Original Article

WT1 protein expression in astrocytic tumors and its relationship with cellular proliferation index

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Abstract

Background: Although Wilms' tumor gene (WT1) was initially known as a tumor marker in Wilms' tumor, nowadays its role is well known in other sorts of malignancy. This study aimed to evaluate WT1 protein expression levels and its association with cellular proliferation in astrocytic brain tumors by immunohistochemical methods.

Materials and Methods: This cross-sectional study performed on 73 randomly selected archived tissue samples of astrocytic brain tumors. Sections were observed after immunohistochemical staining regarding WT1 protein expression and MIB-1 staining index. Tumors were classified based on World Health Organization grading system.

Results: WT1 protein expression was seen in the majority of samples (97.3%) with significantly higher index in high-grade tumors ($P<0.001$). MIB-1 staining index was also significantly higher in high-grade tumors ($P<0.001$). Moreover, a significantly positive correlation was found between WT1 protein expression and MIB-1 staining index (r: 0.64, $P<0.001$).

Conclusion: Astrocytic brain tumors express WT1 protein. It was also found that high-grade tumors are accompanied with higher WT1 protein expression, which is correlated with MIB-1 staining index. WT1 can be used as a marker of malignant cell proliferation and diagnostic tool to differentiate normal astrocytes from neoplastic cells.

Key Words: Astrocytic brain tumor, immunotherapy, MIB-1 staining index, WT1 protein

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INTRODUCTION

The Wilms' tumor gene (WT1) was originally introduced as a tumor-suppressor gene.[1,2]

A transcription factor with zinc figure motifs encoded by this gene suppresses the transcription of some growth factor genes including PDGF-A, CSF-1 and IGF-II as well as growth factor receptor gene IGF-1R.[3]

Its mutation and inactivation was seen in a subset of Wilms' tumor and children who were genetically predisposed to kidney neoplasm.[3,4] Further research showed that wild-type WT1 has oncogenic properties and its continuous over-expression is related to leukemia[5] and several kinds of solid malignancies including lung,[6] colon,[7] breast[8,9] and thyroid cancer.[10,11]

Wild-type WT1 over expression has also been found

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in astrocytic tumors.[1,3,12] WT1 expression has been reported in the majority of patients suffering from malignant astrocytoma.[13] Identification of WT1 protein as a tumor-associated antigen made it an attractive option for immunotherapy against malignancies, particularly brain tumors because it has an important role in regulation of tumorigenesis and it does not express in the normal brain.[4,13-19] In addition, MIB-1 staining index is a marker of tumor cell proliferation and has proven diagnostic value in astrocytic tumors.[20,21]

This study aimed to evaluate WT1 protein expression levels and its association with cellular proliferation in astrocytic brain tumors by immunohistochemical methods.

MATERIALS AND METHODS

Study design

This cross-sectional study was performed at Alzahra Hospital, Isfahan, Iran, on tissue samples obtained from patients who had undergone brain surgery between 2007 and 2010. This study was approved by the ethics committee of Isfahan University of Medical Sciences.

Tissue samples

After evaluation of hematoxylin and eosin (H and E) stained slides, 73 paraffin blocks ((2)) were randomly selected from astrocytic tumor samples of different grades, archived at pathology unit of our center. Samples with unsatisfactory amount of tissue were excluded. Definite diagnosis of different grades of astrocytic brain tumors was made according to World Health Organization (WHO) diagnostic criteria.[22]

Immunohistochemistry

Deparaffinization and antigen retrieval

Four micrometer thickness formalin-fixed tissue sections were cut from each paraffin block and placed on poly-L-lysine covered slides. Sections were heated in dry oven at 60°C for 30–45 min, then immersed in xylene, alcohol, and finally citrate buffer (pH»6).

WT1 staining

Sections were incubated with anti-human WT1 antibody (6F-H2, DAKO, U.S.A.; diluted 1:40)((7)) for 1 h, then following solutions were added for specific time orderly: 0.3% H_2O_2 solution for 10 min, EnVisionä polymer for 25 min, Diaminobenzidine (DAB) for 5 min and hematoxylin for 2 min. Samples were washed after each step by Phosphate Buffered Saline (PBS).

