Zinc Finger DNA Recognition and 17 Amino Acids Residues, a Transcriptor Repressor in WT1: Analysis of Base Specificities by Site Directed Mutagenesis

Rukhshan Khurshid[1], Muhammad Mustansar[*,1], Shamim Akram[2], M. Zamir Ahamad[2] and Atiya Abbasi[2]

[1]*Department of Biochemistry, Fatima Jinnah Medical College, Lahore, Pakistan*

[2]*Department of Biochemistry, Allama Iqbal Medical College, Lahore, Pakistan*

Abstract: The Wilms' tumor suppressor gene encodes a zinc finger DNA binding protein (WT1), which functions as a transcriptional regulator and has been shown to be involved in the progression of breast cancer. Mutation in the zinc finger region of WT1, originally identified in Wilms' tumor patient, has been associated with loss of its DNA-binding activity. We have studied the intra-molecular ionic interactions at active site residues, in particular the four Cys(2)-His(2) pairs of WT1. We have also mutated some of the crucial amino acid residues involved in binding of DNA and observed the effect of these mutations on the binding pattern. Of the five WT1 mutants analyzed, significant change was observed in Cys416Ala416 mutant where reduction in the van der Waal's contact was seen between the amino acids, which may also affect coordination with the Zn ion. Two other mutants His434Asp434 and His434Arg434 also showed a loss of coordination of Zn ion with the mutant residue, Asp434. His434 did not interact directly with A1, which may affect the DNA binding pattern and change the normal functioning of WT1. However no significant change was observed in the Ser415Ala415 mutant.

Keywords: Tumor suppressor protein WT1, 3D structure prediction, mutation, breast cancer.

INTRODUCTION

Cell growth has been shown to be under strict control of a number of positive and negative signals. Studies indicate that an imbalance in these signals has been the major cause of deregulation of the cellular behavior. Recessive oncogenes or tumor suppressor genes encoding important cellular proteins have been identified as the potential negative regulator of cell cycle [1]. Inactivation of tumor suppressor genes, by allelic deletion, loss of expression, mutation or functional inactivation by interacting with oncogene products of DNA tumor viruses or with amplified cellular binding proteins leads to uncontrolled cell growth or malignant formations [2]. Malignant transformations occur through successive mutations in specific cellular genes, leading to the activation of oncogenes and inactivation of tumor suppressor genes [1].

Analyses of primary breast tumors have revealed a large number of dominant and recessive gene alterations encompassing several cellular attributes and activities. It is quite likely that some of these alterations are of a causal nature thus enabling the tumor to attain distinctive malignant phenotypes, such as, dys-regulated proliferation, invasion, angiogenesis, and ability to metastasize [3].

*Address correspondence to this author at the Department of Biochemistry, Fatima Jinnah Medical College, Lahore, Pakistan; Tel: 0333-4971233; 0313-4971233; E-mail: dr_mustansar@yahoo.com

The Wilms' tumor suppressor gene encodes a zinc finger DNA binding protein (WT1) which functions as a transcriptional regulator and has been implicated in the progression of breast cancer [4, 5]. It is found that WT1 protein contributes to breast cancer progression by promoting breast cancer cell proliferation [6]. WTI has been shown to repress the transcription of a variety of target genes like EGFR and c-MYC genes whose products stimulate growth factors, growth factor receptors and other transcriptional elements [7,8]. The tumor suppressor activity of the WT1 gene in regulation of the mammotrophic IGF-IR/IGF-II growth factor system as well as the mammary growth inhibitor TGF-1 makes it a candidate for action in the mammary gland. The regulation by WT1 normally maintains the cells of mammary gland in mitotic arrest and loss or inactivation of WT1 would thus lead to unregulated cell division and tumors [9,10]. Recently it is reported that Wilms' tumor 1 protein and focal adhesion kinase mediate keratinocyte growth factor signaling in breast cancer cells [11].

WT1 encodes nuclear proteins with structural motifs characteristic of transcription factors, including an N-terminal glutamine/proline-rich transregulatory domain and a C-terminal domain with four Kruppel-type Cys_2-His_2 zinc fingers which bind DNA and RNA and are involved in nuclear localization [11]. The discovery of cytoplasmic WT1 in the tumor cells suggests that inactivation of WT1 may occur as a result of restricted

E-ISSN: 2310-9556/16

access to nuclear targets. Recent studies indicate that cytoplasmic retention inhibits the normal regulatory functions of WT1 [8].

Studies also indicate that WT1 can induce apoptosis in embryonal cancer cells, presumably through the withdrawal of required growth factor survival signals, and that EGFR is a physiological target gene for WT1 [7].

Mutations in the zinc finger region of WT1, originally identified in a Wilms' tumor patient, has been shown to abolish the DNA-binding activity suggesting that the WT1 protein may act at the DNA binding site of growth factor-inducible genes and that loss of DNA-binding activity contributes to the tumorigenesis [12].

The present communication highlights the key intra-molecular ionic interactions involving active site residues. The interaction of these residues with DNA appears to orient the N-terminal region of the molecule to plays an important role in transcriptional regulation. Furthermore, mutation of some of the crucial amino acid residues does influence the DNA binding pattern thus contributing to tumor metastasis.

METHODS

3 Dimensional Structure (Model)

3 Dimensional structure (Model) was retrieved from Hydrogen bonding, surface accessibilities, and ion pair in the homology model of WT1 were calculated by HBONDS, ACCESS, and ANACON menus of WHAT IF [13].

DNA Binding Studies

Interactions of WT1 with DNA were studied by superposing the structure of znf268 (protein plus bound DNA) over the 3D model of WT1 using MODELLER Program [14]. The structural coordinates of znf268 were then manually deleted.

