Chapter 15

Functional Roles of Wilms’ Tumor 1 (WT1) in Malignant Brain Tumors

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Abstract

The pleiotropic transcription factor, Wilms’ tumor 1 (WT1), is expressed in the majority of glioblastomas, the most common malignant brain tumors of adulthood. Despite intensive treatment, including surgery and chemoradiotherapy, the prognosis for patients with glioblastoma remains very poor. Encouragingly, immunotherapy targeting WT1 has proven to be effective in recurrent glioblastoma, suggesting that
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this approach may be an important new treatment modality for the disease. However, WT1 appears to function as a context-dependent tumor suppressor or oncogene, and the functional roles of WT1 in the pathogenesis of glioblastoma, and other types of brain tumors, have not been extensively studied. With this in mind, we briefly review WT1 expression data for a range of different brain tumor classes and address the role of WT1 in the regulation of proliferation and apoptosis in glioblastoma. We generated WT1 knockdown glioblastoma cells by using shRNA-expressing lentivirus. Proliferation was reduced and apoptosis increased in WT1 knockdown glioblastoma cells compared with control cells in vitro. Consistent with these data, when WT1 knockdown glioblastoma cells or control glioblastoma cells were intracranially injected into the immunodeficient mice, tumor growth was significantly reduced in WT1 knockdown cells compared with that in control cells. Thus, WT1 is an oncogene that regulates cell proliferation and apoptosis in glioblastoma.

Key words: Ependymoma; Glioblastoma; Medulloblastoma; Meningioma; Oligodendrogloma

Introduction

Wilms’ tumor 1 (WT1) is a pleiotropic transcription factor expressed in various types of hematological malignancies and solid tumors (1–9). WT1 was first defined as a tumor suppressor gene (10–15). However, accumulating evidence suggests that the WT1 can act as an oncogene in some contexts. For example, the growth of a range of different WT1-expressing cancer cell types is inhibited by WT1 antisense oligomer (16, 17) and WT1-specific shRNA (18). Furthermore, overexpression of WT1 promotes cell growth (19–21), migration, and invasion (22). Overexpression of WT1 also inhibits apoptosis (23) and induces tumorigenicity in leukemia (24). However, the functional roles of WT1 in the pathogenesis of malignant brain tumors have not been extensively studied.

Glioblastoma is one of the most common malignant brain tumors. Despite intensive treatment, including surgery, radiation, and chemotherapy, the prognosis is still very poor, and the median survival is only 12–15 months (25). Improved treatments are urgently required to improve the prognosis of glioblastoma patients, and various therapies have been tested or are in development. In this regard, WT1 peptide vaccine immunotherapy has proven to be effective in recurrent glioblastoma (26), suggesting that WT1 is a valid therapeutic target in glioblastoma.

We briefly review the available WT1 expression data for a range of different brain tumor classes and address the involvement of WT1 in the regulation of proliferation and apoptosis in glioblastoma cells. In addition, we also investigated whether WT1 is involved in glioblastoma tumorigenicity in an intracranial xenograft model.
WT1 expression in malignant brain tumors

Glioma

Glioma is one of the most common types of malignant brain tumor. According to the WHO classification of central nervous system tumors, glioma is divided into four different grades depending on the malignant potential. Immunohistochemical analyses demonstrated that WT1 is expressed in many gliomas (5, 26–29) (Figure 1) and expression is variable depending on the tumor grade. In less-aggressive grade II glioma and diffuse astrocytoma, WT1 expression was lower than that in grade IV glioblastoma. Furthermore, Rauscher et al. (29) reported that WT1 expression is a prognostic marker of WHO grade II and III tumors, and WT1 expression is reduced in recurrent tumors.

Oligodendroglioma

Oligodendroglioma is a type of glioma that is thought to originate from brain oligodendrocytes. It occurs primarily in adults (9.4% of all primary brain and central nervous system...
tumors) but also affect children (4% of all primary brain tumors). WT1 is also expressed in oligodendrogliomas, and its expression is elevated in higher grade tumors (29).

Medulloblastoma

Medulloblastoma is the most common type of malignant pediatric brain tumor. A previous report showed that WT1 transcripts were detectable in five of nine primary medulloblastoma tumors (30), but in a separate study, WT1 expression was not detectable by immunohistochemistry (27). It is unclear whether this discrepancy is related to the heterogeneous WT1 expression levels in the four distinct molecular subtypes of medulloblastoma that have been described (31–33). Additional detailed studies are required to address the levels and the significance of WT1 expression in the pathogenesis of the various medulloblastoma subtypes.

