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RESEARCH ARTICLE

Expression of c-Myc and BMI-1 in Iraqi glioma patients

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Abstract

Background: Gliomas are the most common adult brain tumor and are associated with high fatality rates. Despite substantial efforts, the etiology of this cancer remains poorly understood Gliomas are primary brain tumor accounting for about 80% of these tumors. Many environmental and inherited factors can have a role in the progression of these tumors.

Aim: This study aimed to investigate the association of c-Myc and BMI-1 expression with incidence of glioma in Iraqi patients.

Materials and Methods: Fifty paraffin-embedded tissues from glioma patients were used for immunohistochemistry study to evaluate the expression of c-Myc and BMI-1 proteins in these tissues. Monoclonal antibodies for these proteins were used, and the immunoreactive scores were calculated according to staining intensity and the percentage of tumor cells with nuclear staining.

Results: Among the examined fifty samples, there were 36 cases of glioblastoma multiforme (GBM), and 14 cases of anaplastic astrocytoma. For GBM, 77.22% and 69.44% of samples were positive for c-Myc and BMI-1 proteins respectively, while 71.43% and 85.7% of anaplastic astrocytoma samples were positive for the two proteins respectively. There was a significance correlation in the expression of the two proteins.

Conclusion: c-Myc and BMI-1 are highly expressed in glioma tumors and may be involved in the tumerigenesis of this malignancy.

Introduction

Gliomas are primary brain tumors that originate from the supportive cells of the brain, called glial cells, which are the most common cellular component of the brain. Gliomas are the most common tumors of the brain in adults accounting for almost 80% of primary malignant brain tumors and are responsible for approximately 15,000 cancer death in United States each year (Kyritsis *et al.,* 2010). The most common type of gliomas (glioblastoma multiforme) is associated with median survival of only 12-15 months (Prasad and Hass-Kogan, 2009). Causes of this tumor are still not clearly defined, however many environmental factors such as radiation (Melissa *et al*., 2012) and inherited factors (Sima *et al.,* 2012) are well documented. In Iraq, brain tumors are the fifth most common tumor in adults and the second most common in children. They represented about 4.77% among males and 3.3% among females (Iraqi Cancer Registry, 2005).

c-Myc protein is a transcription factor that normally has many functions among which its ability to recruit P-TEFb (the positive transcription elongation factor-β) protein and relieve RNA polymerase pausing and it, thereby, activates transcription (Rahl *et al.,* 2010). The fact that c-Myc is required for the expression of so many genes

suggests that it is the master regulator of cell growth (Conacci-Sorrell *et al*., 2014). However, deregulation of c-Myc production could have devastating sequellae since such disruption has been found in diverse human tumors, and is often associated with advance malignancy and poor prognosis (Vita and Henriksson, 2006). Over-expression of c-myc has been studied in various tumors. In lymphoma, Ott *et al*. (2013) reported that increased the expression of this gene alone leads to cell transformation, while Jamerson *et al*. (2004) showed that dysregulation of c-myc leads to more aggressive breast cancer, but is insufficient to cause tumorigenensis alone.

BMI1 is a transcriptional repressor which belongs to the polycmb group of transcription factors (Liu *et al*., 2005). This protein has a central role in epigenetic silencing of the genes controlling differentiation, self-renewal and proliferation (Valk-Lingbeek *et al*., 2004). It was originally identified due to its ability to fasten c-myc- induced lymphomagenesis (Jacobs *et al*., 1999). Subsequently, it was found to have an Association with various tumors such as breast cancer (Choi *et al*., 2009), Hodgkine lymphoma (Bea *et al*., 2001) and GB (Cenci *et al*., 2012).

The presence study aimed to evaluate the association of c-Myc and BMI-1 expression in brain tumor cells with the incidence and grades of glioma in Iraqi patients.

