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ORIGINAL ARTICLE

CDC25B, Ki-67, and p53 expressions in reactive gliosis and astrocytomas

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Summary

Purpose: *To investigate the expression of CDC25B, which is a member of the cyclin-dependent kinase activating phosphatase family, in diffuse astrocytoma (DA), anaplastic astrocytoma (AA), glioblastoma multiforme (GBM), pilocytic astrocytoma (PA) and reactive gliosis (RG). Also, to study the relationship of the expression level of CDC25B with clinical parameters and with p53 and Ki-67 proliferation index (PI).*

Methods: *Tissues were collected from 36 cases diagnosed with astrocytoma (10 DA, 6 AA, 20 GBM), 10 PA, 10 RG and 10 normal brain tissues for controlling purposes. The sections were immunohistochemically stained with CDC25B, Ki-67 and p53. For each marker, 1000 tumor cells were counted and the ratio of positive tumor cells was calculated.*

Results: *The average CDC2B staining index (CSI) was 0.6% in PA, 0.4% in DA , 7.7% in AA and 25.5% in GBM. The increase of CSI in parallel with the increase of WHO grade was significant (p=0.001). No expressions were identified in RG and normal brain. There was also significant relationship between the tumor size and CSI (p=0.027) and also between Ki-67 PI and CSI (p=0.001). Among the groups with low and high CSI in astrocytoma cases, the disease free survival (DFS) was significantly higher in the low CSI group (p=0.0001).*

Conclusions: *Positive expression of CDC25B in astrocytoma affects the prognosis in an adverse manner. CSI can be used as a diagnostic method and CDC25B may be a possible target molecule for treatment.*

Key words: *astrocytoma, CDC25B, cellular proliferation, cyclin dependent kinases, Ki-67, p53*

Introduction

In eukaryotes, mitosis is set on by activation of CDC2-cyclin B complex, which is in turn activated by its phosphorolysis by CDC25. CDC25 is a member of the cyclin-dependent kinase activating phosphatase family which has three known members: CDC25A, CDC25B and CDC25C. These are believed to act on different points of the cell cycle [1-4]. CDC25A regulates the G1-S complex, while CDC25B and CDC25C are responsible for the activation of CDC2-cyclin B during mitotic division. The role of CDC25B in mitotic division is to regulate the centrosomal microtubule nucleation in the late G2 phase and to start mitosis [5-11]. Overexpression of CDC25B has been reported in certain tumors (aggressive non-Hodgkin's lymphoma, colorectal carcinoma, ovarian carcinoma, non-small cell lung carcinoma) [12-15]. There is only one publication in the literature on the diagnostic and prognostic importance of CDC25B in astrocytic tumors [16]. In this study, it was revealed that the expression of CDC25B increases in parallel with the tumor grade, and adversely affects prognosis.

The purpose of the present study was to investigate the expression of CDC25B in normal brain tissues, in astrocytomas (DA, AA, GBM, PA) and in RG (whose differential diagnosis with low grade astrocytomas is problematic). Also, to study the relationship of the expression level of CDC25B with clinical parameters and with p53 and Ki-67 PI.

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Methods

General case characteristics

For this study, cases diagnosed with astrocytoma from 2000 to 2010 were reviewed and re-graded according to the WHO 2007 brain tumors' classification. Cases with small biopsy material and those involving tumor areas with morphology other than astrocytic component (e.g. oligodendroglioma areas) were excluded from study. Cases with insufficient file information were also excluded. Finally, the study included 36 cases diagnosed with diffuse infiltrating astrocytoma (10 DA, 6 AA, 20 GBM), 10 cases with PA, 10 cases operated for reasons other than tumor, whose histopathological diagnosis matched RG, and 10 normal brain tissues (from autopsy cases who died due to diseases other than brain tumor). The clinical parameters such as age, symptoms at the time of diagnosis, gender, tumor location and DFS survival time were registered and analyzed and investigated in all cases.

