

## Wilms tumor 1 gene, CD97, and the emerging biogenetic profile of glioblastoma

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### Abstract

Glioblastoma multiforme (GBM) is the most common type of primary brain tumor, and current treatment regimens are only marginally effective. One of the most vexing and malignant aspects of GBM is its pervasive infiltration into surrounding brain tissue. This review describes the role of the Wilms tumor 1 gene (*WT1*) and its relationship to GBM. *WT1* has several alternative splicing products, one of which, the KTS+ variant, has been demonstrated to be involved in the transcriptional activation of a variety of oncogenes as well as the inhibition of tumor suppressor genes. Further, this paper will examine the relationship of *WT1* with *CD97*, a gene that codes for an epidermal growth factor receptor family member, an adhesion G-protein–coupled receptor, thought to promote tumor invasiveness and migration. The authors suggest that further research into *WT1* and *CD97* will allow clinicians to begin to deal more effectively with the infiltrative behavior displayed by GBM and design new therapies that target this deadly disease. (<http://thejns.org/doi/abs/10.3171/2014.9.FOCUS14506>)

Glioblastoma multiforme (GBM), a WHO Grade IV glioma, is the most common primary malignancy in the central nervous system. GBM is characterized by high degrees of parenchymal invasion, vascularization secondary to angiogenesis, necrosis, and de-differentiation. Due to its aggressive nature, GBM carries a poor prognosis, with 35.7% survival at 1 year and 4.7% survival at 5 years.<sup>26</sup> Currently, the optimal treatment paradigm is aggressive resection followed by radiotherapy and concomitant chemotherapy.<sup>33</sup> Despite intensive laboratory and clinical research, only moderate advances have been made in improving the quality of life in GBM patients. This suggests that there is a need to build a complete biological profile to uncover novel molecular targets that can be employed in future therapies. Recently, our laboratory and others have shifted their focus to the Wilms tumor 1 gene (*WT1*).<sup>5-8</sup> *WT1* was first isolated in 1990 by Haber et al. with their discovery that an internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms tumor, a pediatric kidney malignancy.<sup>10</sup> *WT1* was initially thought to be a tumor suppressor gene, but subsequent research uncovered its oncogenic role when it was demonstrated that *WT1* can suppress *hTERT* gene expression and telomerase activity in clear cell renal cell carcinoma.<sup>31</sup>

Menssen et al. were the first group to show the expression of *WT1* in human GBM. They reported a high *WT1* expression in 63% of GBM cell lines.<sup>21</sup> In the same year, we also reported the expression of *WT1* in a variety of ovarian and GBM cultured cell lines (Quezado MM, Dechsukhum C, Garrett CT, et al., presented at the United States and Canadian Academy of Pathology Annual Meeting, 2000). Since these initial

descriptions, there has been an expanding interest in connecting *WT1* and its oncological effect with GBM. This paper will review the studies to date that have analyzed the relationship of *WT1* to GBM and describe a novel G-protein coupled receptor, CD97, which may play a role in GBM invasion.

### **Wilms Tumor 1 Gene**

Though *WT1* is absent in normal neural tissue, it has been described in cultured neoplastic glial cell lines, and further work has indicated high *WT1* expression in acute myeloid leukemia and acute lymphoid leukemia.<sup>7,15</sup> *WT1* encodes for a zinc finger family transcription factor located at 11p13 whose physiological expression is associated with normal genitourinary embryologic development.<sup>1</sup> It has been hypothesized that *WT1* may exert its oncological effects in a multifaceted modality via an alternative mRNA splicing variant that involves the insertion of the 3–amino acid sequence of lysine, threonine, serine (KTS) into the active zinc finger region.<sup>28</sup> This is in contrast to the KTS<sup>-</sup> variant, which transcriptionally regulates suppression of at least 7 putative tumor suppressor genes and increases expression of 11 reputed oncogenes.<sup>5</sup> The KTS<sup>+</sup> product, on the other hand, has only a limited role in direct transcriptional regulation; instead it participates in putative splicing factor interactions and has an association with olfactory neuron development.<sup>14</sup>

