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Clinical Study

Elevated E2F7 expression predicts poor prognosis in human patients with gliomas

WenWen Yin [a,1], Bo Wang [a,1], MaoHua Ding [b,1], Yan Huo [a], Hao Hu [a], RuoNan Cai [a], TingTing Zhou [a], ZaoHua Gao [a], ZaoLing Wang [a,*], DeZhi Chen [a,*]

[a] The First People's Hospital of Xuzhou, No.19, North Zhongshan Road, Quanshan District, Xuzhou, Jiangsu Province 221002, China
[b] Department of Neurosurgery, XuZhou Central Hospital, Xuzhou, China

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ABSTRACT

E2F transcription factors have been studied extensively in a broad range of organisms as major regulators of cell cycle, apoptosis, and differentiation. The E2F family includes the atypical member E2F7, which has been rarely studied in gliomas. The aim of this study is to determine the expression status of E2F7 in gliomas, its relationship to clinicopathological features, and patients' outcome. The mRNA levels of E2F7 in the human brain and different grades of gliomas were analysed using datasets from the publically available Oncomine database. One of the most significant co-expression factors, CDK1, together with E2F7, was further validated by immunohistochemistry in 90 different grades of gliomas. Furthermore, univariate and multivariate analyses were performed to identify prognostic variables relative to patient and tumour characteristics and treatment modalities. E2F7 mRNA expression was found to be elevated in gliomas by Oncomine-database analysis. Immunohistochemistry showed an increase in E2F7 labelling index in high- versus low-grade gliomas (62.1 ± 11.8% vs. 18.9 ± 10.2%, $p < 0.0001$). There was a positive correlation between E2F7 and CDK1 immunoreactivity (Spearman $r = 0.446$, $p = 0.037$). Clinicopathological evaluation suggested that E2F7 expression was associated with tumour grade ($p < 0.0001$) and recurrence ($p = 0.025$). In Cox multivariate analysis, pathological classification and recurrence were independent prognostic factors of gliomas, and E2F7 was significantly related to progression-free survival ($p = 0.011$), but not overall survival ($p = 0.062$). Our findings suggested that E2F7 might act as an independent prognostic factor of gliomas and might constitute a potential therapeutic target for this disease.

1. Introduction

Gliomas account for 45–55% of all brain tumours and are the most common tumours of the brain. They can be divided into four grades namely I, II, III and IV based on their biological behaviour and histopathological characteristics [1]. Gliomas are notorious for their highly invasive nature and associated with poor prognosis and survival rates [2]. Despite advances in treatment strategies, the prognosis has only marginally improved, which prompts for further understanding of disease onset and progression [3]. Unfortunately, the potential molecular mechanisms underlying glioma carcinogenesis remain unclear [4]. Hence, identification and characterization of the regulatory molecules involved in glioma development may provide novel therapeutic targets for treatment strategies. The mammalian E2F family of transcription factors is crucial for the regulation of cell proliferation, apoptosis, and differentiation [5,6] and can be broadly classified into two groups. The E2F1, E2F2-E2F3a are potent transcription activating factors, whereas E2F3b, E2F4–E2F8 are thought to function mainly in inhibitor complexes [7]. Apart from the physiological activities, deregulation of some E2F members in various human cancers suggests a common role in the carcinogenic process and possible application as tumour markers. With regard to gliomas, co-expression of E2F2 and P53 enhances the anticancer effect of P53 [8]. E2F1 displays multiple activities that could be involved in either suppressing or promoting tumour development depending on the nature of the other oncogenic mutations that are present [9,10]. More remarkably, the primary role of E2F1 is known as a checkpoint for both apoptosis and cell proliferation [11], and recent studies have shown that E2F7 is considered a master regulator of E2F1 activity [12,13]. The E2F7

* Corresponding authors. Tel.: +86 13852103069 (Z. Wang), tel.: +86 13814429263 (D. Chen).
E-mail addresses: dyyywzl@126.com (Z. Wang), chendezhixz@163.com (D. Chen).
[1] These authors have contributed equally to the manuscript.

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transcription factor is an important cellular regulator that behaves both as an oncogene and suppressor gene under certain circumstances [6,12,14–17]. However, the contribution of E2F7 to gliomas was rarely investigated in depth.

The objective of this study is to investigate the expression pattern of E2F7 in gliomas of different pathological grades using the Oncomine cancer microarray database and immunohistochemical techniques. The relationship between E2F7 protein expression levels in gliomas and tumour progression and overall survival was analysed statistically.

