therapeutic technologies, including surgery, radiotherapy, photodynamic therapy,[2,3] and chemotherapy, survival remains poor, with less than 3% 5-year survival rate for patients with GBM.[4] Recent studies have indicated that age, Karnofsky performance scale (KPS) score, histologic grade, and tumor necrosis are important prognostic factors for gliomas.[5] However, the prognosis of both high-and low-grade tumors remains heterogeneous. Curran et al.[6] demonstrated that the survival of patients with high-grade gliomas ranges from 5–59 months, and some zpatients with low-grade tumors have a poorer outcome than expected.

It is widely accepted that both activation of proto-oncogenes and inactivation of tumor suppressor genes are involved in glioma progression. To understand genetic and molecular pathogenic changes during the development of gliomas, it is therefore important to identify novel diagnostic and prognostic markers.

The chromodomain helicase DNA-binding domain (CHD) protein family includes nine members (CHD 1–9), which all contain two N-terminal chromodomains, a helicase-like ATPase motif associated with nucleosome remodeling, and a less well-defined C-terminal DNA binding domain.[7] As a member of the CHD family, CHD5 was identified in 2002 as a tumor suppressor gene located at 1p36 in the p19/p53 pathway.[8] CHD5 is an unusual CHD member because its expression is found to be limited to the developing brain, adult brain, and adrenal gland, suggesting a role in the development or function of the neural system.[9] Its role in tumorigenesis was originally demonstrated by genetic mapping studies in neuroblastomas. In 2003, Thompson et al.[10] found that CHD5 maps within a small region of deletion on 1p36.3 in human

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neuroblastomas; they also detected low or undetectable CHD5 expression in both neuroblastoma cell lines and tissues. Subsequent cumulative research evidence supports CHD5 as a candidate tumor suppressor gene in more than half of human cancers. For example, Wang et al.[11] reported that the ectopic expression of CHD5 in laryngeal cancer cells led to significant inhibition of growth and invasiveness; Zhao et al.[12] detected low or absent CHD5 expression in lung cancer cell lines and tissues; in epithelial ovarian cancer, Wong et al.[13] found that downregulation of CHD5 was an independent adverse prognostic factor for this tumor. These findings suggest that CHD5 deficiency is a common molecular event in human tumorigenesis.

The CHD5 gene was also found to be significantly deleted in glioma cell lines[14,15]; however, its expression pattern and clinical significance in human malignant glioma remains unclear. To address this question, CHD5 expression in human gliomas and non-neoplastic brain tissues was measured by real-time quantitative polymerase chain reaction (RT–PCR) assay, Western blot, and immunohistochemistry. The association of CHD5 immunostaining with clinicopathological factors and prognosis in glioma patients was statistically analyzed.

2. Materials and methods

2.1. Patients and tissue samples

This study was approved by the Research Ethics Committee of Tangdu Hospital, Fourth Military Medical University, China. Written informed consent was obtained from all patients. All specimens were handled and anonymized according to ethical and legal standards.

A total of 128 formalin-fixed, paraffin-embedded specimens of gliomas resected between 2000 and 2010 were retrieved from the archives of the Pathology Department of Tangdu Hospital, Fourth Military Medical University, China. All slides were re-evaluated according to the WHO classification[1] by two pathologists, with differences resolved by consensus. A total of 76 males and 52 females (1.46:1) were included in the study, with median age 42 years (range 12–71). Among a total of 128 gliomas, 32 were classified as low-grade [18 pilocytic astrocytomas (WHO I) and 14 diffuse astrocytomas (WHO II)], and 96 were classified as high-grade [38 anaplastic astrocytomas (WHO III) and 58 primary glioblastomas (WHO IV)]. None of the patients in this study had received chemotherapy or radiotherapy prior to surgery. Clinicopathological features and treatment strategies of all the patients are shown in Table 1. Paraffin and snap-frozen sections of non-neoplastic brain tissues from 10 patients with intractable epilepsy were also included as controls.