Tissue preparation

Formaldehyde-fixed and paraffin-embedded tissues were sectioned. After deparaffinization and rehydration, each section was immunostained using antibodies for CDC25B (clone 25B03, 1:150), Ki-67 (clone SP6, 1:200) and p53 (clone SP5, 1:100), all from Neomarkers, CA, USA. Tonsillar palatine tissue sections, which are known for their strong nuclear staining, were used as positive control for Ki-67 and CDC25B antibodies, and colon adenocarcinoma sections, which are also known for their strong nuclear staining, were used for anti-p53 antibodies.

Evaluation of sections immunohistochemically stained

For each antibody, immunoreactivity was defined as intense or granular nuclear staining. The level of immunoreactivity in each section was assessed by counting the stained nuclei in approximately 1000 cells in the regions of maximal staining. The staining index SI was defined as the number of positive cells per total cells counted, and was expressed as percentage. For statistical analysis, the immunoreactivity of CDC25B was classified according to the CSI as follows: low expression (<20%) and high expression (≥20%). In very few cells, nuclear staining for CDC25B was accompanied by cytoplasmic staining. However, cytoplasmic staining was not taken into consideration, and only nuclear staining was evaluated. The immunoreactivity of Ki-67 and p53 were classified as low expression (<10%) and high expression (≥10%).

Statistics

Statistical analysis of the data was conducted with the SPSS software (Statistical Package for the Social Science for Windows, version 11.0, SPSS Inc, Chicago, IL, USA). The x^2 test and Student's t-test were used to analyze the association between two categorical variables. DFS was calculated by using the Kaplan-Meier method. In univariate analysis the effects of CSI, Ki-67 and p53 on DFS were assessed using the log rank test and measured from the date of surgery. In multivariate analysis, independent prognostic factors were determined by the Cox proportional hazards model. In all tests, results with a p value <0.05 were considered to be significant.

Results

Clinical parameters

For DA, AA, GBM and PA, the average age at the time of diagnosis was 34.8±3, 44.8±7, 56±2.6 and 16.1±2.9 years, respectively, and the gender distribution (male/female) 6/4, 4/2, 11/9 and 5/5, respectively. The most common symptoms with DA cases were seizures and headache, and the tumor location was in the parietal lobe in most of the cases (N=4; 40%), followed by the occipital lobe. In the AA group, the most common symptoms were seizures and imbalance, and the tumors were located in the frontotemporal and temporal lobes (N=3; 50%). In patients with GBM, headaches, hemiparesis and seizures were the 3 most common symptoms. In this group, the tumors were located mainly in the parietal, temporal and frontal lobes (N=7; 35%). In the PA patients, half of the tumors were in the cerebellum (N=3; 50%). The 3 most common symptoms in this group were headaches, visual impairment and imbalance. The average survival time was 122±15 months for DA, 31±10 months for AA, and 15±3 months for GBM.

Relationship of clinical and immunohistochemical data with CSI

The average CSI was 0.6% in the PA group, 0.4% in the DA group, 7.7% in the AA group and 25.5% in the GBM group (p=0.001) (Figures 1-4 show one sample from each group). No expression was identified in the RG and in the normal brain tissue group. CSI was low in all of DA and AA cases. Only in 2 cases of the PA group there was positive CDC25B expression (CSI 1.1% in the first case and 4.3% in the second case.) The latter of these cases was a 3-year-old boy, and the tumor was located in the brain stem. Although the tumor size was small (2 cm), the patient died on the second postoperative day due to surgical complications. Seventeen GBM cases (85%) showed high CSI, and only 3 cases had low CSI (CSI 3.2%, 16%, and 17%, respectively. In the light of these

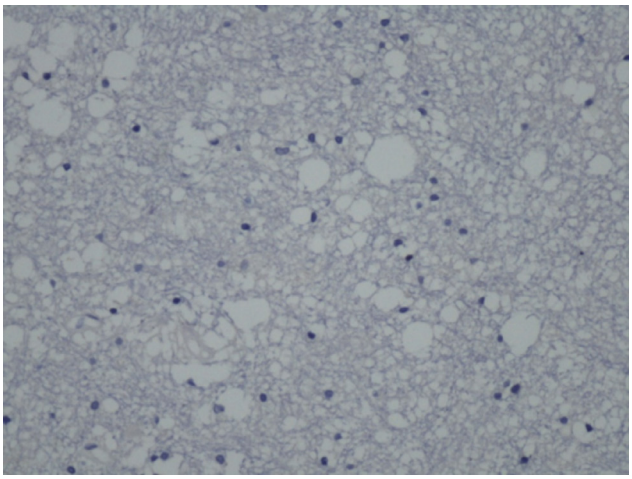

Figure 1. No CDC25B expression in this PA case (CSI 0%; CDC25B immunohistochemistry x200).