2. Materials and methods

2.1. Study subjects

Glioma tissue containing paraffin-embedded sections were obtained from Shannxi Cybrdi Biomedical Research Development Co., Ltd (Xi'an, China), which included 90 samples of glioma tissues with available survival date and clinical information. All tissues were collected with donor consent according to the ethical committee standards. The acquisition and use of human tissue in this study complied with the National Regulations of Clinical Sampling in China. All cases were re-evaluated to confirm the pathological diagnosis and graded by two independent experienced pathologists according to the World Health Organization (WHO) classification of tumour of central nervous system (CNS) updated in 2007 [18]. The mean follow-up period was 51.6 months (range: 7–136 months). The following clinical data were recorded: patient age at surgery, sex, Karnofsky performance status (KPS), and tumour size (mean tumour diameter, MTD defined as the geometric mean of the three diameters on the MRI scan) [19–21]. Extent of resection was assessed in a volumetric fashion according to a surgeon's description and postoperative MRI findings. In this study, the extent of resection was classified as gross total removal (GTR) or subtotal removal (STR). GTR referred to complete removal as described by the surgeon or the absence of the tumour on postoperative MRI. STR referred to 80–99% surgical resection [22–24]. For patients who died within 3 months of the operation, the extent of surgical resection was evaluated based on an extensive review of operation protocols. Postoperative MRI scans were available for all patients who survived longer than 3 months postoperatively. Tumour location was evaluated with contrast-enhanced CT scan or MRI findings in all patients and was divided into supratentorial and infratentorial. The diagnosis of recurrence and progression was confirmed based on imaging and clinical findings at outpatient follow-up. Secondary surgery was the main option for the treatment of recurrent patients in this study, and pathological evaluation was required to separate progression from pseudoprogression. The follow-up was conducted for a minimum of 1 year on an outpatient basis at 3-month intervals for the first 6 months, then every 6 months for the next 2 years, and annually for life thereafter. Information about the status and time of death was obtained with telephone interviews. The clinico-pathological characteristics of patients are summarized in Table 1.

Table 1
Clinicopathologic characteristics of 90 patients with glioma

Features	N (%)
Number of patients	90
Gender	
Male	42 (46.7)
Female	48 (53.3)
Age	
<40 years	38 (42.2)
⩾40 years	52 (57.8)
Tumor location	
Bifrontal	3 (3.3)
Cerebellum	5 (5.5)
Corpus callosum	1 (1.1)
Frontal	42 (46.7)
Fronto-temporal	4 (4.4)
Occipital	1 (1.1)
Parietal	6 (6.7)
Parieto-occipital	1 (1.1)
T3–T4	1 (1.1)
Temporal	21 (23.3)
Temporo-occipital	5 (5.5)
Tumor size (MTD)	
<5 cm	47 (52.2)
⩾5 cm	43 (47.8)
Extent of resection	
Gross total removal	69 (76.7)
Subtotal removal	21 (23.3)
Histology morphology classification	
Astrocytoma	23 (25.6)
Anaplastic astrocytoma	16 (17.8)
Anaplastic ependymoma	5 (5.5)
Anaplastic ganglioglioma	2 (2.2)
Anaplastic oligodendroglioma	3 (3.3)
Anaplastic oligoastrocytoma	2 (2.2)
Fibrillary astrocytoma	5 (5.5)
Glioblastoma	16 (17.8)
Oligodendroglioma	12 (13.3)
Oligoastrocytoma	6 (6.7)
Pathological grade	
WHO I	14 (15.6)
WHO II	32 (35.6)
WHO III	28 (31.1)
WHO IV	16 (17.8)
Recurrence	
Yes	68 (75.6)
No	22 (24.4)
KPS score	
<80	51 (56.7)
⩾80	39 (43.3)
Radiotherapy	
No	63 (70.0)
Yes	27 (30.0)
Chemotherapy	
No	48 (53.3)
Yes	42 (46.7)

MTD = mean tumor diameter, KPS = Karnofsky Performance Scale, WHO = World Health Organization.

2.2. Analysis of oncomine date

E2F7 expression from independent published microarray studies was extracted from the Oncomine database (http://www.oncomine.org). Briefly, the mRNA levels of E2F7 in human brain and glioma tissue were analysed. Four publically available datasets of *E2F7* gene expression were selected for the meta-analysis [25–27]. The Sun's dataset was selected, because it contained the largest number of samples for E2F7 co-expression analysis [26]. All data were log transformed and median centred. Standardized normalization techniques and statistical calculations were provided on the Oncomine website and published.

2.3. Immunohistochemistry

Immunohistochemistry analysis was performed as previously described [28]. Briefly, formalin-fixed, paraffin-embedded specimens were cut into 3-μm sections and were baked at 60°C for 10 min. The sections were deparaffinised in xylene and then rehydrated in a graded ethanol series. The sections were immersed in EDTA buffer (pH 8.0) and were microwaved for antigenic retrieval.