Current Research in Microbiology and Biotechnology
Vol. 5, No. 2 (2017): 1057-1061
Research Article
Open Access

ISSN: 2320-2246

Immunohistochemical expression of Foxp3, T-bet, GATA-3, MAK387 and Bcl-2 in brain tumors (meningioma and glioma) of Iraqi patients

Ali H. Ad'hiah[1]*, Salwa G. Turki[2] and Zaman A. Abed[2]

[1] *Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq.*
[2] *Medical Basic Science Department, College of Nursing, University of Baghdad, Baghdad, Iraq.*

* Corresponding author: Ali H. Ad'hiah; e-mail: dr.a.h.adhiah@gmail.com

Received: 19 March 2017 Accepted: 03 April 2017 Online: 12 April 2017

ABSTRACT

The immunohistochemical expression of some immunological markers in brain tumors (meningioma and glioma) of Iraqi patients was investigated. Forty-two brain tumor tissue samples; meningioma (20 cases) and glioma (22 cases), in addition to 15 control samples were immunohistochemically assessed for Foxp3, T-bet, GATA-3, MAK387, and Bcl-2 expression. Positive expression of Foxp3 was observed in 37.5% of meningioma patients, while it was lower in glioma patients (33.3%). For T-bet, most of meningioma and glioma patients (85.7 and 75.1%, respectively) showed a positive expression, which was mainly observed with the score 1+ (64.3 and 56.3%, respectively). GATA-3 showed a positive expression in 31.3% of meningioma patients, while glioma patients were presented with a lower frequency (23.5%) of positive expression. For MAK387, both meningioma and glioma patients showed an approximated frequency of positive expression (80.0 and 83.3%, respectively). The frequency in meningioma patients was confined to score 1+ (80.0%), while in glioma patients, the frequency was distributed between the three scores, with the highest expression was in score 1+ (49.9%). Finally, both meningioma and glioma patients showed an approximated frequency of Bcl-2-positive expression at scores 1+ and 2+ (30.0 and 33.3%, respectively). However, in glioma patients, 16.7% of cases were observed to have the score +3, while none of meningioma cases had such score. The investigated markers may have a role in brain tumorigenesis, especially T-bet, MAK387 and Bcl-2, which showed an increased percentage of immunohistochemical expression in meningioma and glioma patients.

Keywords: Immunohistochemical markers, Meningioma, Glioma.

1. INTRODUCTION

Meningiomas and gliomas are common brain tumors of adults accounting for 36 and 24%, respectively of primary brain tumors in the United State of America as estimated by the Central Brain Tumor Registry of the United States in 2015 [1]. Their etiology is multifactorial, but it has been established by worldwide cancer analysis that cancer patient survival is well-correlated with a cellular response of the adaptive immune system [2]. In this regard, three broad observations that linked the immune system and the development of brain tumors (BTs) have been highlighted. First, immunocompromised individuals are more prone to develop BTs. Second, an opposite observation depicted that a decreased risk of BT development is observed in patients with a history of autoimmunity. Third, a good prognosis is noticed in patients with malignant BTs who developed post-surgical local infection [3].

The key immune cells for tumor surveillance are T cells, in which T regulatory (Treg) cells exert a critical effect, and an excess of Treg cell activity has been suggested to prevent the immune system from destroying cancer cells and enable tumor cells to escape anti-tumor immunity and facilitate tumorigenesis [4]. These cells are marked by their expression of Foxp3 (forkhead box

protein 3), which is a member of the fox protein family, and it functions as a master regulator (transcription factor) in the development and function of Treg cells [5]. In addition, studies have demonstrated that their ample presence in infiltrates of tumor may risk cancer patients to a reduced rate of survival [6]. Further studies have also depicted the expression of Foxp3 in multiple normal tissues (breast, prostate and ovarian epithelium), while it is down-regulated in the corresponding tumor tissues [7]. Conversely, pancreatic adenocarcinoma, melanoma, hepatocellular carcinoma, bladder cancer, thyroid carcinoma and cervical cancer showed an increased expression of Foxp3, with no expression in the corresponding normal tissues [8]. These findings suggest either a pro- or anti-tumorigenic role of this marker.