RESULTS AND DISCUSSION

Structural Organization

Figure (**1**) presents the schematic representation of WT1 model. The predicted structure of C-terminal region of WT1 comprises of 144 residues folded into 4 helices and 8 beta pleated sheets. The region consists of hydrophilic residues, which are mostly acidic in nature. A group of workers [15] observed that in case of all zinc finger proteins, acidic residues are located in the recognition helix and make a number of water mediated contacts with DNA bases and phosphates.

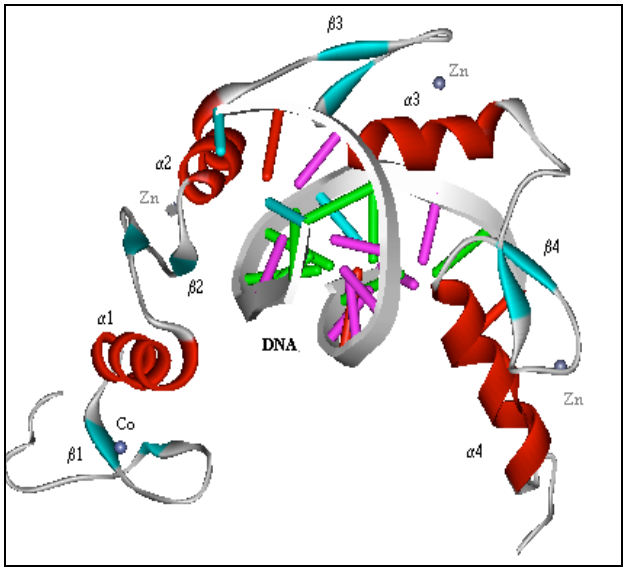


Figure 1: Homology model of the C-terminal fragment of Human Wilm's Tumor protein (WT1) with bound DNA strands based on the crystal structure of a zinc finger protein, znf268, (Pdb id:1aay) and a five-finger protein, GLI (pdb id:2gli).

Human WT1 model shows possible coordination of one cobalt and three zinc atoms with conserved residues i.e. 2 cysteines from each sheet and 2 histidines from each helix. These are held together by a hydrophobic core and by the cobalt/zinc atom.

Active Site of WT1 and its Role in DNA Binding

Analysis of WT1 indicates that like other zinc finger proteins the carboxyl terminal domain contains Cys_2-His_2 Zn–finger motifs which are responsible for DNA and RNA binding. Four such regions could be identified i.e. (i) Cys325, Cys330 and His339, His343 (ii) Cys355, Cys360 and His373, His377, (iii) Cys385, Cys388 and His397, His401 and (iv) Cys416, Cys421 and His434, His435. Analyses of the role of these residues in DNA binding shows that Cys325 and Cys330 does not pair with DNA although Cys330 binds with the cofactor, cobalt (cobalt was used in this study along with zinc and assumed to behave like divalent cation required for the function WT1). Cys330 also make hydrogen bond with Tyr327. Cys355 and Cys360 binds with the cofactor, zinc (Zn201) having a separation distance of 2.98Å and 1.52Å respectively. Cys385 and Cys388 bind with the cofactor, Zn202 having separation distance of 2.09Å and 1.12 Å respectively. Cys416 is also within a distance of 1.32Å with Zn203 and forms hydrogen bond with Lys423. On the other hand,

Cys421 has Zn203 within the radius of 1.65Å. It is worth mentioning that all cysteine residues present in the Zn finger regions of WT1 showed no interaction with DNA bases but maintain the stability with formation of hydrogen bonds.

Among the histidine residues, His339 and His343 showed no pairing with DNA bases. These residues, however, show hydrogen bonds with each other. His 339 also forms a hydrogen bond with Met342. His343 showed coordination with the cofactor, cobalt present within a radius of 2.96Å. On the other hand, His373 (ND1) makes a hydrogen bond with phosphate (O1P) of the nucleotide, G7 and also coordinates with the cofactor (zinc201) which is present within the radius of 3.05Å. Besides, it also forms hydrogen bonds with amino acids, Gln369 (O) and Arg376 (N). His377 does not interact with DNA bases but showed contact with zinc (Zn201) present within the distance of 0.94Å. His377 forms sch:sch and mch:mch hydrogen bonds respectively with Cys355 and Gln374. Although His397 showed no bonding with DNA bases it is the only amino acid at the active center showing ionic interactions with Asp396. Three salt bridges can be seen between His397 (ND1, NE2 and ND1) and Asp396 (OD1, OD1 and OD2) respectively. His401 makes one hydrogen bond each with phosphate (O2P) of G4 ans Thr404 in addition to coordination with the cofactor, zinc (Zn202) residing at a distance of 1.51 Å. His434 showed coordination with Zn203 within the distance of 2.23Å and hydrogen bonds with Glu430 and Met437 but no pairing with any DNA base. On the other hand, His435 showed no contact either with the DNA bases or the metal ions. However it makes mch:mch hydrogen bonds with Leu431 and His 438 and mch:sch hydrogen bond with Arg417.

The primary contact in the protein-DNA complex involves Arg433-guanine and His434–guanine interactions along one strand of the DNA. These interactions appear to stabilize the long side chain of arginine and enhance the specificity of Arg-guanine contacts [16]. In the predicted WT1 model, Arg433 makes water mediated contact with a number of DNA bases like A1, G2, C3 and G60. However, no His434-nucleotide interaction could be seen in the model.

Interestingly a 17 amino acid residues region located in the transcription regulatory domain seems to function as a transcriptional repressor when fused with the DNA binding domain of WT1 [17]. The results of a group of workers [18] indicated that the WT1 17 amino acids isoform exerted its oncogenic functions through modulation of cytoskeletal dynamics.The predicted model of C-terminal region of WT1 shows that this region of the domain (Ser420-Asn436) is highly charged and contains Cys-His-Zn motif responsible for RNA and DNA binding.

Detailed listing of these amino acids and the specific binding interactions with DNA bases is given in Table **1**.

