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Exploring the impact of F270V mutation in the β-tubulin (*Bos Taurus*) structure and its function: a computational perspective

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Abstract Paclitaxel is the most effective chemotherapeutic agent used for the treatment of a broad spectrum of solid tumors. However, observed paclitaxel resistance in clinical trials presents one of the major obstacles for cancer chemotherapy. Most importantly, resistance due to β-tubulin mutations (F270V) has been intensely debated in recent years. Despite all efforts, mechanism of resistance is still not well understood. In this study, computational techniques were employed to uncover the effect of F270V mutation in the β-tubulin structure and its function. The tools such as MuStab, CUPSAT and I-Mutant were employed to address the consequence of F270V mutation in the structural stability of β-tubulin. Further, molecular simulation study was employed to understand the functional impact of β-tubulin mutation. We believe that this study will provide useful guidance for the development of novel inhibitors that are less susceptible to drug resistance.

Keywords F270V mutation · Molecular docking · Molecular dynamics · Paclitaxel

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Introduction

Cancer results from alterations in essential regulatory genes that control cell proliferation, differentiation and survival. Owing to lack of cell cycle regulation, there is an uncontrolled cell proliferation, which ends up into cancer cells (Stewart et al. 2003). Microtubules are the vital components that have a central role in cell division which are major targets in cancer chemotherapy (Shing et al. 2014). β-tubulin (globular protein) is a heterodimer composed of α- and β-tubulin subunits that form the microtubules. The function of microtubules is strongly coupled with their stability. In the mitotic phase of the cell cycle, microtubules are in dynamic equilibrium with β-tubulin dimers by assembling the β-tubulin into microtubules or, conversely, disassembling microtubules to β-tubulin. The interruption in the dynamic equilibrium can induce cell cycle arrest and eventually lead to apoptosis. Therefore, the compounds that disrupt the dynamics of β-tubulin polymerization and microtubule depolymerization would be useful in the treatment of cancer (Jordan et al. 1998). Recently, paclitaxel has emerged as a potential drug for treating several solid tumors including breast, ovarian and non-small cell lung carcinomas (NSCLC) by the disruption of microtubule dynamics (Yin et al. 2010). It is believed that binding of paclitaxel stabilizes the microtubule structure by inducing conformational change. This in turn affects the normal formation of mitotic spindles, chromosome segregation and subsequently leads to mitotic arrest (Gascoigne and Taylor 2009). However, as like with

many cancer chemotherapeutic agents, the emergence of paclitaxel resistant cancer cells has greatly limited its clinical efficacy (Drukman and Kavallaris 2002; Dumontet and Sikic 1999). Cell culture techniques have identified several mechanisms of resistance in patients undergoing therapy (Orr et al. 2003). One resistance mechanism is the over expression of ATP-binding cassette (ABC) transporters. ABC transporters cause drug resistance by actively pumping a variety of drugs, including microtubule agents such as taxanes and vinca alkaloids out cells at the expense of ATP hydrolysis (Hari et al. 2006). The other possible mechanisms of drug resistance are the activation of the enzymes of the glutathione detoxification system and alterations of the genes and the proteins involved into the control of apoptosis (Choi 2005). In addition, the microtubule dynamics could also play a major role in the paclitaxel resistance (Wang and Nogales 2005). Recent evidence indicates that overexpression of heat shock transcription factor 1 enhances the resistance of cancer cells to paclitaxel (Vydra et al. 2013). However, one reported paclitaxel resistance mechanism that has received huge attention in recent years involves β-tubulin mutations (Berrieman et al. 2004). Most importantly, literature evidence indicates that F270V mutation in β-tubulin can affect microtubule stability and interfere with drug-target binding (He et al. 2001). In fact, the resistance is expected to become an increasingly significant problem when more patients are treated with this drug. To overcome these obstacles and to improve cancer therapy, the pharmaceutical industries have focused their efforts on identifying novel compounds that target not only the native β-tubulin but also the mutant one. These efforts could be achieved only by the complete understanding of the structural alterations in its intracellular target, β-tubulin. Keeping this in mind, molecular dynamics (MD) simulation study was initiated with the help of available structures. The results from our analysis aid not only in understanding of the active pocket for paclitaxel but are also helpful in gaining a much clearer picture of the key residues in β-tubulin.