A Wilms' tumor sample was stained based on the above method and considered as the positive control.

MIB-1 staining

Sections similar to those selected for WT1 staining were recruited for MIB-1 staining, incubated with anti Ki-67 antibody (DAKO, U.S.A.; diluted 1:80)((7)) for 1 h. Other steps were performed on the same serial steps used for WT1 immunohistochemistry.

A lymph node sample was stained according to the above method for positive control.

Sample evaluation

All specimens were observed under light microscope at high magnification. Only samples in which endothelial cells showed WT1 expression were included the study. WT1 index was determined as the sum of WT1-positive relative frequency score and WT1 staining score.

WT1 staining score was considered as 0 (negative: no staining), 1 (weak: mildly increased staining compared to the normal glial cells), 2 (intermediate: staining at intensity between 1 and 3), and 3 (strong: significantly increased staining in tumor cells compared with normal glial cells) (1) ((3)).

Relative frequency of WT1-positive tumor cells was scored as 0 (0%), 1 (<25%), 2 (25–75%), and 3 (>75%).[3]

Regarding MIB-1 staining index, the number of positively stained tumor cell nuclei in every 1000 tumor cell nuclei was calculated.

Statistical analysis

Data were analyzed by SPSS 16; spearman correlation test, Kruskal-Wallis test and Mann-Witney U test were applied as they were appropriate. $P<0.05$ was considered as significant statistical difference.

RESULTS

According to the WHO grading system, 4 types of brain astrocytic tumors were found among studied samples including pilocytic astrocytoma, diffuse astrocytoma, anaplastic astrocytoma and gliobastoma. Majority of cases were glioblastoma (49.31%). Based on immunohistochemical staining, 71 of 73 (97.3%) studied primary astrocytic tumor samples expressed WT1 protein in the cytoplasm. Only 1 sample with glioblastoma and 1 with pilocytic astrocytoma had no WT1 protein expression. High WT1 index (WT1 index: 6) was found significantly more in gliobastoma samples (87.5%) ($P<0.001$); also MIB-1 staining index was significantly higher in gliobastoma in comparison with diffuse and pilocytic astrocytoma ($P<0.001$)

Table 1: Distribution of various tumor grades and relevant indices

WHO grading system		n (%)	MIB-1 staining index	WT1 index=0	WT1 index=1	WT1 index=2	WT1 index=3	WT1 index=4	WT1 index=5	WT1 index=6
Low grade										
Grade I	Pilocytic astrocytoma	13 (17.80)	0.54 ± 0.51	1	0	1	3	2	5	1
Grade II	Diffuse astrocytoma	19 (26.02)	1.21 ± 0.71	0	0	7	9	3	0	0
High grade										
Grade III	Anaplastic astrocytoma	5 (6.84)	6.40 ± 7.63	0	0	0	0	1	2	2
Grade IV	Glioblastoma	36 (49.31)	12.28 ± 11.12	1	0	0	2	3	9	21
Total		73 (100)	6.90 ± 9.68	2	0	8	14	9	16	24

Frequencies are presented as number (percentage) (n (%)).MIB-1 staining index: MIB-1 staining index presented as mean ± SD. WT1 index: WT1 protein expression score presented as number of cases.

Table 2: Comparison of mean rank of WT1 index between different grades of astrocytic brain tumors

Mean rank of WT1 index		*P*-value	
Pilocytic astrocytoma (29.81)			
Diffuse astrocytoma	(15.26)	0.01*	
Anaplastic astrocytoma	(47.00)	0.07	
Glioblastoma	(49.68)	0.001*	<0.001
Diffuse astrocytoma (15.26)			
Pilocytic astrocytoma	(29.81)	0.01*	
Anaplastic astrocytoma	(47.00)	<0.001*	
Glioblastoma	(49.68)	<0.001*	
Anaplastic astrocytoma (47.00)			
Pilocytic astrocytoma	(29.81)	0.07	
Diffuse astrocytoma	(15.26)	<0.001*	
Glioblastoma	(49.68)	0.60	
Glioblastoma (49.68)			
Pilocytic astrocytoma	(15.26)	0.001*	
Diffuse astrocytoma	(15.26)	<0.001*	
Anaplastic astrocytoma	(47.00)	0.60	

*Difference is statistically significant

[Table 1 and Figures 1, 2].