T-bet (T-box protein expressed in T cells) was firstly described in 2000 in a report examining the effects of T-bet on the differentiation of T helper (Th)1 cells, which direct type 1 immune responses and IFN-γ production *in vivo*. In addition to IFN-γ regulation, T-bet significantly suppresses the expression of IL-2, and at the same time modulates IL-2 and Th2 cytokines in an IFN-γ-independent manner; resulting in Th2 cell development attenuation [9]. T-bet also plays a role in inhibiting cancer metastasis by regulating the function of natural killer (NK) cells, and T-bet expression by NK cells has been suggested to be essential to protect against tumor metastasis and important for NK-mediated cross-talk between innate and adaptive immune systems in response to metastatic disease [10].

GATA-3 is one of the GATA transcription family. It exerts a prominent role in promoting and directing proliferation, development and differentiation of many types of cells and tissues. Additionally, GATA-3 is a master regulator for Th2 differentiation and development, highly expressed in Th2 CD4+ T cell, and plays a significant role in early development of thymocytes [11]. GATA-3 has also been shown to promote the secretion of some cytokines (IL-4, IL-5 and IL-13) from Th2 cells, and it is also involved in inducing Th0 differentiation towards Th2 and suppressing the differentiation towards Th1 cells [12]. Furthermore, it has been shown that mutations, loss/over expression or alteration of GATA gene can contribute to the development of cancer in human [13].

MAK387 is a marker of macrophages, which can be differentiated into either pro-inflammatory M1 macrophages or anti-inflammatory M2 macrophages. The former expresses a series of pro-inflammatory cytokines, and effector molecules, such as IL-12, IL-23, TNF-α, IFN-γ and MHC class I and II molecules. In contrast, M2 macrophages express a number of anti-inflammatory molecules, such as IL-10 and TGF-β, and in most tumors, such cells infiltrate the tumor in order to provide a microenvironment of tumor growth immunosuppression [14]. Tumor associated macrophage (TAM) has been associated with poor survival rate in some human cancers, and poor prognosis in advance stages of cancer, such as breast, lung, thyroid, and bladder cancers [15]. In contrast, TAMs have been associated with a high survival rate in colorectal, stomach, lung and endometrial cancers [16]. Collectively, these results suggest that TAMs can have either positive or negative effects on tumor growth depending on the specific tissue type, tumor location, and tumor stage.

Bcl-2 (B-cell lymphoma 2) is a protein of Bcl-2 family that regulates apoptosis, by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) it [17]. It is located in the endoplasmic reticulum, nuclear membranes and mitochondria, and regulates the pathway of apoptosis by controlling the permeabilization of the outer mitochondrial membrane leading to the release of cytochrome C into the cytoplasm, formation of the apoptosome and an increase in effector caspase activities; thereby resulting in apoptosis [18]. Bcl-2 is specifically regarded as a landmarked anti-apoptotic protein and it is therefore grouped under oncoproteins [19].

Based on the aforementioned significance of the presented markers, the present study was designed to examine the immunohistochemical expression of Foxp3, T-bet, GATA-3, MAK387 and Bcl-2 in BTs (meningioma and glioma) of Iraqi patients.

2. MATERIALS AND METHODS

2.1 Subjects

The study was approved by the ethics committee of Iraqi Ministry of Health. Forty-two BT tissue samples of patients were investigated. They were admitted to the Specialized Surgical Hospital and Neurological Disorder Hospital in Baghdad for surgical operation to respect their BT. Based on a clinical evaluation by the consultant surgeons at the hospital and a histopathological examination, the patients were distributed into two clinical groups; meningioma (20 cases) and glioma (22 cases). Control brain tissues included 15 cadavers (less than 20 hours postmortem) from Forensic Medical Institution in Baghdad. Age and gender distribution of investigated cases are given in table 1.

Table 1: Age and gender distribution of brain tumor patients and controls.