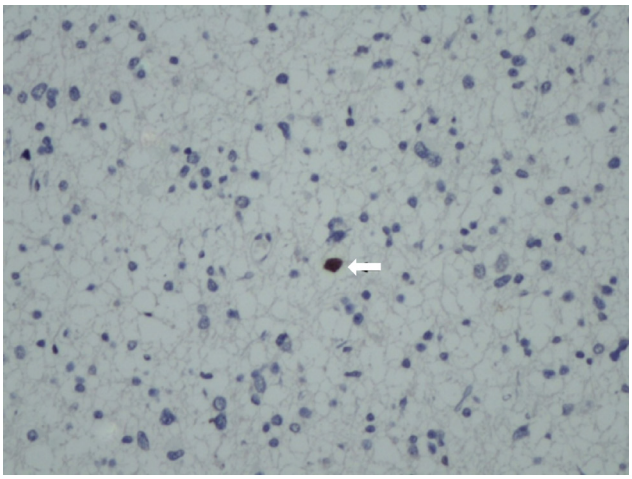

Figure 2. Only one cell (arrow) expresses CDC25B in this DA case (CSI 0.8%; CDC25B immunohistochemistry x200).

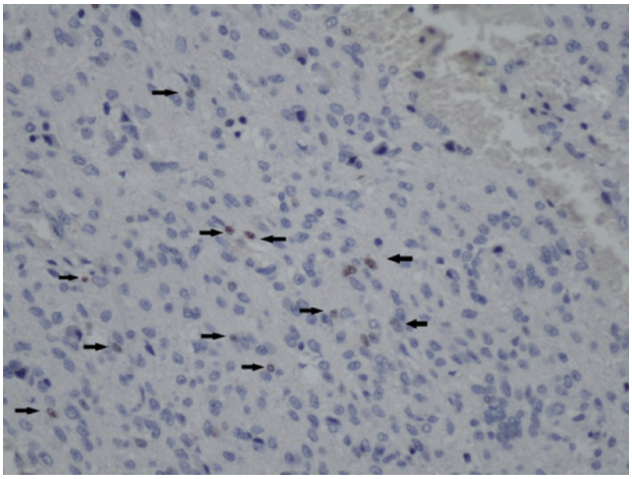

Figure 3. AA case: some cells (arrows) show CDC25B expression (CSI 7.5%; CDC25B immunohistochemistry x200).

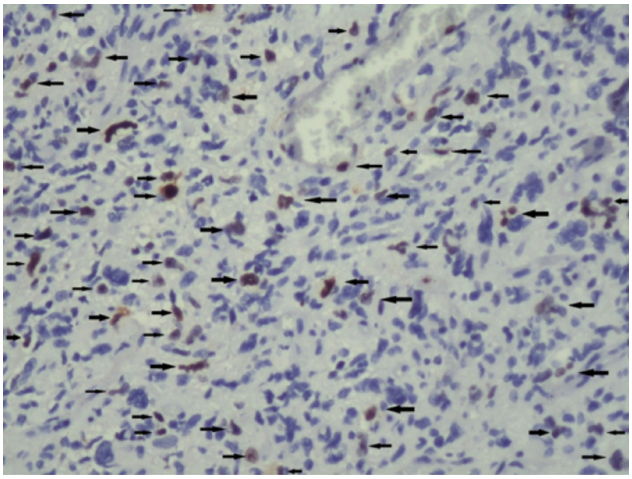

Figure 4. Many of the glioblastoma cells (arrows) stained for CDC25B (CSI 45%; CDC25B immunohistochemistry x200).