Table 1: Detailed Listing of 17 Amino Acids and their Binding with DNA Bases

Amino acids	Metal/Bases
Ser420	Zn203
Cys421	Zn203
Gln422	Zn203
Lys423	Zn,203 A1(C5)
Phe425	G2(OIP)
Ala426	C3(OIP)
Arg427	C3,G4,T5(N3C4O4 C5C5MC6) C57(N4),A58(C6N6N1)
Ser428	C56(O,OIP.C2O5O6) C57(N4)
Glu430	G2 (C8,C2,O5) C3(O1P,O2P,C5,N4)
Val432	C57(P,O1P,O2P)
Arg433	A1(C2N3C4)G2(N7) C3(N4C4) G60(O6)
His434	Zn203

The zinc finger binds in the major groove of DNA and wraps part way around the double helix. Each finger has a similar relation to the DNA and makes its primary contacts in a 3 base pair subsite [16]. A study [19] demonstrated that the recognition helices of the individual fingers are inserted into major groove of DNA at 3 base pair intervals. DNA recognition is mediated through base contacts with the side chains of amino acid located at 4 positions of the recognition helix (i.e. amino acid at positions 1, 2, 3 and 6). Hydrogen bonds are formed when amino acids at these positions contact the nucleotide bases in the DNA major groove. Each helix is oriented such that position 1 on the helix amino terminal interacts at the 3' end of the binding site. The residue at position 1 usually contacts the 3 bases of each DNA triplet. Position 3 contains residue, which recognizes the middle base of the triplet. Position 2 seems to play an auxiliary role in DNA recognition. Although residues at positions 1, 2, 3 and 6 are directly implicated in DNA binding other residues in zinc finger protein and also control DNA affinity. A group of workers [15] have refined the Zif268 protein-

DNA complex and made a detailed analysis of zinc finger-DNA interactions. Human WT1 shares 62% sequence homology with Zif268 with all 3 fingers of the C-terminal region being conserved. We have analyzed the role of these residues in DNA binding with WT1.

Role of Helix Residues 1, 2, 3 and 6 in DNA Binding

In the helix 1, Lys, Ser and His occupy 1st, 2nd and 3rd position respectively. Among these both Lys, Ser form hydrogen bonds with Met342. His present at the 3rd position make a hydrogen bond with His343 and is stacked against guanine 4, thymine 5 and guanine 6 giving rise to van der Waal interactions. However, no base pairing of these amino acid residues has been observed. Also histidine-thymine interactions contribute significantly towards site specific recognition and substitution of uracil at this position results in reduced binding [20]. In Zif268 protein, helices 1 and 3 have glutamic acid, instead of histidine at the 3rd position.

In helix 2, Asp368 present at position 2 makes three hydrogen bonds with Arg366 immediately preceding the helix. In helix 3 and 4, Aspartic acid residues (396 and 429) present at position 2 do not interact with Arg394 and Arg427. Analysis of DNA protein interactions in helix 2 shows that Asp368 stacks with T51 (O5) whereas in helix 3, Asp396 forms a hydrogen bond with DNA base, C55 (N4). In the helix 4, Asp429 (OD2) interacts with DNA base, C3 (N4) (Figure **2**). Among Arginine residuess, Arg366 (NH1) of helix 2 forms two hydrogen bonds with T11 (O4), A52 (N1) and A52 (N7). Arg366 (NE) also makes a hydrogen bond with A52 (N6). Arg394 in helix 3 showed no pairing with DNA bases whereas Arg427 in helix 4 shows two H-bonds with G4 and one with T5 (Figure **3**).

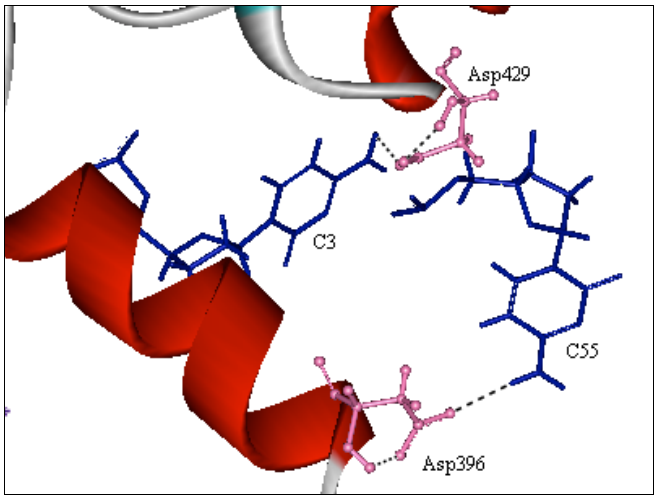


Figure 2: Hydrogen bonded interactions between amino acid residues (Asp396 and Asp429) and DNA bases (C3and C55) present at position 2 of helix 3 and 4. Amino acid residues are shown in ball and stick (pink) and DNA bases in stick (blue) configuration.

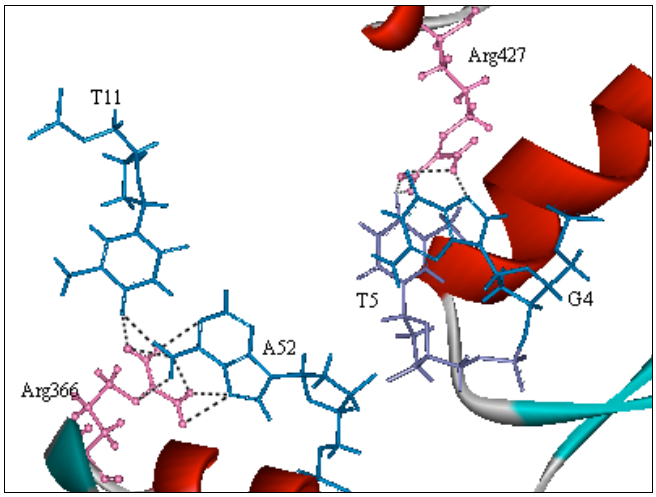


Figure 3: Hydrogen bonded interactions between amino acid residues (Arg366 and Arg427) and DNA bases (A52, T11, G4 and T5) present at position 1 of helix 2 and 4. Amino acid residues are shown in ball and stick (pink) and DNA bases in stick (blue) configuration.