### **GBM and Wilms Tumor**

When *WT1* expression was first reported in brain tumors, it was hypothesized that testing for *WT1* expression might not have clinical significance.<sup>21</sup> The first laboratory investigation that focused on *WT1* expression in central nervous system malignancies, including GBM, found *WT1* expression in most of these tumors; yet there were no mutations affecting the zinc fingers of the gene product in tumors expressing *WT1*.<sup>9</sup> Though this appeared to indicate that *WT1* expression might not have clinical relevance to the molecular etiology of brain tumors, these results nonetheless ignited a novel interest in the relationship of *WT1* to central nervous system tumors. A few years later, Oji et al. were able to show that the *WT1* protein expression was significantly greater in high-grade tumors than in low-grade tumors and that treatment with *WT1* antisense oligomers specifically inhibited GBM cell lines.<sup>24</sup> This study, coupled with later confirmation from immunohistochemical tests that demonstrated *WT1* expression in GBM cells, high-lighted its potential as a target for immunotherapy.<sup>12,23</sup> This differential expression of *WT1* in high-grade brain tumors has recently been validated in an in vivo study analyzing human gliomas, which included 442 glioblastomas, 303 astrocytomas, 41 oligodendrogliomas, and 43 oligoastrocytomas.<sup>27</sup> The results showed that *WT1* expression in brain tumors increased with WHO grade, older age, and absence of *IDH1* mutation.<sup>27</sup>

The introduction of a *WT1*-based vaccine in clinical trials was first described by Oka et al. in 2004.<sup>13,25</sup> The authors reported the effects of the vaccine on breast cancer, lung cancer, and leukemia in 26 patients, reporting tumor regression and no toxicity as a result of the drug. The same group recently published data

on a Phase II clinical trial that included 21 patients who received WT1 peptide vaccination for recurrent GBM.<sup>15</sup> Preliminary data showed that patients with WT1/HLA-A\*2402 positivity who received WT1 immunotherapy showed a favorable outcome when compared with patients treated with a standard chemotherapy protocol.<sup>15</sup> In a follow-up study analyzing the same GBM patient population, the authors reported that *WT1* expression can be used as a prognostic marker in determining progression-free survival after immunotherapy and that patients with intermediate *WT1* expression levels may have the best outcomes.<sup>4</sup>

One possible mechanism of action of a WT1-based vaccination could lie in its suppression of WT1's inactivation of tumor suppressor gene *p53* (*TP53*). This hypothesis was initially tested in p53-null Saos-2 osteosarcoma cells, and it was revealed that WT1 inhibits p53-mediated apoptosis, which is normally induced by chemotherapy, radiation, and overexpression of wild-type p53.<sup>20</sup> Clark et al. continued to investigate this relationship between *WT1* and *p53* in GBM by examining the effect of *WT1* expression and *WT1* silencing on p53-mediated cell death and response to radiotherapy.<sup>6</sup> Results showed that *WT1* silencing led to GBM cells becoming susceptible to radiation-induced death, suggesting a potential target to improve responses to radiotherapy. The relationship of *WT1* silencing and chemotherapy was tested in a separate study by Chen et al. that revealed similar results; *WT1* silencing resulted in increased chemotherapeutic response.<sup>3</sup> It was reported in follow-up work that there was an interaction between the KTS<sup>+</sup> isoform of WT1 and wild-type p53, revealing that the potential oncogenic function of *WT1* in GBM may depend on this splicing variant.<sup>7</sup>

Additional avenues whereby WT1 promotes tumorigenesis was found in *WT1*'s relation with Wilms tumor 1-associated protein (WTAP, also known as pre-mRNA-splicing regulator WTAP). WTAP is a nuclear protein that was isolated through a yeast 2-hybrid screen and has been reported to be overexpressed in GBM cell lines.<sup>16,19</sup> Jin et al. suggested that WTAP may regulate migration and invasion of GBM cells by controlling epidermal growth factor signaling.<sup>16</sup> The link between *WT1* and cancer cell invasion was initially reported by Jomgeow et al. in TYK ovarian cancer cell lines, where in vitro experiments demonstrated that *WT1* overexpression led to an increase in cell invasion.<sup>17</sup> Kijima et al. demonstrated that GBM cell lines with downregulated *WT1* expression had decreased tumorigenicity in an intracranial in vivo assay.<sup>18</sup> Further, they suggested that *WT1* may play a role in apoptosis as cell lines transfected with anti-*WT1* shRNA showed upregulation of apoptosis-related genes when compared with control cell lines—supporting a previous report that *WT1* may be involved in apoptosis in GBM.<sup>34</sup> These preliminary studies suggest that the oncogenic role *WT1* may play in GBM cell proliferation, invasiveness, and survival involves suppressed apoptosis.