Mean rank of WT1 index was significantly different between various grades of astrocytic brain tumor (P<0.001) [Table 2].

In addition, significant positive correlation was found between MIB-1 staining index and WT1 protein expression index (r: 0.64, P<0.001) [Figure 3].

DISCUSSION

The emphasis of previous studies on the role of WT1 protein as a tumor marker led us to conduct this study to evaluate WT1 status in primary brain tumors. This role was confirmed for astrocytic tumors; present study showed that similar to the mentioned kinds of malignancy, majority of astrocytic brain tumors also express WT1 protein. It was also found that high-grade tumors are accompanied with higher WT1 protein expression. These findings are confirmed by the study of *T. Hashiba et al.* in which high WT1 protein expression

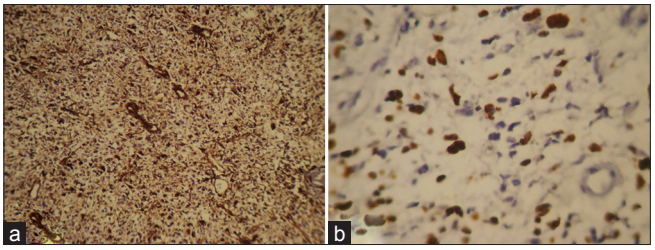

Figure 1: Strong WT1 protein expression (a) and high proportion of ki-67 positive nuclei (MIB-1 staining) (b) in a case of glioblastoma multiforme

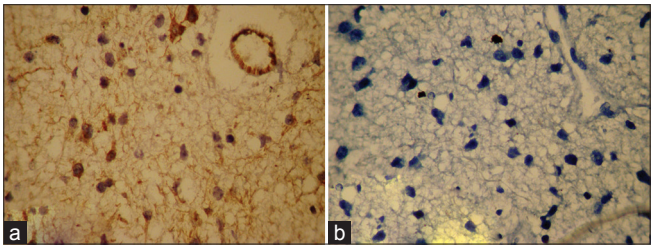

Figure 2: Weak WT1 protein expression (a) and low percentage of ki-67 positive nuclei (MIB-1 staining) (b) in a case of diffuse fibrillary astrocytoma

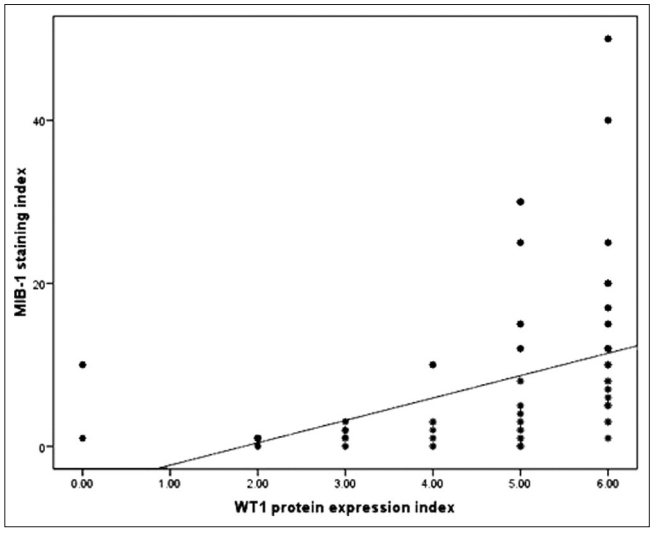

Figure 3: Scatter plot and regression line showing the positive correlation between MIB-1 staining index and WT1 protein expression index

is considered to be due to probable involvement of WT1 gene in proliferation or transformation of astrocytic tumors.[1] Another study, which reported a diagnostic value for WT1 in neuroepithelial tumors,[23] also approved the positive correlation between WT1 protein expression and tumor grade. As well, WT1 was suggested to have a role in tumorigenesis and progression of brain tumors.[23]