Materials and methods

Data set

The crystal structures of β-tubulin (*Bos taurus*) used in our analysis were obtained from the Protein Data Bank

(PDB). The corresponding PDB code is 1TVK (Xu et al. 2012) and the mutant structure was generated using Swiss-PDB viewer (Guex and Peitsch 1997). Paclitaxel was used as the drug molecule for our studies. The SMILES notation for paclitaxel was retrieved from PubChem (NCBI) (Feldman et al. 2006) and submitted to CORINA for deducing the 3D structure of paclitaxel molecule (Gasteiger et al. 1990). All the water molecules and the heteroatoms were removed before performing MD simulation.

Recognition of ligand binding site residues of β-tubulin

Protein ligand interactions are the most fundamental to all biological mechanisms. These interactions are highly specific and are the consequence of distinct molecular interaction properties of the binding sites. Therefore, the analysis of binding site residues are certainly important for the understanding of ligand affinity and ultimately for the molecular underpinnings of protein functions. In the present investigation, we employed SITECOMP server (Lin et al. 2012) to obtain the binding site locations in β-tubulin structure. The SITECOMP program uses molecular interaction fields (MIFs) for the analysis. The PDB Code 1TVK was submitted into the program to get the binding residues information in the β-tubulin structure. Moreover, literature evidence (Ganesh et al. 2004) was also used to validate the obtained results.

Protein stability analysis

First, we studied the impact of F270V mutation in the β-tubulin structural stability. The protein stability change upon F270V mutation was analyzed using three different approaches with increasing resolution. Initially, MuStab program was employed (Teng et al. 2010). It uses machine learning technique for predicting protein stability changes upon amino acid substitutions. The biochemical features, structural features and various biological features were considered. For instance, amino acid composition, conformational parameters for alpha helix, beta sheet, and average buried area in folded state, polarity and number of codons for amino acid as a classifier were the main parameters utilized to predict protein stability changes due to single amino acid variations.

Subsequently, Cologne University Protein Stability Analysis Tool (CUPSAT) was employed for the prediction and analysis of protein stability changes upon point mutations. In the CUPSAT approach, coarse-grained atom potentials and torsion angle potentials were used to predict protein stability upon point mutations (Parthiban et al. 2006). Finally, the I-Mutant program was also employed for investigating the impact of F270V mutation in β-tubulin stability. It is an artificial neural network based system to predict the direction towards which the mutation shifts the stability of the protein instead of directly estimating the relative stability changes upon protein mutation (Raghav and Sharma 2013). It is also believed that I-Mutant is one of the most reliable predictors existing in the literature (Khan and Vihinen 2010).

Protein ligand docking

Autodock (Morris et al. 2009) examines ligand conformations comprehensively and estimates the binding affinity of the drug molecule. Initially, polar hydrogen was added to the structure of β-tubulin. Subsequently Kollman charges and Gasteiger charges were assigned to all atoms and rotable bonds were assigned, using Autodock Tools. The free binding energies between β-tubulin and paclitaxel were estimated, using atom affinity potentials pre-calculated, using AutoGrid4 on grid maps. Grid center was focused on the active site and $60 \times 60 \times 60$ frame lattice with grid positioning of 0.375 Å (Rathinasamy et al. 2010) were determined. For molecular docking, Global And Local Search (GA-LS) method was used (Iman et al. 2011). For GA different parameters were followed: a maximum number of 250,000 energy evaluations; a maximum number of generations of 27,000; mutation and crossover rates of 0.02 and 0.8, respectively. Pseudo-Solis & Wets parameters were used for local search and 300 iterations of Solis & Wets local search were imposed. Both Autogrid and Autodock computations were performed on Cygwin (Iman et al. 2013) and ten independent docking runs were performed for each native and mutant β-tubulin protein. Autodock evaluates the ligand binding through the conformational search space using a Lamarckian genetic algorithm (Morris et al. 2009). Final Autodock result was analyzed using Autodock tools, graphical user interface of Autodock.