Table 1
Clinicopathological features of 128 patients with gliomas

Features	WHO I	WHO II	WHO III	WHO IV
No. of patients	18	14	38	58
Mean age (year)	38.6	45.9	43.1	44.2
Gender				
Male	12	6	25	33
Female	6	8	13	25
KPS				
>80	15	11	9	15
<80	3	3	29	43
Surgery				
Gross total resection	18	14	28	38
Partial resection	0	0	9	15
Biopsy	0	0	1	5
Adjuvant treatment				
Radiotherapy	0	0	30	12
Chemotherapy	0	1	0	6
Radiotherapy and chemotherapy	0	0	5	28

KPS = Karnofsky Performance Scale score, WHO = World Health Organization grade.

All patients were followed for a minimum of 5 years, or until death. Overall survival was calculated from the date of the initial surgery to death. Patients who died of diseases not directly related to their gliomas or due to unexpected events were excluded from this study. In addition, 20 glioma specimens [five pilocytic astrocytomas (WHO I), three diffuse astrocytomas (WHO II), three anaplastic astrocytomas (WHO III), and nine primary glioblastomas (WHO IV)] were snap-frozen in liquid nitrogen and stored at −80 °C following surgery for real-time quantitative RT-PCR assay and Western blot analysis.

2.2. Real-time quantitative RT-PCR

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After purification, complementary DNA (cDNA) was synthesized from 10 μg total RNA using the First-Strand cDNA Synthesis Kit (ReverTra Ace-a, FSK-100; Toyobo, Osaka, Japan) according to the manufacturer's protocol. The primers were designed as follows: for human CHD5, forward primer, 5'- AGT TCC GTG TGA GGA TGA AC -3', and reverse primer, 5'- TCA AGG CTG ACG TGT TCA AG -3'; for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward primer, 5'- CCC ACT CCT CCA CCT TTG AC-3', and reverse primer, 5'-ATG AGG TCC ACC ACC CTG TT-3'. The real-time RT-PCR was conducted by using the SYBR Green Realtime PCR master mix (QPK-201, Toyobo). The cDNA fragments were denatured at 95 °C for 15 s, annealed at 58 °C for 15 s, and extended at 72 °C for 45 s, for 40 cycles with the ABI PRISM 7500 Quantitative PCR system (Applied Biosystems, Foster City, CA, USA). Each sample was examined in triplicate and the PCR products produced were non-neoplasticized to GAPDH, which served as internal control.

2.3. Western blot analysis

The tissues were harvested by scraping and lysed using modified radioimmunoprecipitation assay buffer (50 mM Tris–HCl, pH 7.4), 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and Complete Protease Inhibitor Cocktail (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The protein content was determined according to Bradford's method, with bovine serum albumin used as a standard. Equal amounts of protein were separated electrophoretically on 7.5% SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Roche, Basel, Switzerland).

Membranes were probed with a monoclonal rabbit anti-human CHD5 antibody (1:1000 dilution; Abcam). CHD5 expression levels were determined by incubating the membranes with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (1:1000 dilution) and enhanced chemiluminescence reagent (Pierce, Minneapolis, MN, USA), according to the manufacturers' suggested protocols. Membranes were then stripped and reprobed with an anti-β-actin mouse monoclonal antibody (1:1000 dilution: Sigma; St Louis, MO, USA) as a loading control. Densitometry was performed using ImageJ software (http://rsb.info.nih.gov/ij/) from National Institutes of Health (NIH; Bethesda, MD, USA). We evaluated the expression of CHD5 as an optical densitometry (OD) ratio that was scored as the densitometry of CHD5 relative to the densitometry of β-actin.

2.4. Immunohistochemistry assay

Formalin-fixed, paraffin-embedded, sectioned tissues (4 μm thick) were immunostained using the Labelled Streptavidin Biotin