But is WT1 a tumor-specific marker in brain neoplasm as well as other mentioned sorts of cancer? Yes! Another study by Schittenhelm *et al.,* which compared normal astrocytes with neoplastic astrocytes, found that in contrast to normal and reactive astrocytes, neoplastic cells express WT1 protein frequently.[24]

In contrast, a recent study found WT1 not to be a reliable marker for differentiation of reactive from neoplastic astrocytes. However, this different finding is attributed to different tissue fixation and immunohistochemistry methodology.[25]

Proliferative activity is known as a reliable marker to assess tumor biology and has established power in the diagnosis of astrocytic tumors.[20,21] MIB-1 is a marker commonly used to denote proliferative activity. We found that along with the progression of tumor grade, MIB-1 staining index demonstrates a continuous increase, which verifies higher proliferative activity in high-grade tumors.[1] Previous studies support this finding where MIB-1 is reported to be correlated with tumor grade as well as with mitotic activity.[1,26,27] Low MIB-1 staining index has been considered to be associated with slower tumor growth.[28]

Based on values we have found, MIB-1 staining index can be used to differentiate glioblastoma from pilocytic and diffuse astrocytoma more reliably.

In spite of higher WT1 protein expression and MIB-1 staining index in high-grade tumors, WT1 index was surprisingly significantly lower in diffuse astrocytoma (grade 2) than pilocytic type (grade 1); Such a finding has been reported in previous studies as well and could be considered as one of the several unclear issues related to the role of WT1 gene in astrocytes, which needs further specific investigations to clarify.[1]

In addition, the significant positive correlation between WT1 protein expression and MIB-1 staining index implies that WT1 protein expression might be related to both tumor cell proliferation and tumor grade. Albeit, this correlation has been attributed to higher WT1 protein expression in areas of high cell proliferation,[1] but since we did not observe any specific pattern in the expression of WT1 protein, it could be inferred that higher WT1 protein expression is not necessarily confined to the high proliferation areas. Therefore, this correlation might be more complex than a direct correlation limited to specific sites and could be related to a common underlying condition, which regulates both of these indices; further studies are needed to shed light on the basis of this correlation.

CONCLUSION

In summary, we can conclude that proven roles of WT1 in malignancies could be attributable to brain astrocytic tumors; WT1 can be used as a marker of malignant cell proliferation and invasion as well as a diagnostic tool to differentiate normal astrocytes from neoplastic cells. Moreover, over-expression of WT1 in astrocytic brain tumors, especially high-grade types, suggests that this kind of malignancies could be a suitable target for cancer immunotherapy.

REFERENCES

1. Hashiba T, Izumoto S, Kagawa N, Suzuki T, Hashimoto N, Maruno M, *et al.* Expression of WT1 protein and correlation with cellular proliferation in glial tumors. Neurol Med Chir (Tokyo) 2007;47:165-70; discussion 70.
2. Cilloni D, Gottardi E, De Micheli D, Serra A, Volpe G, Messa F, *et al.* Quantitative assessment of WT1expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. Leukemia 2002;16:2115-21.
3. Oji Y, Suzuki T, Nakano Y, Maruno M, Nakatsuka S, Jomgeow T, *et al.* Overexpression of the Wilms' tumor gene W T1 in primary astrocytic tumors. Cancer Sci 2004;95:822-7.
4. Gessler MP, Cavenee W, Neve RL, Orkin SH, Bruns GA. Homozygous deletions in Wilms' tumours of a zinc-finger gene identified by chromosome jumping. Nature 1990;343:774-8.
5. Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, *et al.* WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. Blood 1994;84:3071-9.
6. Oji Y, Miyoshi S, Maeda H, Hayashi S, Tamaki H, Nakatsuka S, *et al.* Overexpression of the Wilms' tumor gene WT1 in de novo lung cancers. Int J Cancer Res Clin Oncol 2002;100:297-303.
7. Oji Y, Yamamoto H, Nomura M, Nakano Y, Ikeba A, Nakatsuka S, *et al.* Overexpression of the Wilms' tumor gene WT1 in colorectal adenocarcinoma. Cancer Sci 2003;94:712-7.
8. Loeb DM, Evron E, Patel CB, Sharma PM, Niranjan B, Buluwela L, *et al.* Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. Cancer Res 2001;61:921-5.
9. Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, *et al.* High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. Clin Cancer Res 2002;8:1167-71.
10. Oji Y, Miyoshi Y, Koga S, Nakano Y, Ando A, Nakatsuka S, *et al.* Overexpression of the Wilms' tumor gene WT1 in primary thyroid cancer. Cancer Sci 2003;94:606-11.
11. Menssen HD, Bertelmann E, Bartelt S, Schmidt RA, Pecher G, Schramm K, *et al.* Wilms' tumor gene (WT1) expression in lung cancer, colon cancer and glioblastoma cell lines compared to freshly isolated tumor specimens. J Cancer Res Clin Oncol 2000;126:226-32.
12. Nakahara Y, Okamoto H, Mineta T, Tabuchi K. Expression of theWilms' tumor gene productWT1 in glioblastomas and medulloblastomas. Brain Tumor Pathol 2004;21:113-6.
13. Clark AJ, Dos Santos WG, McCready J, Chen MY, Van Meter TE, Ware JL, *et al.* Wilms tumor1 expression in malignant gliomas and correlation of