Groups		Gender	Number	Age Mean ± S.E. (Years)
Patients (No.= 42)	Meningioma (No.= 20)	Males	6	57.1 ± 2.3
		Females	14	44.1 ± 2.9
	Glioma (No.= 22)	Males	14	40.6 ± 3.8
		Females	8	35.3 ± 5.1
Controls (No.= 15)		Males	15	29.1 ± 3.9

2.2 Methods

The tissues samples were embedded in paraffin for immunohistochemical examination. The paraffinized embedded sections of BTs or normal tissues were cut into 5µm thickness. The sections were applied on Fisher-brand positively charged slides. Immunohistochemical staining was performed by the envision method. The expression of Foxp3, T-bet, GATA-3, MAK387 and Bcl-2 in tissue samples was examined, as previously described (20). The primary antibody was mouse monoclonal anti-human and the secondary antibody was Novolink polymer detection system (Leica, United Kingdom). The slide was examined by a light microscope (40X), and after comparing the tested sections with negative and positive control slides, the score of reaction (0, 1+, 2+ and 3+) was assigned according to the laboratory protocol of King's College Hospital (London). In score 1+, the positive cells (stained) represented 10 - 29% of total cells. Score 2+ involved 30 - 50% positive cells, while more than 50% positive cells were grouped under score 3+. Score 0 was considered for negative sections.

2.3 Statistical analysis

The expression of each marker was given as a percentage frequency, and no statistical was carried out because the expression in control samples was not detected.

3. RESULTS AND DISCUSSION

The tissue expression of Foxp3, T-bet, GATA- 3, MAK387 and Bcl-2 were evaluated in brain tumor patients (meningioma and glioma) and controls (normal brain). It is worth to mention that the 15 brain tissue samples of controls (cadavers) showed a negative immunohistochemical expression of the investigated markers; therefore their results were not given.

Positive expression of Foxp3 was observed in 37.5% of meningioma patients, while it was lower in glioma patients (33.3%). In terms of scores, glioma patients with score 1+ represented the highest frequency (26.7%), and a similar observation was made in meningioma for scores 1+ and 2+, but the frequency was lower (18.8 and 18.7% respectively). For T-bet, most of meningioma and glioma patients (85.7 and 75.1%, respectively) showed a positive expression, which was mainly observed with the score 1+ (64.3 and 56.3%, respectively). GATA-3 showed a positive expression in 31.2% of meningioma patients, while glioma patients were presented with a lower frequency (23.5%) of a positive expression, and most of the expression had 1+ score (25.0 and 17.6%, respectively). In the case of MAK387, both meningioma and glioma patients showed an approximated frequency of positive expression (80.0 and 83.3%, respectively). However, the frequency in meningioma patients was confined to score 1+ (80.0%), while in glioma patients, the frequency was distributed between the three scores, with the highest expression was in score 3+ (49.9%). Finally, both meningioma and glioma patients showed an approximated frequency of Bcl-2-positive expressions at scores 1+ and 2+ (30.0 and 33.3%, respectively). However, in glioma patients, 16.7% of cases were observed to have the score 3+, while none of meningioma cases had such score (Table 2).

Table 2: Percentage frequency of Foxp3, T-bet, GATA-3, MAK387 and Bcl-2 immunohistochemical tissue expression in brain tumor patients (meningioma and glioma).

Marker	Percentage Frequency of Immunohistochemical Expression									
	Meningioma (No. = 20)					Glioma (No. = 22)				
	Positive				Negative	Positive				Negative
	Total	Score				Total	Score			
		1+	2+	3+			1+	2+	3+	
Foxp3	37.5	18.8	18.7	0.0	62.5	33.3	26.7	6.6	0.0	66.7
T-bet	85.7	64.3	14.3	7.1	14.3	75.1	56.3	6.3	12.5	24.9
GATA-3	31.2	25.0	6.2	0.0	68.8	23.5	17.6	5.9	0.0	76.5
MAK387	80.0	80.0	0.0	0.0	20.0	83.3	16.7	16.7	49.9	16.7
Bcl-2	60.0	30.0	30.0	0.0	40.0	83.3	33.3	33.3	16.7	16.7