## CD97

Recent research at our institution has focused on another molecule that appears to be involved in promoting the invasiveness of GBM through a relationship with *WT1*, as an apparent target of *WT1* transcriptional activation.<sup>5</sup> The CD97 molecule, also known as TM7LN1, is a member of the epidermal growth factor–7 transmembrane (EGF-TM7) cell receptor subfamily that mediates cell-cell interactions. CD97 is a part of a 6-member family of adhesion G-protein coupled receptors that have been primarily reported as expressed on the surface of leukocytes.<sup>29</sup> Structurally, CD97 resembles all other EGF-TM7 receptors, consisting of an extracellular alpha unit, a transmembrane beta unit composed of 7 domains, and an intracellular C-terminus. The alpha unit is composed of 5 EGF-like domains and a single Arg-Gly-Asp motif, which acts as a binding site for several classes of integrins. Alternative splicing of the mRNA transcript of the alpha subunit allows 3 isoforms to be generated—EGF (1–5), EGF (1,2,5), and EGF (1,2,3,4,5)—which allows binding heterogeneity.<sup>30</sup> CD97 has 3 known ligands, which include CD55/decay accelerating factor (DAF), chondroitin sulfate, and alpha-beta integrin. CD55/DAF serves as an inhibitor of the complement system and binds to the EGF (1,2,5) domain.<sup>11,22,30</sup> Chondroitin sulfate proteoglycans are components of the extracellular matrix that have a role in cell adhesion, growth, receptor binding, and the migration of cells and binding to EGF (1,2,3,4,5) domains.<sup>29</sup> Integrins, which are transmembrane receptors involved in cell-to-cell adhesion, migration, and signaling, bind to EGF (1,2,5) and EGF (1,2,3,4,5).<sup>29</sup> The capability of these various isoforms to bind to ligands with complex functions makes CD97 an intriguing therapeutic target for cancer treatment, because it raises the possibility that an exogenous small molecule might be developed that could inhibit its function.

CD97 was first described in thyroid carcinomas, showing a high expression in undifferentiated anaplastic carcinomas.<sup>2</sup> Since this initial description in thyroid cancer, further work has found CD97 to be highly expressed in pancreatic cancers, colon cancers, and oral squamous cell carcinoma.<sup>32</sup> Chidambaram et al. were the first to describe CD97 in GBM.<sup>5</sup> They showed that *CD97* expression was significantly downregulated in all 3 GBM cell lines they analyzed after suppression of *WT1* expression. They observed that decreasing the endogenous expression of CD97 decreased the ability of cells to invade through Matrigel, suggesting that CD97 might promote cellular invasiveness. Safaee et al., using human GBM cell lines prepared at their institution, revealed through siRNA knockdown that CD97 may play a role in invasiveness and migration but not proliferation.<sup>30</sup> They reported that patients whose tumors overexpressed CD97 had a significantly shorter survival time when compared with those who had tumors that showed a downregulation of *CD97* using data from the Cancer Genome Atlas database.<sup>30</sup> The analysis included 212 patients and revealed that patients with upregulation of *CD97* had a median survival of 250 days compared with a median of 500 days for patients with downregulated expression. This preliminary study suggests that upregulation of *CD97* in GBM patients may confer a poorer prognosis, and future studies will help further elucidate this relationship.

## Conclusions

When *WT1* was first reported in brain tumors it was hypothesized that it was unlikely to be of clinical significance, but as research has evolved *WT1* has been shown to play a significant role in GBM tumor biology. *WT1* promotion of tumor invasiveness, proliferation, and survival through its interaction with p53, WTAP, and CD97 all contribute to its multifaceted oncogenic role. Molecular genetics is opening exciting new avenues in the understanding of difficult-to-treat cancers such as GBM. In an effort to capitalize on this ongoing revolution, this paper has summarized the current knowledge concerning *WT1* and GBM and this gene's potential role in tumor biology. Moving forward, the authors call upon researchers to continue to elucidate the role of the KTS<sup>+</sup> *WT1* variant in tumorigenesis, further define the interplay between *WT1* and *CD97*, and continue to develop novel therapies using these new targets. Future studies that will help further develop this emerging field of glioblastoma biology include: larger experiments that use human tissue to clinically correlate *WT1* and *CD97* expression and patient out-comes, identification of molecules that can inhibit CD97 to see if there is a survival advantage that could lead to therapeutic options for GBM patients, investigate *WT1* and *CD97* expression in recurrent GBM, and a study focusing on the microenvironment changes during GBM progression with and without treatment interventions to further investigate the multiple effects of CD97 in invasion and growth.

## Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Broaddus, Somasundaram. Acquisition of data: Somasundaram, Opalak. Analysis and interpretation of data: Somasundaram, Ardanowski, Opalak. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Broaddus. Study supervision: Broaddus, Fillmore.

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Abbreviations used in this paper: DAF = decay accelerating factor; EGF-TM7 = epidermal growth factor–7 transmembrane; GBM = glioblastoma multiforme; shRNA = short hairpin RNA; WTAP = Wilms tumor 1–associated protein (pre-mRNA-splicing regulator WTAP); WT1 = Wilms tumor 1.