Studies by Erickson and coworkers indicate that the hydrogen bond between the aspartic acid at position 2 and arginine at position 1 orients the arginine side chain thus increasing the specificity of arginine-guanine interaction [15].

In the case of WT1, helices 2 and 4 have glutamine (Gln369) and glutamic acid (Glu430) respectively at the 3rd position of the helix. Gln369 showed no interaction with DNA bases. It however forms two H-bond with Arg366. Glu430 also showed no direct interaction with DNA base. Instead, it forms a hydrogen bond with Arg427 which stacks with the base, C3. Furthermore, glutamic acid/glutamine, in analogy with histidine at the corresponding position in helix 2, contacts the base cytosine present at the center of the GCG triplet recognized by these fingers [19, 20]. These interactions are expected to stabilize the conformation of residues of the helix and enhance the specificity of contacts made by arginine at position 1.

In Zif268 protein, helices 1 and 3 have an arginine at position 6 of the helix. In contrast, WT1 has Met342 at 6th position in helix 1, which shows no base pairing. Thr400 is at the 6th position of helix 3. It forms a water mediated contact with O1P of G4. Arg372 and Arg433

are present at the 6th position of helix 2 and 4 respectively. Arg372 makes two contacts, one with N4 of C9 and the other with O6 of G54. Arg433 shows four hydrogen bonds, three with various nitrogen atoms of A1, and one with N4 of C3 (Figure **4**). This is consistent with the findings [20], which observed water mediated arginine-guanine interactions and visualized its possibly contribution towards the specificity of these positions.

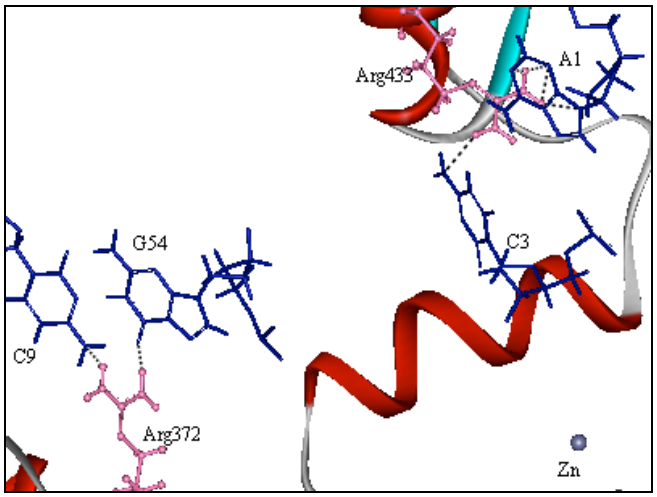


Figure 4: Hydrogen bonded interactions between amino acid residues (Arg372 and Arg433) and DNA bases (C9, G54, C3 and A1) present at position 6 of helix 2 and 4.

Linker Sequence (TGEKP)

NMR spectroscopy of DNA binding proteins reveal a highly specific DNA induced helix capping at the C-terminus. Capping involves residues TGEPK in the linker sequence, between the fingers [21]. These zinc finger linkers seem to play a significant role in DNA binding specificity. It has been observed that substitution of 3 amino acids in the linker sequence result in a 50 fold reduction in DNA specificity, concomitant with an eight fold reduction in affinity [22]. In contrast to this, X-ray crystallographic study [23] suggests that the linker sequence plays only a passive role in DNA recognition.

In WT1, the linker sequences are positioned at 317-322, 348-352, 378-383 and 409-413 of helix, 1, 2, 3 and 4 respectively (Table 3.2.6) and with the exception of helix 1 (317-322), all other threonine residues are present in the coils which join helices with sheets. These linker sequences following each helix provide substantial binding energy to DNA complexes of zinc finger 1-3 of TF111A and the 4 zinc fingers of Wilm's tumor suppressor protein. Furthermore, these sequences are highly conserved in Cys(2)-His(2) zinc finger protein. Comparison of the canonical linker structure of the zinc finger-DNA complexes with the NMR structure of the TGEKP linkers connecting finger 1 and 2 in Zif268, in the absence of DNA, reveals that additional stabilization in the DNA complexes arises most likely from the hydrogen bonding between the backbone amide of Glu3 and the side chain of Thr1 in the linkers [24]. In the predicted WT1 model, however, there is no bonding between Thr317 and Glu319 in the first linker sequence (317-322). Instead, Thr317 forms hydrogen bonds with Ser313. Other hydrogen bonds like Ser318(OG)-Ser318(O), Lys320(NZ)-(O)Arg333 were also observed in this linker region.

Table 2: Hydrogen Bonding in the Linker Sequence

Linker Sequence	H-Bonding with Amino Acid Residues	H-Bonding with DNA Bases
TSEKR(317-321)		
Thr317	Thr317(N)-(O)Ser313 (2.62Å)	-
Ser318	Ser318(N)-(O)Ser318 (2.59Å)	-
Glu319	-	-
Lys320	Lys320(N)-(O)Arg333 (2.75Å)	-
Arg321	-	-
TGEKP(348-352)		
Thr348	Thr348(N)-(O)Ser344 (3.39Å) Thr348(OG1)-(O)Arg345 (2.70Å)	-
Gly349	Gly349(N)-(O)Arg345 (2.87Å)	-
Glu350	-	-
Lys351	-	C8 (O1P,O5)
Pro352	-	-
TGVKP(378-382)		
Thr378	Thr378(N)-(O)Gln374 (3.09Å) Thr378(OG1)-(O)Arg375 (3.19Å)	-
Gly379	Gly379(N)-(O)Arg375 (2.62Å)	-
Val380	Val380(N)-(OG1)Thr378	-
Lys381	-	-
Pro382	-	-
TSEKP(409-413)		
Thr409	Thr409(OG1)-(O)Thr409 (2.62Å)	-
Ser410	-	-
Glu411	-	-
Lys412	Lys412(N)-(O)Ser410 (2.65Å)	-
Pro413	-	-

In the linker region, Thr348-Pro352, hydrogen bonding is observed between Thr348- Ser344 but no hydrogen bond could be seen between Thr348 and Glu350. On the other hand, Gly349 and Thr348 form hydrogen bonds with Arg345 whereas Lys351 showed

hydrogen bonds with the DNA base, C8 (atoms, O1P and O5).