The present study suggests that T lymphocyte play a key role in anti-tumor immunity, and the two transcription factors (Foxp3 and GATA-3), and the other three immunological markers (T-bet, MAK-387 and Bcl-2) may have a role in brain tumor progression and development. However, Foxp3 and GATA-3 showed a low expression in both tumor types, and approximately 30% of the patients were observed to have a positive expression. Similar findings have also been presented and a low expression of Foxp3 in low grade glioma and oligodendroglioma was observed [21]. However, no Foxp3 accumulation in benign meningioma and pituitary adenomas has been reported [22]. In contrast, high level of Foxp3 expression was observed in different grades of glioma. Therefore, it was suggested that the elevation of Foxp3 parallels tumor grades [23]. Further findings suggest that the abundance of Foxp3 cells in tumor site is expected to be associated with unfavorable clinical prognosis, and many studies reported that increased Treg infiltration in tumor site predicted reduced survival in cancer bearing patients [24]. In this regard, Foxp3 has been described to facilitate tumorigenesis by enabling tumor cells to escape anti-tumor immunity and inhibits T cell proliferation [5].

The second transcription factor (GATA-3) of present study might be the first presentation in BT, but such

marker has been emerged as a sensitive and relatively specific immunomarker for other cancers; for instance, breast and urothelial carcinoma [25]. However, GATA-3 assessment revealed low expression in both tumor types, and in agreement with such finding, a down-regulation of GATA-3 expression was also observed in cervical cancer during carcinogenesis; whereas, pancreatic cancer cell lines and primary pancreatic cell cultures showed high expression of GATA-3 [26]. GATA-3 was also significantly correlated with the expression of TGF-β in pancreatic cancer cell lines, and a down-regulation of GATA-3 mRNA upon TGF-β exposure was noticed, whereas in TGF-β-unresponsive cell lines persisted at high levels; an observation that suggests that GATA-3 plays a central role in human pancreatic cancer. In contrast, GATA-3 showed negative correlation with TGF-β expression and caused down-regulation of GATA-3 mRNA upon TGF-β exposure [27]. The high expression of TGF-β may negatively affect the expression of GATA-3, which is the master regulator for Th2 differentiation and development and involved in initiating and maintaining anti-cancer immune responses by regulating NK cell generation and function, Treg cell function, type-2 innate lymphoid cells generation, as well as tumorigenesis [28].

In addition to GATA- 3, T-bet was also examined for the first time in BTs, and its expression was distributed for both tumor types at all scores and the highest score was 1+. It is an important finding, especially if we consider that around 70% of patients were positive for such marker, but no further evidence that supports such finding; however, there is an evidence based on a clinical evaluation suggests that increased survival rate in cancer patients is positively correlated with an increase in the expression of type 1 effector T cell genes, especially those of the T-box master transcription factor regulator; T-bet [33,34]. In addition, T-bet-deficient mice were reported to have a higher tumor load compared to wild-type mice [35].

Macrophage is one of immune cells that contribute to tumor growth and progression. MAK387 is expressed in macrophages, squamous, transitional epithelia and some cancers types, and recruited in central nervous system inflammation [14]. To our knowledge, MAK387 has not been evaluated as a macrophage marker in BT, and it was highly expressed in meningioma and glioma. The elevation of MAK387 positive expression was also detected in other type of cancers. In urothelial bladder cancer, an over-expression of MAK387 was associated with poor prognostic survival and a greater risk of death [29,30]. An association between a high MAK387+ macrophage count and poor outcome has also been reported in breast cancer, especially high tumor grade [31].