Molecular dynamics stimulation

The native and mutant type's β-tubulin and paclitaxel docked complexes were used as starting structure for performing MD simulation. The GROMACS package 4.6.3 was (Elengoe et al. 2014) implemented with the GROMOS43a1 force-field that was used to run MD simulation (Van Der Spoel et al. 2005). The system was solvated in cubic 0.9 nm, using periodic boundary conditions and the SPC water model (Meagher and Carlson 2005). The PRODRG server (Schuttelkopf and Van Aalten 2004) was utilized to prepare ligand topology file. 1000 steepest descent energy minimization steps were carried out with no constraints to minimize the systems energy. After energy minimization, the system was equilibrated at constant temperature and pressure. The equilibrated complex structures were then exposed to MD simulations for 20,000 ps and the integration time was set to 2 fs. The atom-based cutoff of 8 Å method was used to treat the van der Waal's interactions. The particle-mesh Ewald algorithm was used to treat the long-range electrostatic interactions (Darden et al. 1999). For Lennard–Jones interaction 0.9 nm cutoff was employed. During the stimulations, all bond lengths containing hydrogen were controlled by using Lincs algorithm (Lindahl et al. 2001). For structural analysis the trajectory snapshots were stored at every pico-second. Potential energy, intramolecular H bonds, root mean square fluctuation (RMSF), radius of gyration (Rg) and solvent accessible surface area (SASA) details were analyzed, using GROMACS conveniences g_energy, g_hbond, g_rmsf, g_gyrate and g_sas respectively. Graphing, advanced, computation and exploration (GRACE) program was utilized in the MD data analysis.

Results and discussions

Protein stability analysis

Identifying the change in the stability of the protein structure allows the biologist to quickly annotate the functions of the protein. Hence, initially we investigated the stability of β-tubulin protein upon mutation (F270V) using MuStab, CUPSAT and I-Mutant. MuStab program predicts whether the mutation increases or decreases the stability of the protein.

Most importantly, it gives the prediction confidence. The prediction results clearly indicate that F270V mutation in β-tubulin structure affect the structural stability of the protein. It is noteworthy to mention that prediction confidence of 88.21 % was observed for the F270V mutation. Subsequently, we used I-Mutant and CUPSAT program to predicts the free energy change for F270V mutation in β-tubulin structure. The values are −0.41 and −1.45 kcal/mol respectively. The negative value indicates the destabilizing effect of F270V mutation in β-tubulin structure (Entwistle et al. 2008). These results hypothetically depict that the mutation, Phenylalanine into Valine at position 270, was significantly affects the structural stability of the β-tubulin (Venselaar et al. 2010; Orr et al. 2003). Moreover, the conservation score is also examined in our analysis, using ConSurf program. This service is available at http://consurftest.tau.ac.il. The results indicate that native type residue is more conserved (Score 7) than mutant type residue (Score 4) in the β-tubulin structure. Thus, mutation at this conserved position damages the protein structure and its function as well.

Molecular docking studies

The docking procedure implicates the prediction of ligand conformation and orientation within a targeted binding site. In general, the docking study is essential for the precise prediction of activity of drug molecule against the target structure. In this study, GROMACS package 4.6.3 was used to minimize the structure of native and mutant protein. One thousand steps of steepest descent energy minimization were carried out for the β-tubulin proteins. Initially, the binding site residues information in the structure of β-tubulin were obtained, using SITECOMP server and the results were validated using available literature evidence (Ganesh et al. 2004). Subsequently, flexible docking studies were performed using Autodock program. The binding free energies of the native and mutant types of β-tubulin paclitaxel complex determined by Autodock were −8.11 and −5.31 kcal/mol, respectively. The binding energy difference indicates that the incompetent binding of paclitaxel with mutant (F270V) protein. Moreover, LIGPLOT analysis was carried out to study the possible reasons for the decreased binding affinity of paclitaxel with mutant structure. This result is shown in Fig. 1. The dotted lines and red

arc represents the hydrogen and hydrophobic contacts in the complex structures respectively. It is evident from the figure that 5 residues in native type protein such as Arg282, Gly360, Arg276, His227 and Asp224 are involved in the hydrogen bonding with paclitaxel. On the other hand, only four hydrogen bonding interactions were observed in the mutant structure. For instance, Arg282 did not involve in the drug binding process. This is mainly because of the differences in size, charge and hydrophobicity-value of the original native-type residue and newly introduced mutant residue. In fact, the Valine is smaller than Phenylalanine and this might lead to the loss of interactions. Furthermore, the differences in the properties of native and mutant type residue alter the conformations of the binding pocket in the β-tubulin structure. For instance, Arg282 moved away from the cavity in the mutant type (6.1 Å), whereas it was found closer to the drug molecule in the native type (2.1 Å). This is shown in Fig. 2. In addition, mutation introduces a more hydrophobic residue at this position. Hence, the displacement of residue alongside hydrophobic make up of the binding site is certainly a critical factor for the Paclitaxel resistance. These evidences also bring to the hypothesis that interaction formed by the Arg282 residue is certainly important for the effective binding of paclitaxel with β-tubulin. It is noteworthy to mention that the results obtained from this study correlate well with the available experimental evidence (Contini et al. 2012) (Table 1).