Table 3: Comparison of Surface Accessibility (Å^2) of the Wild Type and Mutated Residues and those in the Vicinity of the Mutated Residues in Four WT1 Mutants

Amino Acid	Wild Type (Ser415)	Mutant (Ala415)
Cys416	0.00	0.00
Ser/Ala415	7.96	7.62
His434	8.96	0.53
Cys/Ala416	0.00	0.00
Glu430	14.83	0.00
His/Asp434	8.96	0.53
Glu430	14.83	0.00
His/Arg434	8.96	4.43

In the linker sequence, Thr378-Pro382, Thr378 shows 2 hydrogen bonds: Thr378(N)-(O)Gln374 and Thr378(OG1)-(O)Arg375 whereas Gly379 forms water mediated contact with phosphate (O1P) of G6 and Val380 (N) makes a hydrogen bond with side chain OG1 of Thr378. Lys381 also form water mediated contact with phosphates (O1P, O2P) of DNA bases, T5 and G6.

In the linker sequence, Thr409-Pro413, the side chain OG1 of Thr409 shows a hydrogen bond with O within the same residue i.e. Thr409. Ser410 forms a hydrogen bond with Lys412 but no interaction could be seen with DNA.

A study [15] described the role of lysine residues in the linkers. They found that Lys381 located in the linkers between fingers 1 and 2, forms a pair of water mediated contacts with the 5' phosphate of base 5 whereas Lys412 (in the linkers between finger 2 and 3) makes a similar water mediated contact with the 5' phosphate of base 2. In the predicted structure of WT1 Lys351, located in the linkers between finger 1 and 2, makes water mediated contacts with O5 of G8 and O1P of C8. Lys381, located in the linkers between fingers 2 and 3, also shows a pair of water mediated contacts with base, T5. Lys412 (in the linkers between finger 3 and 4) however, does not interact with any base. A group of workers [23] found that the mutation of corresponding lysine derived from TF111A reduces its affinity for DNA approximately by seven folds indicating that the lysine residue in fact forms water mediated phosphate contacts. These studies also clearly indicate that sequence capping the helix does recognize the DNA and facilitates its specific binding with zinc finger.

Mutational Studies

It is well known that alterations in the sequence of the purine and pyrimdine bases in a gene due to change, removal or insertion of one or more bases can lead to an altered gene product which in turn results in a mutated protein [25,26]. Mutations in the WT1 gene product have been detected in both sporadic and familial Wilms' tumors, suggesting that alterations in WT1 may disrupt its normal function as a transcriptional regulator [27,28].

In the present study we have modeled four WT1 mutants and analyzed the accompanying changes in the WT1 model.

Lys371Ala371 Mutant

Lys371 is present on the surface (accessibility = 47.04 Å) of the WT1 molecule. It stacks against thymine, T51 (C5 and O5 atoms) and forms a water-mediated contact with side chain hydroxyl (OG) of Ser367. Mutation of Lys371 with Ala does not affect the internal structure of the protein (Table 3.2.7a). Analysis of the mutant protein shows that Ala371 also stacks against the same DNA base but with a slightly altered distance. The hydrogen bond seen between Lys371 and Ser367 could not be traced in the mutant model. A Study [29] indicate that mutation within finger 2 and 4 abolished sequence specific binding of WT1 with the DNA bases. Thus mutation of the corresponding lysine containing peptide could lead to a reduction in affinity for DNA [15]. The observation on Lys Ala is however supported by the analysis [30] which shows that surface mutations do not cause a significant change in the internal structure of protein. However, replacement of a basic polar residue with a non-polar one would lead to a reduction in polarity. The Lys Ala mutation thus requires further analysis.

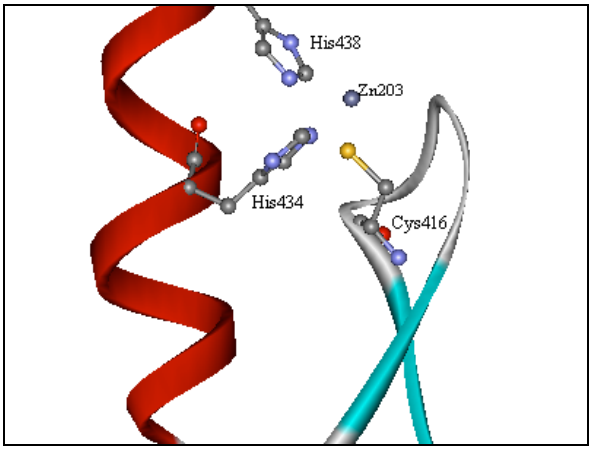
Ser415Ala415 Mutant

Ser415 is located near the active center of WT1 and forms water mediated contact with phosphate of DNA base, guanine [22]. In the predicted model of WT1, Ser415 establishes two such (number 516 and 568) contacts. Mutation of Ser with Ala results in the loss of one of these binding sites (Table **3**). The replacement of a relatively polar residue Ser, with a non-polar residue Ala could account for reduced interaction,