Finally, given the significant role of Bcl-2 in controlling cellular immortality that can lead to tumor formation by blocking apoptosis, we examined the expression of this protein in both types of BT. In glioma, the study revealed a high percentage of positive cells (83.3%) for Bcl-2. Elevated expression of bcl-2 has also been demonstrated to have a significant relationship with a predictive survival in glioma patients, but not with tumor grade [32]. Bcl-2 overexpression has been shown to result in a retardation of the apoptotic process via prevention of cytochrome C release and caspase activation, and ceramide formation (waxy lipid molecules in cell membrane) [33]. In addition, an overexpression of anti-apoptotic members has been more often found in low-grade than in high-grade gliomas and repeatedly associated with a more favorable prognosis [32]. A further study reported that high-grade glioma showed a high expression of bcl-2, and the authors proposed that Bcl-2 functions in the malignant phenotype of glioma cells to enhance migration and invasion by altering the expression of a set of metalloproteinases and their inhibitors of human glioma cells [33]. However, few studies have investigated the role of Bcl-2 expression in determining the biologic behavior of meningiomas. In present study, meningioma tumors showed lower percentage of positive cells (60%) than in glioma tumors (83.3%), but similar findings were reported by others. It was found that Bcl-2 is more common in atypical and malignant meningiomas than in low-grade tumors. In contrast, others reported no association between Bcl-2 expression and tumor grade or recurrence, and the high expression of Bcl-2 in benign meningioma, has been reported to have predictable value of tumor recurrence [34,35]. In addition and from the point view of genetics, a study in Chinese Han population showed that *BCL2*$_{-938}$ single nucleotide polymorphism is significantly associated with susceptibility to glioma, and the authors suggested that this polymorphism might be used as molecular markers for evaluating the risk of glioma [36].

4. CONCLUSION

The significance of the investigated immunological markers was presented in meningioma and glioma, and they are possibly involved in brain tumorigenesis, especially, T-bet, MAK387 and Bcl-2, but further investigations are certainly required.

ACKNOWLEDGMENTS

The authors express their gratitude for the consultant surgeons and their assistant staff at the Specialized Surgical Hospital and Neurological Disorder Hospital in Baghdad for their cooperation in obtaining the tumor biopsies.

5. REFERENCES

1. McNeill KA. Epidemiology of Brain Tumors. Neurol Clin 2016; 34:981–98.
2. Galon J, Costes A, Sanchez-cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313:1960–4.
3. Walker DG. Immunology of brain tumors and implications for immunotherapy. 3rd ed. Edinburgh: AH Kaye and ER Laws, 2012.
4. Igney FH, Krammer PH. Immune escape of tumors: apoptosis resistance and tumor counterattack. J Leukoc Biol 2002; 71:907–20.
5. Martin F, Ladoire S, Mignot G, et al. Human FOXP3 and cancer. Oncogene 2010; 29:4121–9.