Ependymoma

Ependymoma is a neuroepithelial malignancy of the central nervous system, which occurs in both children and adults. Although Idowu et al. (34) and Yeung et al. (35) reported that WT1 is expressed in ependymoma by immunohistochemistry, the significance of WT1 expression in ependymoma pathogenesis remains to be determined.

Meningioma

Meningioma is a common and predominantly benign intracranial tumor, which is classified as grade I according to the WHO classification of central nervous system tumors. Less than 10 percent of meningioma cases are classified as malignant WHO grade II or III tumors. The expression of WT1 in meningioma is controversial. Singh et al. (36) reported that WT1 is not expressed in WHO grade I meningiomas by immunohistochemistry, while Iwami et al. (37) showed that at the mRNA level, WT1 is expressed in many meningioma samples irrespective of WHO grade. Furthermore, Iwami et al. (37) reported that WT1 could be a therapeutic target for skull base meningioma.

In summary, WT1 is expressed in many different classes of intracranial tumors, including gliomas, oligodendrogliomas, ependymomas, and meningiomas. However, in most cases, the significance of WT1 expression in the pathogenesis of brain tumor remains unclear. In part, this is related to the fact that WT1 expression will need to be assessed in many more representatives of the various histological and/or molecular brain tumor subtypes to generate the statistically robust conclusions. At present, there are more than 100 histological subtypes of brain tumors according to the WHO classification, many of which are rare, and of which only a specific subset predominates in children, and WT1 expression data are limited or not available. In addition, consensus will be required to determine the WT1 detection method that is most appropriate for the comparison of data across laboratories.

The available data suggest that the expression of WT1 in some major brain tumor classes, most notably glioma, is likely to play an important role in tumor initiation and progression.
**WT1 in malignant brain tumors**

Based on this, the impetus is provided to assess WT1 expression in all brain tumor types to determine the validity of WT1 as a therapeutic target across the brain tumor spectrum.

**Functional roles of WT1 in glioblastoma pathogenesis**

Most studies investigating the functional roles of WT1 in the pathogenesis of malignant brain tumors have focused on glioblastoma. In other types of brain tumors, the functional roles of WT1 have not been assessed. Previous reports suggest that WT1 is involved in driving cell proliferation (38) and inhibiting apoptosis (18, 38) in glioblastoma. In an earlier study conducted in our laboratory (38), we transduced two glioblastoma cell lines, U87MG and U251, with lentivirus carrying WT1 shRNA to address the effect of WT1 knockdown on cell proliferation. We found that cell proliferation was significantly reduced (Figure 2A and 2B), suggesting that WT1 is involved in proliferation of glioblastoma cells. We also examined the effect of WT1 on glioblastoma progression in vivo by transducing U87MG cells with WT1 shRNA or control shRNA followed by intracranial injection into the immunodeficient Rag2−/− gamma c−/− mice. There was a significant difference in survival between the mice injected with U87MG cells transduced with WT1 shRNA and those injected with control U87MG cells. We also found that all the mice inoculated with U87MG cells transduced with control shRNA died of glioblastoma within 40 days, whereas none of the mice injected with WT1-shRNA-treated U87MG cells died of glioblastoma by the same time point (Figure 2C). These data demonstrated that WT1 knockdown significantly inhibited glioblastoma growth in vivo.

Immunohistochemical analysis of formalin-fixed, paraffin-embedded tumor sections from mice inoculated with U87MG cells transduced with WT1 shRNA or control shRNA revealed that the Ki67 proliferation index was higher in control tumors compared with those transduced with WT1 shRNA (Figure 2D). Consistent with the in vitro data, these findings also suggest that WT1 drives cell proliferation and tumor formation in vivo.