Molecular dynamics analysis

As ligand binding is a microscopic event that takes place in mere millionths of a second, a complete understanding of the mechanics and energetic of binding are unattainable, using current experimental techniques. This issue could be addressed with the aid of MD simulations study (Durrant and McCammon 2011). In the present study, MD simulations were performed for native and mutant β-tubulin protein and paclitaxel complexes, using GROMACS package that execute the GROMOS96 force field parameters. The data such as Potential Energy, Hydrogen bond, RMSF, Rg and SASA details were analyzed from the MD trajectory file. Potential energy graphs along the MD simulation showed that the potential energy of the proteins stabilizes since the first ps of simulation (Fig. 3a), with negligible variation in potential energy,
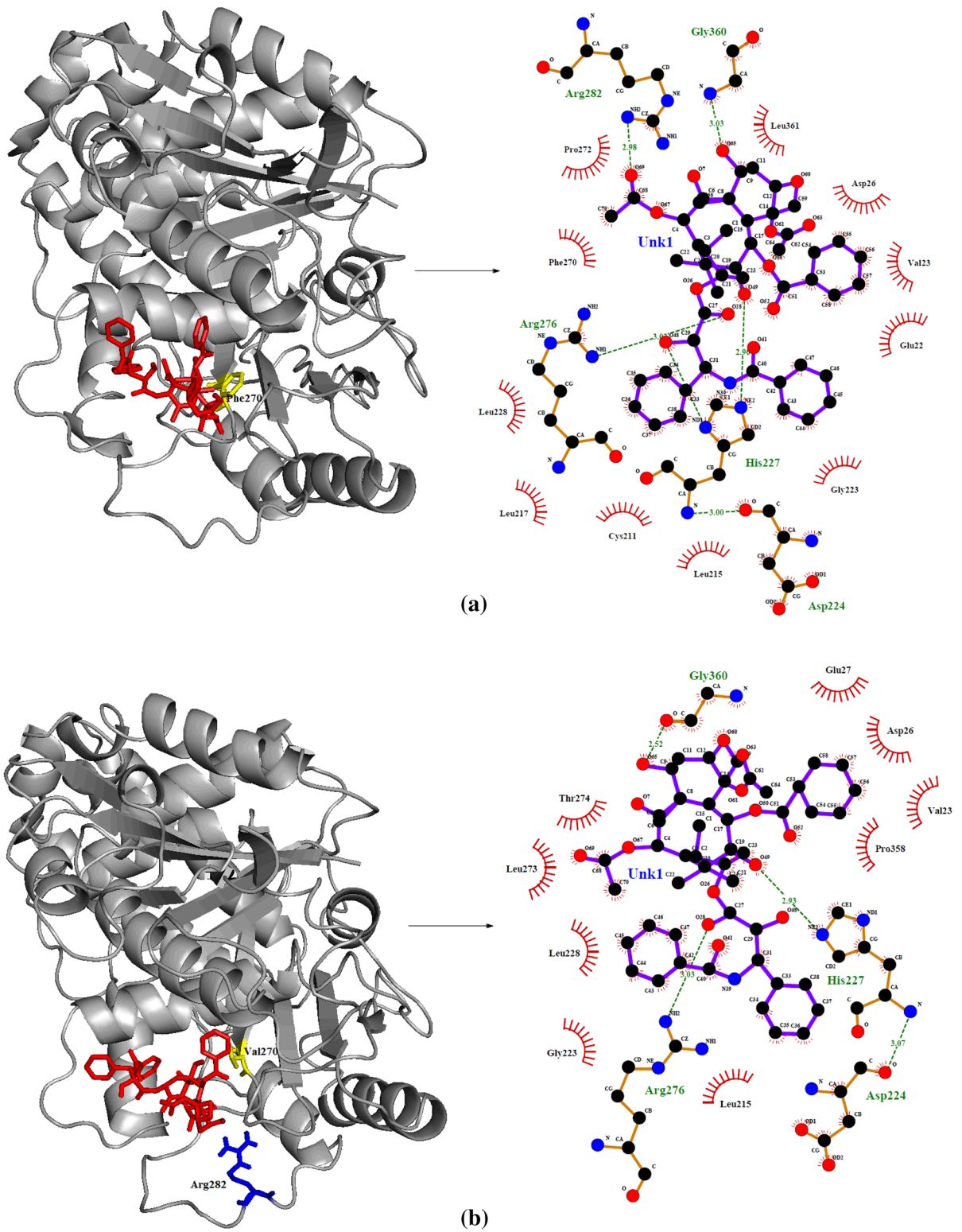
Fig. 1 Representation of intermolecular interactions in **a** native and **b** mutant (F270V)—paclitaxel complexes

