Wilms’s Tumor Gene Mutations: Loss of Tumor Suppressor Function: 
A Bioinformatics Study

Rukhshan Khurshid¹, Uzma Jabbar²*, Mazhar Mushtaq³, Asmat Saleem⁴ and Attiya Abbasi⁵

¹Department of Biochemistry, Shalamar Medical and Dental College, Pakistan
²Department of Biochemistry, Fatima Memorial Medical and Dental College, Pakistan
³Department of Basic Medical Sciences, Sulaiman Al Rejhi University, Saudi Arabia
⁴International Center for Chemical and Biological Sciences, University of Karachi, Pakistan
⁵Department of Chemistry, University of Karachi, Pakistan

*Corresponding author: Uzma Jabbar, Department of Biochemistry, Fatima Memorial Medical and Dental College, Pakistan

ABSTRACT

Introduction: Mutation in the Wilms’s Tumor (WT1) gene product has been detected in both sporadic and familial cases suggesting that alteration in WT1 may disrupt its normal function. The study aims to find the protagonist amino acid in WT1 proteins by mutating these residues with other amino acids.

Material and Methods: The 3D modeling approach by MODELLER 4 was utilized to build a homology of WT1 proteins. Quality of the WT1 model was verified by predicting 10 models of WT1 and hence selecting the best one. Stereochemistry of model was evaluated by PROCHECK. Mutational studies were done by WHAT IF. Five human WT1 mutations were modeled which were Lys371→Ala371, Ser415→Ala415, Cys416→Ala416, His434→Asp434 and His434→Arg434.

Result: Based on active side of WT1 protein and its role in DNA binding mutation. No significant change was observed when Lys371 was mutated to Ala371, Ser415 was mutated to Ala415. Significant change was observed in Cys416 mutated to Ala416. In mutant Ala416, loss of coordination with the metal ion Zn was also predicted. In case of Mutants His434→Asp434, there was a loss of coordination of metal ion (Zn203) with mutant Asp434. In case of mutant His434→Arg434, there was a loss of Zn203 coordination with Arg434. His434 does not interact directly with any DNA base, whereas mutated Arg434 is predicted to interact directly with DNA base.

Conclusion: It is concluded that mutation of amino acid residue Cys416→Ala416, His434→Asp434 and His434→Arg434 may lose the proto-oncogenic function of WT1.

Keywords: WT1 protein, MODELLER9.0, Mutation, Active side residues

Introduction

WT1, is a protein, which in humans is encoded by the WT1 gene on chromosome 11p13. The WT1 is responsible for the normal kidney development. Mutations in this gene are reported to develop tumors and developmental abnormalities in the genitourinary system. Conversion of proto-oncogenic function to oncogenic in WT1 has also been documented cause of various hematological malignancies.

Multifaceted protein of WT1 gene has transcriptional factor activity [1]. It regulates the expression of insulin-like growth factor and transforming growth factor system, implicated in breast tumorigenesis [2]. A main function of WT1 is to regulate transcription, which control the expression of genes involved in the process of proliferation and differentiation [3]. In wide range of tumor, WT1 is shown to be predisposing factor for cancer, therefore it has become hot target in research to find out its inhibitor which can be safely used as a treatment of cancer. It can induce apoptosis in embryonic cancer cell, presumably through the withdrawal of required growth factor survival signal [4]. WT1 is involved in the normal tissue homeostasis and as an oncogene in solid tumors, like breast cancer [5]. Increased expression of WT1 is related with poor prognosis in breast cancer [6]. A number of hypotheses are postulated for the relationship of WT1 with tumorigenesis. According to one of the hypothesis, elevated levels of WT1 in tumors may be related with increased proliferation because normally WT1
have a role with apoptosis [7,8]. Another study proposed that WT1 can alter many genes of the the family of BCL2 [9,10] and also have a role to regulate with Fas-death signaling pathway [11]. Furthermore, it is suggested that WT1 can encourage cell proliferation by up-regulation of protein cyclin D1 [12].