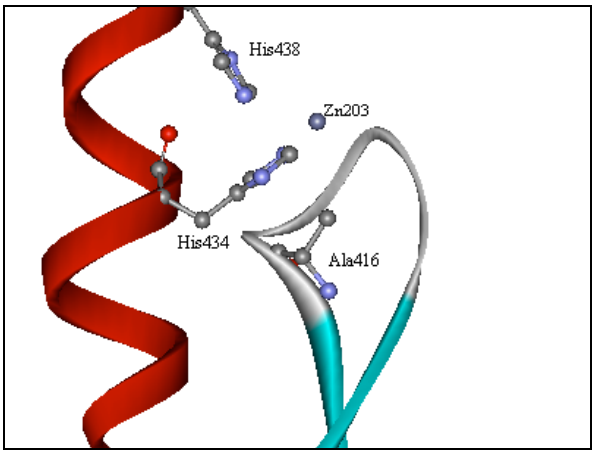
which is also evident by the slight decrease in the accessibility of Ala (Ser415= 7.96 Å^2, Ala415=7.61Å^2).

Cys416Ala416 Mutant

Cys416 is located at the domain interface with its polar side chain completely buried (0.00 Å^2). Replacement of this amino acid may account for considerable changes in the interior of the protein molecule (Table **3**). 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 (1.8 Å) of Cys416 (Figure **5**). Although the mutated residue i.e. Ala is also buried (0.00 Å^2) in the interior of protein significant change is observed in the surrounding of the mutated residue. Only a few atoms of His434 (CD2 and NE2) and His438 (CE1) were seen in the vicinity, which may reduce the van der Waal's contacts between the respective amino acids. The loss of coordination with the metal ion, zinc is also observed as the distance increases form 1.32 Å to 3.12 Å. Thus Cys416 seems to play a vital role by virtue of its interaction with other amino acid residues as well as metal coordination.



(**a**)



(**b**)

Figure 5: Wild Type (Cys416) and mutated (Ala416) WT1. Distance between Zn and Cys416 is increased in mutated (Ala416) model. Cys416 is predicted to be found in the vicinity of His434 and His438 which are implicated in catalysis (**a**) while Ala416 can only interact with His434 and not with His 438 in the mutated model (**b**).

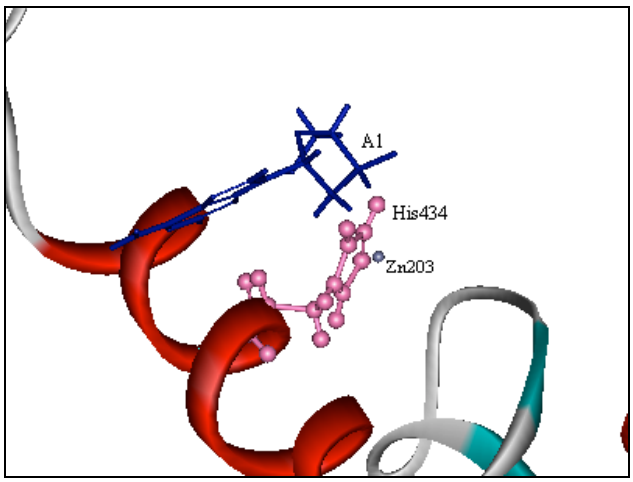
His434Asp434 and His434Arg434 Mutants

His434 is also present at the active center of WT1. Two mutants, His434Asp434 and His434Arg434 have been studied by molecular modeling (Table **3**). In the case of His434Asp434 mutation, the water-mediated contact is lost. The distance between mutated Asp and zinc (Zn203) also increased form 1.51 Å to 3.57 Å suggesting a possible loss of coordination with the metal ion. In the case of His434Arg434 the mutated residue Arg434 moves from a considerably buried to a relatively exposed environment (2.28 Å^2 to 5.35 Å^2). Presence of positively charged Arg on the surface may lead to additional interactions with other proteins or with surrounding water molecule. His434 does not interact directly with any DNA base whereas mutated Arg434 shows direct interaction with Adenine, A1 (atoms O4 and O5) (Figure **6**). Thus mutation of His434 with Arg might result in interaction with DNA. It is worth mentioning that mutations in Zn^{2+}-coordinating histidine residues can cause structural disruption of zinc finger leading altered transcriptional efficiency [23].

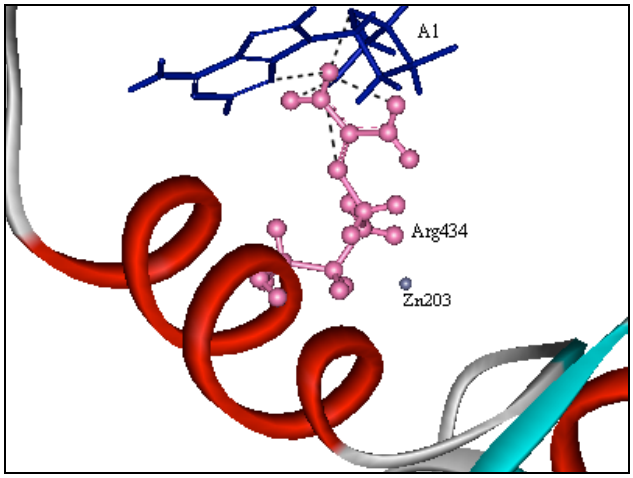
CONCLUSION

Wilms' tumor gene, WT1 encodes a zinc finger protein. The protein is made up of several motif where each motif folds around a central zinc ion to form an independent mini-domain where adjacent zinc fingers combine to form a DNA binding domain responsible for "gripping" the DNA.

WT1 is present in normal breast tissue and appears to be developmentally regulated whereas a high percentage of breast tumor cells express little or no WT1 protein. In normal tissue, WT1 is present in the nucleus. Its absence in tumors *in situ* suggests that altered WT1 expression may coincide with the first appearance of tumor cells possibly reflecting an event related to its cause. The cytoplasmic localization of WT1 suggests its functional inactivation in a subset of tumors.