We also investigated the differences in mRNA expression of selected apoptosis-related genes in U87MG and U251 cells transduced with WT1 shRNA or control shRNA by real-time PCR. We found that apoptosis-related genes such as MAP3K5, PIK3CA, and p53 were upregulated in both U87MG and U251 WT1 knockdown cells compared with those in control cells. Extending these findings, we examined the differences in apoptosis between glioblastoma cells transduced with WT1 shRNA and control shRNA using an Annexin-V-Fluos kit. We found that the number of apoptotic cells was higher in U87MG and U251 WT1 knockdown cells compared with that in U87MG cells transduced with control shRNA (Figure 3A and 3B). These results showed that apoptosis was promoted in both U87MG and U251 WT1 knockdown cells, suggesting that WT1 drives glioblastoma tumorigenicity by regulating apoptosis.
In addition to our report, roles of WT1 in glioblastoma pathogenesis have been described in several studies (5, 39–41). Oji et al. (5) reported that the growth of U87MG cells was inhibited by a WT1 antisense oligomer consistent with WT1 being involved in the regulation of cell proliferation in glioblastoma. Tatsumi et al. (18) found that WT1 inhibited apoptosis in the A172 glioblastoma cell line. Clark et al. (39) reported that WT1 regulated cell proliferation in U251 cells and that U251 WT1 knockdown cells showed significantly lower tumorigenicity in a subcutaneous nude mouse model. However, contrasting data were presented in several other studies using different glioblastoma cell lines. Chidambaram et al. (40) reported that silencing of WT1 reduced the invasiveness of U1242 glioblastoma cells but had no effect on the U1242 cell proliferation in vitro. Clark et al. (39) found that transduction of T98G cells with WT1 shRNA had no effect on apoptosis compared with those transduced with control shRNA in vitro. These results suggest that the regulation of cell proliferation and apoptosis
WT1 in malignant brain tumors

In summary, the functional roles of WT1 in glioblastoma pathogenesis remain controversial although the weight of evidence is consistent, with WT1 being involved in regulating cell proliferation and apoptosis in at least some glioblastoma. Clearly, more work is required to comprehensively address the functional roles of WT1 in the initiation and progression of glioblastoma and the many other types of malignant brain tumor that have not yet been adequately studied.

Immunotherapy targeting WT1 peptide for malignant glioma

Cancer vaccination is one of the immunotherapeutic strategies that have been developed to target many solid tumors. Recently, a large number of tumor-associated antigens, including WT1, were identified and assessed as potential candidates as cancer vaccines. Tumor antigen epitopes associated with human leukocyte antigen (HLA) class I molecules were
recognized by cytotoxic T lymphocytes, providing a potential mechanism for direct tumor cell killing. Furthermore, recent studies reported that systemic immunotherapy can induce an antitumor response within the immunologically privileged brain. These findings suggest that the peptide-based cancer immunotherapy could be a potent therapeutic strategy for the treatment of malignant brain tumors.

Izumoto et al. (26) carried out a phase II clinical trial of WT1 peptide vaccine immunotherapy for recurrent glioblastomas and found that the approach was effective in this context. To improve the efficacy of the treatment, we have been assessing a possible combination of WT1 peptide vaccine immunotherapy with temozolomide, a standard chemotherapeutic agent for newly diagnosed malignant glioma patients (42). We found that the combination therapy was tolerable without serious side effects. We are now moving on to the phase II clinical trial of combination WT1 peptide vaccine immunotherapy and temozolomide chemotherapy for newly diagnosed glioblastoma. This represents one example of combined immunotherapy with chemotherapeutic or other immunotherapeutic modalities, such as antiangiogenic agents or checkpoint inhibitors. Although WT1 peptide vaccine immunotherapy for malignant brain tumors has promise, more extensive studies are needed to determine the clinical efficacy of this approach.

Conclusions

WT1 expression in malignant brain tumors varies depending on the tumor type. WT1 functions as an oncogene in at least some glioblastomas, in part, by regulating cell proliferation and apoptosis. Overall, these findings suggest that WT1 is a valid molecular target for the treatment of glioblastoma and potentially a range of other malignant brain tumors.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

Acknowledgment

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References


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http://dx.doi.org/10.1056/NEJMoa043330

http://dx.doi.org/10.3171/JNS/2008/108/5/0963


http://dx.doi.org/10.1007/s10014-010-0265-9

http://dx.doi.org/10.1136/jclinpath-2013-202114


http://dx.doi.org/10.1200/JCO.2009.27.4324

http://dx.doi.org/10.1038/nrneurol.2012.78

http://dx.doi.org/10.1007/s00401-011-0922-z

http://dx.doi.org/10.1097/PAS.0b013e318159a2b4