data, the increase in CSI in parallel with the increase in WHO grade was statistically significant (p=0.001). A high CSI was identified in 16 of 21 cases above the age of 45 years (76.2%), and a low CSI was identified in 24 of 25 of cases under 45 years (96%) (p=0.0001). In the GBM group, 2 of the 3 cases with low CSI were under the age of 45 (37 and 39 years old), which was remarkable. In 17 GBM cases with high CSI, 9 (52.9%) were male and 8 (47.1%) female, whereas in the low CSI group, 17 of the cases (58.6%) were males, and 12 (41.3%) females. The relationship between gender and CSI was not statistically significant (p=0.708). Nine of 14 cases (64.3%) with tumor size ≥5cm showed high CSI, and 24 of 32 cases (75%) with tumor size <5cm had low CSI. A statistically significant correlation (p=0.027) between tumor size and CSI was registered. In 15 of 19 cases (78.9%) with high Ki-67 PI CSI was also found to be high, and in 25 of 27 cases (92.6%) with low Ki-67 PI CSI was also low (p=0.001). A high CSI was identified in 6 of 14 cases (42.9%) with high p53 expression, and a low CSI was found in 11 of 32 cases (34.4%) with low p53 expression. Contrary to Ki-67, there was not significant relationship between p53 and CSI (p=0.829). In the DA group the average DFS of cases with high CSI was 15 months vs 79 months in cases with low CSI (p=0.018). These data are summarized in Table 1.

Ki-67 proliferation index

In the DA cases there was a significant increase in the Ki-67 PI with increasing grade of disease (p=0.001). The average Ki-67 PI was 3.7% in DA, 5.4% in AA, and 25.5% in GBM. No Ki-67 expression was identified in the normal brain tissue. In RG, the average Ki-67 PI was 2.9%.

Table 1. CDC25B staining index (CSI) and its relation with clinicopathological data

	CDC25B protein expression				
	Low (<20%)		*High (≥ 20%)*		
	N	*%*	*N*	*%*	*p-value*
Total	29	63	17	37	
Age (years)					0.0001
≥ 45	5	23.8	16	76.2	
<45	24	96	1	4	
Gender					0.708
Male	17	65.4	9	34.6	
Female	12	60	8	40	
WHO grade					0.001
I	10	100	0	0	
II	10	100	0	0	
III	6	100	0	0	
IV	3	15	17	85	
Ki-67 PI					0.001
≥ 10	4	21.1	15	78.9	
<10	25	92.6	2	7.4	
p53 expression SI					0.829
≥10	8	57.1	6	42.9	
<10	21	65.6	11	34.4	
Tumor diameter (cm)					0.027
≥5	5	35.7	9	64.3	
<5	24	75	8	25	

PI: proliferation index, SI: staining index

p53 expression

No p53 expression was identified in the normal brain tissue and in RG and PA cases. The average p53 SI was 13.5% in DA, 17.8% in AA, and 10.9% in GBM (p=0.136).

Discussion

There is only one publication in the literature on the diagnostic and prognostic importance of CDC25B in astrocytic tumors [16]. Nakabayashi et al. analyzed the DA cases as a patient group, and concluded that CDC25B expression increases in parallel to the grade of astrocytoma, affecting the prognosis adversely. They identified low expression of CDC25B in all of 21 DA cases, and a high expression of CDC25B in 10 of 17 AA cases, and 18 of 19 GBM cases. Similarly, we identified low expression in all DA cases while finding high CDC25B expression in most of the GBM cases (17 of 20 cases). However, the most important difference in our study was in the AA group, where all cases were identified with low CSI (average CSI=7.7%). The ratio is higher in comparison to the DA cases (average CSI=0.4%) and significantly lower in comparison to the GBM cases (average CSI=25.5%). In both studies, the percentage value determining the low and high CSI groups was common (20%). However, in the study of Nakabayashi et al., the CDC25B staining occurred in the cytoplasm. In our study clone 25B03 as CDC25B antibody was used, and only nuclear staining was accepted as positive. The staining with CDC25B antibody both in the control sections prepared from tonsillar palatine and in the tumor sections was nuclear. Cytoplasmic staining was observed in very few tumor cells at a negligible level, only to accompany nuclear staining. Cytoplasmic staining appears to be higher in neoplastic gemistocytic astrocytes. Different staining patterns in the two studies were associated with the use of different epitopes.