(**a**)



(**b**)

Figure 6: Wild Type (His434) and mutated (Arg434) WT1. Distance between Zn and His434 is increased in mutated model. Arg434 is predicted to bind DNA base A1 (**a**) while His434 in the original model (**b**) showed no bonding with DNA base.

Phosphorylation of WT1 by protein kinase may result in the cytoplasmic retention of WT1 protein and in turn lead to transcriptional suppression of number of growth factors like the over expression of IGF-IR common to breast cancer.

Analysis of the WT1 model provides a framework for understanding the process of DNA recognition by zinc finger proteins and suggests a useful base for the design of novel DNA binding proteins. The WT1 protein-DNA complex shows the normal zinc finger protein structure with four Cys(2)-His(2) pairs found in the C-terminal/Zn finger region. Among these all Cysteine bind with the cofactor but shows no pairing with DNA. Among histidine residues His373 makes a hydrogen bond with phosphate of nucleotide G7. His401 also makes a H-bond with phosphate of G4.

In the predicted structure of WT1 Asp396 and Asp429 present at position 2 of helix 3 and helix 4 respectively, make H-bonds with DNA base C55 and C3 respectively. Among the arginines present at position 1 of each helix, Arg366 of helix 3 and Arg427 of helix 4 makes a number of H-bonds with T11, A52 and T5, G4 respectively. These amino acids widen the major groove to make it deeper. Also in helix 1, His339 is present at the 3rd position and is stacked against G4, T5 and G6 making van der Waal's interaction which may play a significant role in site-specific recognition. In helix 2 and 4, glutamine (Gln369) and glutamic acid (Glu430) are present at the 3rd position of helix respectively. Gln369 (OE1) and Glu430 (OE1) form H-bond with Arg366 and Arg427 respectively. These interactions may help to stabilize the conformation of the residues of the helix. Thr400 present at the 6th position of helix 3 makes a water mediated contact with O1P of G4. Arg372 and Arg433 are present at the 6th position of helix 2 and 4 respectively. Arg372 interacts with C9 and G54 whereas Arg433 interacts with A1 and C3.

Furthermore a 17 amino acid residue sequence patch inserted between the transcription regulator domain appears to functions as transcription repressor, when fused with DNA binding domain of WT1. This domain contains Cys-His-Zn motif responsible for DNA and RNA binding. The zinc fingers bind in the major groove of DNA and wraps part way around the double helix. DNA recognition is mediated through base contacts with the side chains of amino acid located at 4 positions of the recognition helix (i.e. amino acid at positions 1,2, 3 and 6. Hydrogen bonds are formed when amino acids at these positions contact bases in the DNA major groove.

Like other zinc finger proteins WT1 also possesses linker sequence in each helix. The linker sequence caps the helix, recognizes the DNA and facilitates the specificity of DNA binding with zinc finger. All these sequences are stabilized by H-bonds within the linker residues as well as with other amino acids. Only Lys351 makes a H-bond with DNA base C8.

Analysis of the 5 WT1 mutants showed no significant change in the case of Lys371→Ala and Ser415→Ala mutants whereas significant change was observed in Cys416→Ala mutant where reduction in

the van der Waal's contact was seen between the amino acids, which may also affect coordination with the metal ion Zn. Also the two other mutants His434→Asp and His434→Arg also showed a loss of coordination of metal ion (Zn203) with mutant Asp434. In the Asp434 mutant, Glu430 moves from a relatively exposed to completely buried environment. Also His434 does not interact directly with any DNA base, whereas the Arg434 mutant interacts directly with A1 which may effect the DNA binding pattern.

REFERENCES

[1] D Grander. How do mutated oncogenes and tumor suppressor genes cause cancer? Med Oncol. 1998; 15: 20-6. http://dx.doi.org/10.1007/BF02787340

[2] X Gao, KV Honn. Recessive oncogenes: current status. Pathol Oncol Res. 1995; 1: 7-22. http://dx.doi.org/10.1007/BF02893578

[3] SH Dairkee, HS Smith. Genetic analysis of breast cancer progression. J Mammary Gland Biol Neoplasia 1996; 1: 139-51. http://dx.doi.org/10.1007/BF02013638

[4] L Yang, Y Han, F Suarez Saiz, MD Minden. A tumor suppressor and oncogene: the WT1 story. Leukemia. 2007; 21 (5): 868-76.

[5] Y Oji, Y Miyoshi, E Kiyotoh, S Koga, Y Nakano, A Ando, N Hosen, A Tsuboi, M Kawakami, K Ikegame, Y Oka, H Ogawa, S Noguchi, H Sugiyama. Absence of mutations in the Wilms' tumor gene WT1 in primary breast cancer. Jpn J Clin Oncol. 2004; 34: 74-7. http://dx.doi.org/10.1093/jjco/hyh012

[6] P Zapata-Benavides, M Tuna, G Lopez-Berestein, AM Tari. Downregulation of Wilms' tumor 1 protein inhibits breast cancer proliferation. Biochem Biophys Res Commun. 2002; 295(4): 784-90. http://dx.doi.org/10.1016/S0006-291X(02)00751-9

[7] DM Cook, MT Hinkes, M Bernfield, FJ. 3rd Rauscher. Transcriptional activation of the syndecan-1 promoter by the Wilms' tumor protein. Oncogene 1996; 13: 1789-99.

[8] GB Silberstein, K Van Horn, P Strickland, CT Roberts Jr, CW Daniel. Altered expression of the WT1 Wilms tumor suppressor gene in human breast cancer. Proc Natl Acad Sci USA 1997; 94: 8132-8137. http://dx.doi.org/10.1073/pnas.94.15.8132

[9] CE Patek, MH Little, S Fleming, C Miles, JP Charlieu, AR. Clarke, K Miyagawa, S Christie, J Doig, DJ Harrison, DJ Porteous, AJ Brookes, ML Hooper, and ND Hastie A zinc finger truncation of murine WT1 results in the characteristic urogenital abnormalities of Denys-Drash syndrome. Genetics. 1999; 96: 2931-2936.