Material and Methods

Patient Characteristics

Patient attending hospitals to undergo surgical resection of brain tumors from January 2012 to January 2013 were eligible for this study. Five hospitals in Iraq were included: Neurosurgery Hospital, Neuroscience Hospital, AL-Kadimiya Teaching Hospital, AL- Fallujah Hospital, and Private Nursing Home Hospital. Ethical approve to conduct the research was sought and obtained from these hospitals. Selection of patients was accomplished with the assistance of surgeons in the hospitals. Fifty paraffin-embedded brain tissue related to the patients were enrolled in this study (mean age between patient group 31.85±1.95 years, ranged 1-72 years). All were confirmed as brain tumor glioma by the histopathological examination. Hematoxylin and eosin slides were prepared for the paraffin embedded blocks and were examined by a histopathologist for histological typing, staging and grading of the tumor. Among the 50 specimens, 36 were GBMs and 14 were anplastic astrocytoma. Section for resection margin was used as malignant-free-controls for the immunohistochemistry study.

Immunohistochemistry

Fifty brain tumor paraffin-embedded blocks from glioma patients were used in this study. Adequate section (6μm thick) from each block was prepared on positively charged slides for detection of c-Myc and BMI1 proteins using immunohistochemistry technique. As previously described (Cenci *et al*., 2012). The sections were deparaffinized by heating slides in a vertical position in oven for 1 hour followed by washing in xylenes (20 min) and serial dilutions (100%, 95%, 75%, and 50%) with ethanol (each steps 10 min), The sections were blocked for endogenous peroxidase (3% H2O2 for 20 min BioGenex). Antigen retrieval was performed using Citra Plus antigen retrieval solution (BioGenex) for 2 min at 97 °C in microwaves. Protein blocks (Chemicon, Temecula, CA, USA) were applied to the sections prior to application of a primary antibody. Slides were incubated with monoclonal antibody against c-Myc and BMI1 (1:100 dilution, Chemicon, Millipore, USA for each protein) for 1 hour at room temperature. And negative controls of tumor sections stained in the absence of primary antibody were also included. All slides were incubated overnight at 4°C. The slides were then stained with a secondary antibody (1:17.5; BioGenex), peroxidase-labeled streptavidin (BioGenex), and 3, 3′-diaminobenzidine (DAB) (Innovex Biosciences) was used as a chromogen. Hematoxyline stain was used as a counterstain. All samples were stained more than once and the result was highly reproducible.

Evaluation of Immunostaining

Immunostaining tissue slides were evaluated independently by two histopathologists, who were blinded to the patients' characteristic and clinical signs. Cases with disagreement were discussed using multi-headed microscope until agreement was achieved. The expression of c-Myc and BMI1 was measured by counting the number of tumor cells with brown staining under light microscope (400x). Percentages of stained tumor cells were obtained for each section and scoring system that included an evaluation of both the percentage of tumor cells and staining intensity was employed (Brabletz *et al*., 2000). Staining intensity was graded as no staining (value 0, as in normal brain epithelial), weak staining (value1), moderate staining (value 2) or strong staining (value 3). The percentages of tumor cells with nuclear staining were assigned scores as follows: 1, <5%; 2, 5-20%; 3, 21-50% and 4, > 50%. Multiplication of the intensity value and the percentage value gave multiplication values from 0 to 12. The multiplication values were grouped in into four immunoreactive scores defined as 0 (negative; multiplication values 0, 1), 1(low; multiplication values 2, 3), 2 (medium; multiplication values 4, 6), or 3 (high, multiplication values 8, 9, 12).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, version 14) was used for statistical analysis**.** Chi- square was used for comparing the immunostaining. Correlation test was used to calculate r values among HCMV proteins and c-Myc expression. A *p*-value 0.05was considered statistically significant.

Results

The 50 paraffin-embedded brain sections were catagerorized by a pathologist into 36 glioblastoma multiform (GBMs) and 14 anaplastic astrocytoma. All the restriction margins of the sections gave negative staining results for both c-Myc and BMI-1 proteins (figure 1).