A group of workers hypothesized that WT1 has been observed in the vasculature of some tumour types [13] and its expression may be related with angiogenesis especially in endometrial cancer [14]. Another hypothesis based on the fact that WT1 is a main regulator of the epithelial/mesenchymal balance and may have a role in the epithelial-to-mesenchymal transition of tumor cells [3]. Expression of WT1 is higher in Estrogen Receptor (ER) positive than in ER negative tumors. It is therefore possible that WT1 not only interact with ER alpha, but it may orchestrate its expression [15]. A study, on triple negative breast cancers [7] has shown that high WT1 levels associate with poor survival due to increased angiogenesis [16,17], altered proliferation/apoptosis [10,11], and induction of cancer-epithelial-mesenchymal transition [4]. In breast tumors, WT1 is mainly related with a mesenchymal phenotype and increased levels of CYP3A4 [18]. A mutation in the zinc finger region of WT1 protein has been identified in the patients that abolished its DNA binding activity [19]. A study also observed that the mutation in the WT1 gene product has been detected in both sporadic and familial cases suggesting that alteration in WT1 may disrupt its normal function [20]. Bioinformatics approaches are being utilized to resolve the biological problems. Efforts start with the prediction of 3D structures. To achieve the aim, study was designed to view 3D structure of WT1, tumor suppressor protein predicted by homology modeling and to study the role of crucial residues in WT1 proteins by mutating these residues with other amino acids.

Material and Methods

Structure Prediction

3D structure of WT1 was taken as target of human WT1. Figure 1 shows the normal interaction of WT1 with DNA strands based on the crystal structure of a zinc finger protein.

The binding of protein-DNA complex involves four zinc finger binding domain in the C-terminal/Zn finger region of WT1. These are;

(i) Cys325, Cys330 and His339, His343: figure 2; yellow highlighted.
(ii) Cys355, Cys360 and His373, His377: figure 3; yellow highlighted.
(iii) Cys385, Cys388 and His397, His401: figure 4; yellow highlighted.
(iv) Cys416, Cys421 and His434, His435: figure 5; yellow highlighted.

Results and Discussion

The study was largely based on active side of the WT1 and its role in DNA binding mutation. Zinc finger binding domain interact selectively and non-covalently. This zinc finger-binding domain is the classical zinc finger domain, in which two conserved cysteine and histidine co-ordinate a zinc ion at the active site.

Cys416→Ala416 MUTANT

Significant change was observed in Cys416 mutated to Ala416. In mutant Ala416, reduction in the Van der Waal’s contact between the amino acids. Loss of coordination with the metal ion Zn was also predicted (Figure 6 A and B).
Cys416 is located at the domain interface with its polar side chain completely buried (0.00 Å). Replacement of this amino acid may account for considerable changes in the interior of protein (Table 1). We have predicted the possible changes that arise due to the mutation of Cys to Ala by molecular modeling experiments. Amino acids, Pro419, Ser420, Cys421, His434 and some atoms of His438 (ND1, NE2, CD2 and CE1) are present near Cys416. Zinc (Zn203) is also present in the vicinity (1.82 Å) of Cys416 (Figure 6 A and B). The mutated residue, Ala is also predicted to remain buried (0.00 Å) in the interior of protein. Significant change is observed however, in the surrounding area of the mutated Ala416. Only a few atoms of His434 (CD2 and NE2) and His438 (CE1) were seen in the surrounding. This may reduce the Van der Waal's contacts between the respective amino acids. The loss of coordination with the metal ion, zinc was also predicted as the distance is increased from 1.82 Å to 3.12 Å. It is therefore predicted that Cys416 plays a vital role in the interaction with other amino acid residues as well as in the metal coordination. It is observed that there is a possibility of loss of these interactions in case of Cys416 replacement.

Figure 2-5: Amino Acid sequence of human Wilms Tumor protein. Highlighted C-terminal fragment are the zinc binding domain of the protein.
His434→Arg434 MUTANTS

In case of mutant His434→Arg434, there was a loss of zn203 coordination with Arg434. His434 does not interact directly with any DNA base, whereas mutated Arg434 is predicted to interact directly with DNA base, A1. This suggests that change might effect on the DNA binding pattern, Figure 7 A and B.

In case of mutation of His434→Arg434, the distance between the mutated Arg and zinc (Zn203) was increased from 2.28 Å to 5.00 Å suggesting that there could be a loss of coordination with the metal ion. Mutational studies proved that hydrogen bonding network close to the zinc-binding motif plays a significant role in stabilizing the coordination of the zinc metal ion to the protein [23]. The mutated amino acid, Arg434 also moved considerably form buried to relatively exposed environment (2.28 Å to 5.35 Å). Presence of positively charged Arg on the surface could account for additional interaction of the protein with other proteins or with the surrounding water molecules. His434 does not interact directly with any DNA base whereas mutated Arg434 is predicted to interact directly with DNA base Adenine, A1. (Figure 7 A and B). This suggests that the change might cause the DNA binding pattern.
Comparison of surface accessibilities (Å) of the wild type and mutated residues and those in the vicinity of the mutated residues in the five WT1 mutants