6. Takenaka M, Seki N, Toh U, et al. FOXP3 expression in tumor cells and tumor-infiltrating lymphocytes is associated with breast cancer prognosis. Mol Clin Oncol 2013;1:625–32.
7. Wang L, Liu R, Li W, et al. Somatic single hits inactivate the X-linked tumor suppressor FOXP3 in the prostate. Cancer Cell 2009; 16:336–46.
8. Tan B, Anaka M, Deb S, et al. FOXP3 over-expression inhibits melanoma tumorigenesis via effects on proliferation and apoptosis. Oncotarget 2014; 5:264–76.
9. Oh S, Hwang ES. The role of protein modifications of T-bet in cytokine production and differentiation of T helper cells. J Immunol Res 2014;2014:589672.
10. Moretta L, Ferlazzo G, Bottino C, et al. Effector and regulatory events during natural killer-dendritic cell interactions. Immunol Rev 2006; 214:219-28.
11. Pai SY, Truitt ML, Ting CN, et al. Critical roles for transcription factor GATA-3 in thymocyte development. Immunity 2003;19:863-75.
12. Yagi R, Zhu J, Paul WE. An updated view on transcription factor GATA3-mediated regulation of Th1 and Th2 cell differentiation. Int Immunol 2011; 23:415-20.
13. Zheng R, Blobel GA. GATA Transcription Factors and Cancer. Cancer 2010; 1:1178–88.
14. Hao NB, Lü MH, Fan YH, et al. Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol 2012; 2012:948098.
15. Jung KY, Cho SW, Kim YA, et al. Cancers with higher density of tumor-associated macrophages were associated with poor survival rates. J Pathol Transl Med 2015; 49:318–24.
16. Forssell J, Öberg Å, Henriksson ML, et al. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. Clin Cancer Res 2007; 13:1472–9.
17. Chipuk JE, Moldoveanu T, Llambi F, et al. The BCL2 family reunion. Mol Cell. 2010; 37:299-310.
18. Brunelle JK, Letai A. Control of mitochondrial apoptosis by the Bcl-2 family. J Cell Sci 2009; 122:437–41.
19. Hardwick JM, Soane L. Multiple functions of BCL-2 family proteins. Cold Spring Harb Perspect Biol 2013; 5: pii: a008722.
20. Taylor, C.R., Rudbeck L, editor. Immunohistochemical staining methods. 6th Ed. Dako Denmark A/S, 2013.
21. Heimberger AB, Abou-Ghazal M, Reina-Ortiz C, et al. Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. Clin Cancer Res. 2008;14:5166–72.
22. Jacobs JFM, Idema AJ, Bol KF, et al. Regulatory T cells and the PD-L1/PD-1 pathway mediate immune suppression in malignant human brain tumors. Neuro Oncol 2009;11:394–402.
23. el Andaloussi A, Lesniak MS. CD4+ CD25+ FoxP3+ T-cell infiltration and heme oxygenase-1 expression correlate with tumor grade in human gliomas. J Neurooncol 2007; 83:145–52.
24. Miyara M, Sakaguchi S. Natural regulatory T cells: mechanisms of suppression. Trends Mol Med. 2007;13:108-16.
25. Gulmann C, Paner GP, Parakh RS, et al. Immunohistochemical profile to distinguish urothelial from squamous differentiation in carcinomas of urothelial tract. Hum Pathol 2013; 44:164–72.
26. Gulbinas A, Berberat PO, Dambrauskas Z, et al. Aberrant gata-3 expression in human pancreatic cancer. J Histochem Cytochem 2006; 54:161–9.
27. Wang Y, Misumi I, Gu A-D, et al. GATA-3 controls the maintenance and proliferation of T cells downstream of TCR and cytokine signaling. Nat Immunol 2013; 14:714-22.
28. Hoyler T, Klose CS, Souabni A, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. Immunity 2012; 37:634-48.
29. Lee K, Min HJ, Jang EJ, et al. In vivo tumor suppression activity by T cell-specific T-bet restoration. Int J Cancer 2010; 127:2129–37.
30. Bahria-Sediki IB, Yousfi N, Paul C, et al. Clinical significance of T-bet, GATA-3, and Bcl-6 transcription factor expression in bladder carcinoma. J Transl Med 2016; 14:144.
31. Mukhtar R, Moore AP, Tandon VJ, et al. Elevated levels of proliferating and recently migrated tumor-associated macrophages confer increased aggressiveness and worse outcomes in breast cancer. Ann Surg Oncol 2012; 19:3979–86.
32. Qiu B, Wang Y, Tao J, et al. Expression and correlation of Bcl-2 with pathological grades in human glioma stem cells. Oncol Rep 2012; 28:155-60.
33. Wick W, Wagner S, Kerkau S, Det al. BCL-2 promotes migration and invasiveness of human glioma cells. FEBS Lett 1998; 440:419–24.
34. Abramovich CM, Prayson RA. Apoptotic activity and bcl-2 immunoreactivity in meningiomas: association with grade and outcome. Am J Clin Pathol 2000; 114:84–92.
35. Abdelzaher E, El-Gendi SM, Yehya A, et al. Recurrence of benign meningiomas: predictive value of proliferative index, BCL2, p53, hormonal receptors and HER2 expression. Br J Neurosurg 2011; 25:707-13.
36. Li W, Qian C, Wang L, et al. Association of BCL2-938C>A genetic polymorphism with glioma risk in Chinese Han population. Tumour Biol 2014; 35:2259–64.

*****