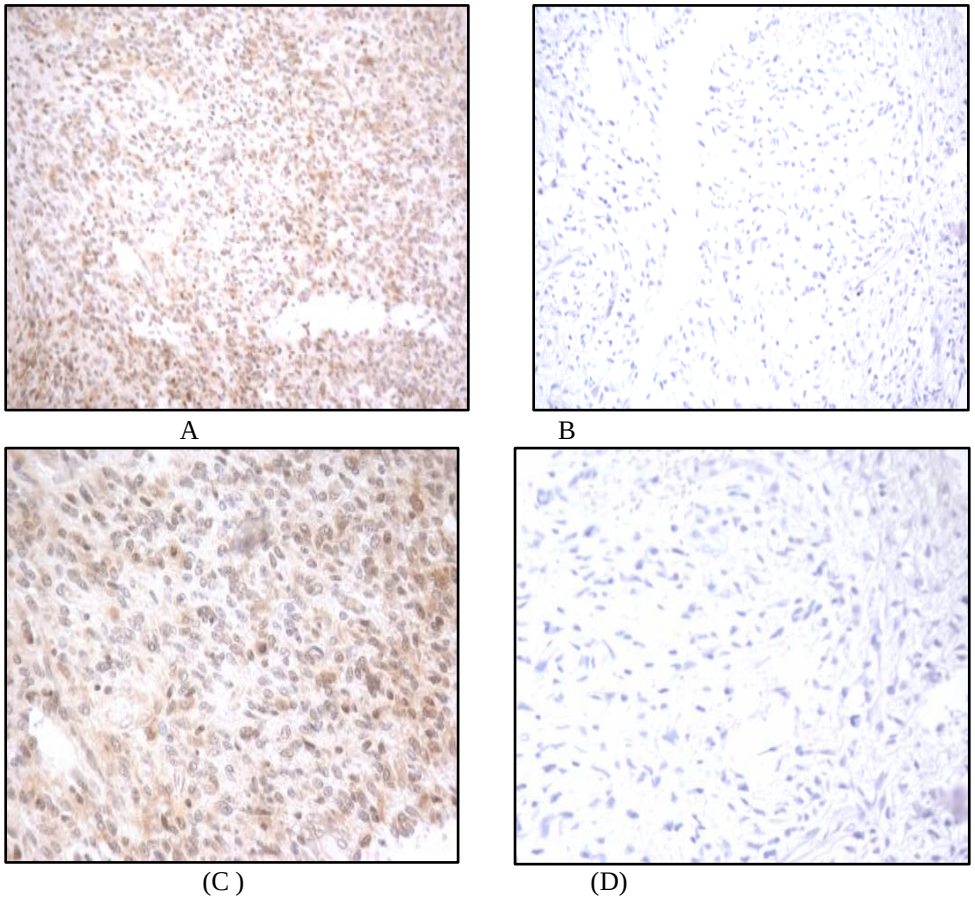

Figure 1: Immunohistochemical staining of C-Myc and BM11 in malignant Glioma (A) Immunohistochemical Stain of Glioma section with C-Myc (B) Immunohistochemical stain of glioma section without antibody (control) (C) Immunohistochemical stain of Glioma section with BM1-1 protein (D) Without antibody (control**)(Magnification power X400)**

Out of 36 GBM samples, 26 (77.22%) and 25 (69.44%) were positive for c-Myc and BMI-1 respectively with no significant difference (table 1). Immunoscores showed close results regarding the expression of these two proteins except for high score where c-Myc had 19.23% compared to 8% for BMI-1 protein with significant difference ($p<0.05$).

For 14 samples of anaplastic astrocytoma, 10 (71.43%) and 12 (85.71%) were positive for c-Myc and BMI-1 proteins respectively with no significant difference. Again, immunoscore revealed a great similarity in the expression of the two proteins at different immunoreactive scores (table 2). Correlation test revealed significant association in the expressions of the two proteins both in GBM ($r=0.772$, $p=0.034$) and anaplastic astrocytoma ($r=0.62$, $p=0.042$).