In addition to diffuse infiltrating astrocytomas, we studied PA, RG and normal brain tissue samples in our study. In the PA group the CSI was 0.6%. No expression was identified in the RG group and in the normal brain tissue. Distinguishing reactive gliosis from low grade astrocytoma cases is easy in the presence of original morphological findings; however, in some cases, insufficient biopsy material (stereotactic biopsies) and failure to reflect original morphological findings to the biopsy samples may lead to problems. CDC25B staining in such cases, accompanied by clinical and radiological findings, is considered as a finding in favor of astrocytoma.

We found statistically significant relationship between DA cases in terms of expression of CDC25B (p=0.001). Our study revealed high CSI in 76.2% of the cases aged 45 years and older, and low CSI in 96% of the cases under 45 years old. In DA cases, there was a strong relationship (p=0.0001) between age and CSI; however, this seems to be affected by the fact that the majority of the GBM group patients were older than 45 years. Only 3 patients were less than 45 years old in the GBM group, and 2 of them had a low CSI, which was remarkable. Similar to the other study, there was not statistically significant relationship between CSI and gender (p=0.708). We identified a statistically strong relationship between cases with tumor sizes ≥5cm and <5cm and CSI (p=0.027). As CDC25B is a protein regulating the cell cycle, it is not surprising that tumors with high expression of CDC25B are greater in size.

In the DA group, the cases with high CSI had an average survival time of 15 months, while the cases with low CSI had an average survival time of 79 months. In our study, among the groups with low and high CSI in astrocytoma cases, the DFS was significantly higher in the low CSI group (p=0.0001). It was high in 17 of 20 GBM cases

(85%) and low in only 3 cases (15%). However, in the GBM group, the difference in survival of patients with low or high CSI was incidental and was not statistically significant (r=-0.415, p=0.069). Although CDC25B is a prognostic factor in astrocytoma cases, larger series are required to validate it as a prognostic indicator in GBM.

Literature suggests an average Ki-67 PI of 1.1% in PA cases, <4% in DA cases, from 5 to 10% in AA cases, and from 15 to 20% in GBM cases [17-20]. In our study, Ki-67 PI was 1% in the PA group, 3.7% in the DA group, 5.4% in the AA group and 25.5% in the GBM group. The slightly high ratio in the GBM group can be associated with the genetic differences between populations. There was a statistically significant relationship between high Ki-67 PI and high CSI (p=0.001). In their study, Watanabe et al. suggested that tumor progression in astrocytoma is directly proportionate to the immunohistochemical p53 accumulation, which develops in relation to clonal proliferation of mutant p53 [21]. In our study, similar ratios of immunohistochemical p53 accumulation in DA, AA and GBM (13.5, 17.8, and 10.9%, respectively) are in contrast with the results of the Watanabe et al. study. Our findings suggest that our GBM cases were not histologically progressing to GBM (secondary GBM) but were de novo (primary) GBM.

It is known that p53 mutation does not play a role in the development of PA [21,22]. In our study, the lack of p53 staining in all of PA cases is in agreement with the literature. A high CSI was found in 6 of 14 cases (42.9%) with high p53 expression, while a high CSI was identified in 11 of 32 cases (34.4%) with low p53 expression. Contrary to the Ki-67 PI, no statistically significant relationship was registered between p53 expression and CSI (p=0.829).

In addition, CDC25B may be a possible target molecule in the treatment of astrocytomas. It is known that inactivation of CDCs efficiently prevents progression throughout the mitotic cell cycle. Efforts are currently under way to synthesize specific small-molecule CDC25 inhibitors that might have anticancer properties. nsc 95397, a protein tyrosine phosphatase antagonist, was reported to be a potent CDC25 inhibitor. This protein potently inhibited the growth of human hepatoma and breast cancer cells *in vitro* [23]. The combination of biochemical and genetic data on the function of these regulators will be instrumental to define new targeted therapies for inhibiting proliferation in cancer cells [24].

In conclusion, the expression of CDC25B in astrocytoma increases as the tumor grade increases, affecting the prognosis in an adverse manner. CDC25B can be used as a diagnostic method in biopsy samples where original findings of grading are absent and in small stereotactic brain biopsy materials which are diagnostically problematic. We believe that inhibition of CDC25B may act therapeutically in cases of astrocytomas.

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