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Wild type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu370</td>
<td>12.93</td>
<td>13.28</td>
</tr>
<tr>
<td>Lys/Ala371</td>
<td>47.04</td>
<td>33.66</td>
</tr>
<tr>
<td>Cys416</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ser/Ala415</td>
<td>7.96</td>
<td>7.62</td>
</tr>
<tr>
<td>His434</td>
<td>8.96</td>
<td>0.53</td>
</tr>
<tr>
<td>Cys/Ala416</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Glu430</td>
<td>14.83</td>
<td>0.00</td>
</tr>
<tr>
<td>His/Asp434</td>
<td>8.96</td>
<td>0.53</td>
</tr>
<tr>
<td>Glu430</td>
<td>14.83</td>
<td>0.00</td>
</tr>
<tr>
<td>His/Arg434</td>
<td>8.96</td>
<td>4.43</td>
</tr>
</tbody>
</table>

**Lys371→Ala371 and Ser415→Ala415 MUTANT**

No significant change was observed when Lys371 was mutated to Ala371, and Ser415 was mutated to Ala415. It is observed in this mutation that the change that arise in the overall structure and surrounding amino acid residues (Table 1). Lys371 is present on the surface (accessibility = 47.04 Å) of the WT1 molecule. It was observed that the internal protein structure was not affected considerably, as Lys371 is present on the outer most surface of the protein. In the original model, Lys371 stacks against thymine. It also forms a water-mediated contact with side chain hydroxyl of Ser367. Although, Ala371 also stacks against the same DNA base but the distance is slightly altered. The hydrogen bond between Ala371 and Ser367 has not been predicted in the mutated model. It has been demonstrated that mutation within finger 2 and 4 abolished sequence specific binding of WT1 to DNA bases [19]. The mutation of the corresponding lysine in a peptide could reduce its affinity for DNA seven folds [27]. On the other hand, it is reported [28] that a surface mutation would not cause a significant change in the internal structure of protein. However, the replacement of a basic polar residue with a non-polar one could account for a reduction in polarity. The modeling studies of Lys to Ala mutation do not however support this finding and require further analysis.

Mutation of Ser415→Ala415 in the WT1 model (Table 1). Ser415 is located near the active center of WT1. It has been demonstrated that Ser415 makes a water-mediated contact with phosphate of DNA base, guanine [20]. In our predicted model of WT1, Ser415 makes two water (numbers 516 and 568) mediated contacts. Mutation of this Ser with Ala resulted in the loss of one of these contacts leading to the loss of binding. The replacement of relatively polar residue, Ser to a non-polar one, Ala could account for this reduced interaction. This is also evident by a slight decrease in the accessibility of Ala (Ser415 = 7.96 Å; Ala415 = 7.61 Å).

**His434→Asp434 MUTANTS**

In case of mutants His434→Asp434, there was a loss of coordination of metal ion (zn203) with mutant Asp434. Glu430 move from relatively exposed to completely buried environment. His434 is also present at the active center of WT1. We predicted two mutants; His434→Asp434 and His434→Arg434 mutants by molecular modeling (Table 1). In case of His434→Asp434 mutation, the water mediated contact is lost. The distance between mutated Asp and zinc (Zn203) was also increased from 2.28 Å to 3.57 Å suggesting that there could be a loss of coordination with the metal ion as well. The amino acid Glu340 that is present near His434 also moved considerably form relatively exposed to completely buried environment (14.83 Å to 00.0 Å).

**Conclusion**

It is concluded that mutation of amino acid residue Cys416→Ala416, His434→Asp434 and His434→Arg434 of WT1 may lose its function to regulate the function of genes by binding to specific parts of DNA. Besides the mutation of above-mentioned amino acid residue, the role of WT1 in cell growth, cell differentiation, apoptosis and tumor suppressor function is also lost.

**Conflict of Interest**

Authors declares no conflict of interest.

**References:**


27. Fukasawa KM, Hirise J, Hata T, Ono Y (2010) In rat dipeptidyl peptidase III, His568 is essential for catalysis, and Glu507 or Glu512 stabilizes the coordination bond between His455 or His450 and zinc ion," Biochimica et Biophysica Acta 1804: 2063–2069.