?KTS isoforms with p53 status. J Neurosurg 2007;107:586-92.
14. Izumoto S, Tsuboi A, Oka Y, Suzuki T, Hashiba T, Kagawa N, *et al.* Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. J Neurosurg 2008;108:963-71.
15. Morita S, Oka Y, Tsuboi A, Kawakami M, Maruno M, Izumoto S, *et al.* A phase I/II trial of a WT1 (Wilms' tumor gene) peptide vaccine in patients with solid malignancy: Safety assessment based on the phase I data. Jpn J Clin Oncol 2006;36:231-6.
16. Oka Y, Udaka K, Tsuboi A, Elisseeva OA, Ogawa H, Aozasa K. Cancer immunotherapy targeting Wilms' tumor gene WT1 product. J Immunol 2000;164:1873-80.
17. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Halber DA, *et al.* Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 1990;60:509-20.
18. Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, *et al.* The candidate Wilms' tumour gene is involved in genitourinary development. Nature 1990;346:194-7.
19. Armstrong JF, Pritchard-Jones K, Bickmore WA. The expression of the Wilms' tumour gene, WT1, in the developing mammalian embryo. Mech Dev 1993;40:85-97.
20. Johannessen A, Torp SH. The clinical value of Ki-67/MIB-1 labeling index in human astrocytomas. Pathol Oncol Res 2006;12:143-7.
21. Prayson RA. The utility of MIB-1/Ki-67 imunostaining in the evaluation of central nervous system neoplasms. Adv Anat Pathol 2005;12:144-8.
22. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, *et al.* The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114:97-109.
23. Schittenhelm J, Beschorner R, Simon P, Tabatabai G, Herrmann C, Schlaszus H, *et al.* Diagnostic value of WT1 in neuroepithelial tumours. Neuropathol Appl Neurobiol 2009;35:69-81.
24. Schittenhelm J, Mittelbronn M, Nguyen TD, Meyermann R, Beschorner R. WT1 expression distinguishes astrocytic tumor cells from normal and reactive astrocytes. Brain Pathol 2008;18:344-53.
25. Bourne TD, Elias WJ, Lopes MB, Mandell JW. WT1 is not a reliable marker to distinguish reactive from neoplastic astrocyte populations in the central nervous system. Brain Pathol 2010;20:1090-5.
26. Giannini C, Scheithauer BW, Burger PC, Christensen MR, Wollan PC, Sebo TJ, *et al*. Cellular proliferation in pilocytic and diffuse astrocytomas. J Neuropathol Exp Neurol 1999;58:46-53.
27. Kamiya M, Nakazato Y. The expression of cell cycle regulatory proteins in oligodendroglial tumors. Clin Neuropathol 2002;21:52-65.
28. Machen SK, Prayson RA. Cyclin D1 and MIB-1 immunohistochemistry in pilocytic astrocytomas: A study of 48 cases. Hum Pathol 1998;29:1511-6.

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