[10] DA Haber, RL Sohn, AJ Buckler, J Pelletier, KM Call, DE Housman. Alternative splicing and genomic structure of the Wilms tumor gene WT1. Proc Natl Acad Sci USA 1991; 88: 9618-22. http://dx.doi.org/10.1073/pnas.88.21.9618

[11] XP Zang, JT Pento, AM Tari. Wilms' tumor 1 protein and focal adhesion kinase mediate keratinocyte growth factor signaling in breast cancer cells. Anticancer Res 2008; 28: 133-7.

[12] FJ Rauscher 3d, JF Morris, OE Tournay DM, Cook, T Curran. Binding of the Wilms' tumor locus zinc finger protein to the EGR-1 consensus sequence. Science 1990; 250: 1259-62. http://dx.doi.org/10.1126/science.2244209

[13] A Sali, TL Blundell. Comparative protein modeling by satisfaction of spatial restraints. J Mol Biol. 1993; 234: 779-815. http://dx.doi.org/10.1006/jmbi.1993.1626

[14] RA Laskowski, MW McAurthur, DS Moss, JM Thornton. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Cryst. 1993; 26: 283-291. http://dx.doi.org/10.1107/S0021889892009944

[15] M Elrod-Erickson, TE Benson, CO Pabo. High resolution structures of varient Zif268-DNA complexes: Implications for understanding zinc finger-DNA recognition. Structure. 1998; 6: 451-64. http://dx.doi.org/10.1016/S0969-2126(98)00047-1

[16] NP Pavletich, CO Pabo. Zinc finger-DNA recognition: Crystal structure of a Zif268-DNA complex at 2.1Å. Science 1991; 252: 809-817. http://dx.doi.org/10.1126/science.2028256

[17] ZY Wang, QQ Qiu, J Huang, M Gurrier, TF Deuel. Products of alternatively spliced transcript of Wilm's tumor suppressor gene WT1, have altered DNA binding specificity and regulate transcription in different ways. Oncogene 1995; 10: 415-22.

[18] T Jomgeow, Y Oji, N Tsuji, Y Ikeda, K Ito, A Tsuda, T Nakazawa, N Tatsumi, N Sakaguchi, S Takashima, T Shirakata, S Nishida, N Hosen, M Kawakami, A Tsuboi, Y Oka, K Itoh, H Sugiyama. Wilms' tumor gene WT1 17AA(-)/KTS(-) isoform induces morphological changes and promotes cell migration and invasion in vitro. Cancer Sci. 2006; 97(4): 259-70. http://dx.doi.org/10.1111/j.1349-7006.2006.00169.x

[19] AC Jamieson, SH Kim, JA Wells. In vitro selection of zinc fingers with altered DNA-binding specificity. Biochemistry. 1994; 33: 5689-5695. http://dx.doi.org/10.1021/bi00185a004

[20] AH Swirnoff, J Milbrandt. DNA binding specificity of NGFI-A and related zinc finger transcription factors. Mol Cell Biol 1995; 15: 2275-2287. http://dx.doi.org/10.1128/MCB.15.4.2275

[21] JH Laity, HJ Dyson, PE Wright. DNA-induced alpha-helix capping in conserved linker sequences is a determinant of binding affinity in Cys(2)-His(2) zinc fingers. J Mol Biol 2000; 295: 719-27. http://dx.doi.org/10.1006/jmbi.1999.3406

[22] RF Ryan MK Darby. The role of zinc finger linkers in P43 and TFIIIA binding to 5S rRNA and DNA. Nucleic Acid Res. 1998; 26: 703-709. http://dx.doi.org/10.1093/nar/26.3.703

[23] Choo and Klug Selection of DNA binding sites for znf using randomly randomized DNA reveals codon interaction. Proc Natl Acad Sci USA 1994; 91: 11168-72. http://dx.doi.org/10.1073/pnas.91.23.11168

[24] J Nardelli, T Gibson, P Charnay. Zinc finger DNA recognition, analysis of base specificity by site directed mutagenesis. Nucleic Acid Res. 1992; 20: 4137-44. http://dx.doi.org/10.1093/nar/20.16.4137

[25] Murray RK, Granner DK, Mayer PA, Rodwell VW. Harper's Biochemistry. 25th ed. Appleton and Lange USA 2000; 788-90.

[26] Wardlaw GM. Contemporary Nutrition. Issue and Insight. 4th ed. McGraw Hill, Boston. 2000; pp. 250.

[27] Rosenfeld R, Margalit H. Zinc fingers: Conserved properties that can distinguish between spurious and actual DNA binding motif. J Biomol Struct Dyn. 1993; 11: 557-70. http://dx.doi.org/10.1080/07391102.1993.10508015

[28] Gupta S, Joshi K, Wig JD, Arora SK. High frequency of loss of allelic integrity at Wilms' tumor suppressor gene-1 locus in advanced breast tumors associated with aggressiveness of the tumor. Indian J Cancer. 2009; 46(4): 303-10. http://dx.doi.org/10.4103/0019-509X.55550

[29] Hejtmancik JF. The genetics of cataract: Our vision becomes clearer. Am J Hum Genet. 1998; 62: 520-25. http://dx.doi.org/10.1086/301774

[30] Nakagama H, Heinrich G, Pelletier J, Housman DE. Sequence and structural requirements for high-affinity DNA binding by the WT1 gene product. Mol Cell Biol. 1995; 15: 1489-98. http://dx.doi.org/10.1128/MCB.15.3.1489

Received on 26-10-2015 Accepted on 12-12-2015 Published on 10-05-2016

DOI: http://dx.doi.org/10.20941/2310-9556.2016.04.1