Table 1: The percentage and immunoreactive score of c-Myc and BMI1 proteins in gliblastoma multiforme.

Proteins No=36	c-Myc No (%)	Immunoreactive score		
		Low No (%)	Medium No (%)	High No (%)
c-Myc	26 (77.22)	11 (42.3)	10 (38.46)	5 (19.23)
BMI-1	25(69.44)	13(52)	10 (40)	2 (8)

Table 2: The percentage and immunoreactive score of c-Myc and BMI1 proteins in anaplastic astrocytoma.

Proteins No=14	c-Myc No (%)	Immunoreactive score		
		Low No (%)	Medium No (%)	High No (%)
c-Myc	10(71.43)	4 (40)	4 (40)	2 (20)
BMI-1	12 (85.71)	5 (41.67)	4 (33.33)	3 (25)

Discussion

Results of the immunohistochemical study revealed that c-Myc was expressed in 77.22% of GMB and 71.42% in anaplastic astrocytoma. These results are in part consistent with other previous studies that showed higher expression of c-Myc in GBM cases (Orian *et al*., 1992; Faria *et al*., 2006; Cenci *et al*., 2012). Howevere, the highest expression recoded in these studies was 75%. Among brain tumors, Herms *et al.* (1999) investigated the c-Myc expression in 46 GBs adult patients using *in situ* hybridization. C-myc protein was found to be expressed in 78% of tumor cells. In addition, increased expression of *c-myc* has been related to more aggressive medulloblastoma associated with shorter survival and greater therapy resistance (Faria *et al*., 2010; Zitterbart *et al.*, 2011). In the same context, Cenci *et al.* (2012) demonstrated a causative link between increase c-Myc and the occurrence of anaplasia in medulloblastoma which indicates the role of this protein in malignancy.

c-Myc oncogene is one of the most important and frequently regulated gene in different human tumors (Meyer and Penn, 2008). Elevated expression occurs through multiple mechanisms in tumor cells, including: gene amplification, chromosomal translocation, SNPs in regulatory regions, mutation in the upstream signaling pathways and mutations that enhance the stability of the proteins (Pomerantz *et al*., 2009; Wright *et al*., 2010). Since the usual function of this protein is its role as transcription factor, presence of such protein in tumor cells but not in normal cell is regarded as a strong evidence for its association with the tumor. It is reasonable to say that there may be an over expression of certain locus in the genome enhanced by this protein. Determination of such locus is a logical aim but it is very hard to achieve. However, one of the suspected locus is the *bmi-1*.This gene is located on chromosome 10p11.23 (NC_000010.10), extends over 10kb and comprises 10 exons.

The high percent of tumor cells expressing BMI-1(BMI-1 was expressed in 69.44% of GMB and 85.7% in anaplastic astrocytoma) is an expected result. That is because the over expression of this protein was documented in many tumors and frequently associated with poor prognosis (Wang *et al*., 2008; Vrzalikiva *et al*., 2008). There are several hypothesized mechanisms by which this protein can influence tumor progression, the most of which is affecting the central tumor suppressor genes (Rb and p53) by suppressing the $p16^{Ink4a}$/$p19^{Arf}$ locus (Molofsky *et al*., 2005).

The correlation between the two proteins appeared significance. Thus on locus of c-Myc protein may be the *bmi-1* gene. This result was previously reported by Cenci *et al*. (2012) who found a significant correlation in the expression of c-Myc and BMI-1. This finding suggest that BMI-1 expression in glioma patients is concurrent with c-Myc activation, in line with notion that c-Myc is able to directly induce upregulation of BMI-1 (Guo *et al*., 2007), which is in turn collaborates with c-Myc oncogene in mediating key properties of GB cells (Abdouh *et al*., 2009). Recently, Wang *et al*. (2013) demonstrated that this upregulation is through the binding of c-Myc to the E-box element (-181) within the promoter region of *bmi-1*gene.

The current study added an evidence for the central role of both c-Myc and BMI-1 protein in the occurrence of glioma.

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