Fig. 2 Distance between Arg282 and paclitaxel in **a** native and **b** mutant (F270V) paclitaxel complex structures

(a)

(b)
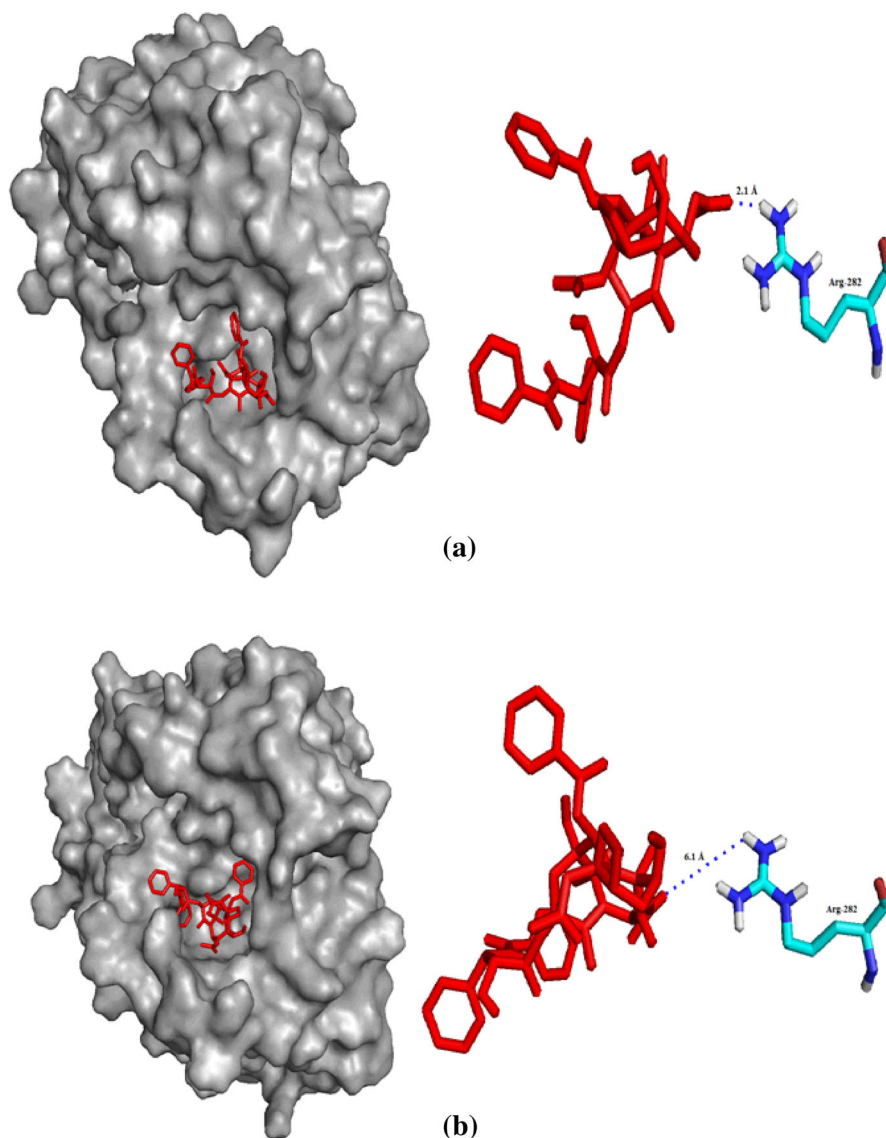
Table 1 Analysis of free binding energy of paclitaxel with native and mutant (F270V) type β-tubulin protein

S.no.	β Tubulin-paclitaxel complex	Intermolecular energy (kcal/mol)	Total internal energy (kcal/mol)	Torsional free energy (kcal/mol)	Unbound systems energy (kcal/mol)	Binding free energy (by autodock) (kcal/mol)
1.	Native	−13.18	−5.82	+5.07	−5.82	−8.11
2.	Mutant	−10.38	−8.66	+5.07	−8.66	−5.31

indicating that the potential energy remains constant, thus suggesting structural stabilization. Furthermore, the intramolecular hydrogen bond details were analyzed during the MD simulation study. The result is shown in Fig. 3b. It is clear from the figure that native

type protein can maintain maximum of 375 intramolecular hydrogen bonds, whereas the mutant type protein can maintain only only 350–360 hydrogen bonds during the simulation. Most importantly, the frequency of occurrence of hydrogen bonding interaction
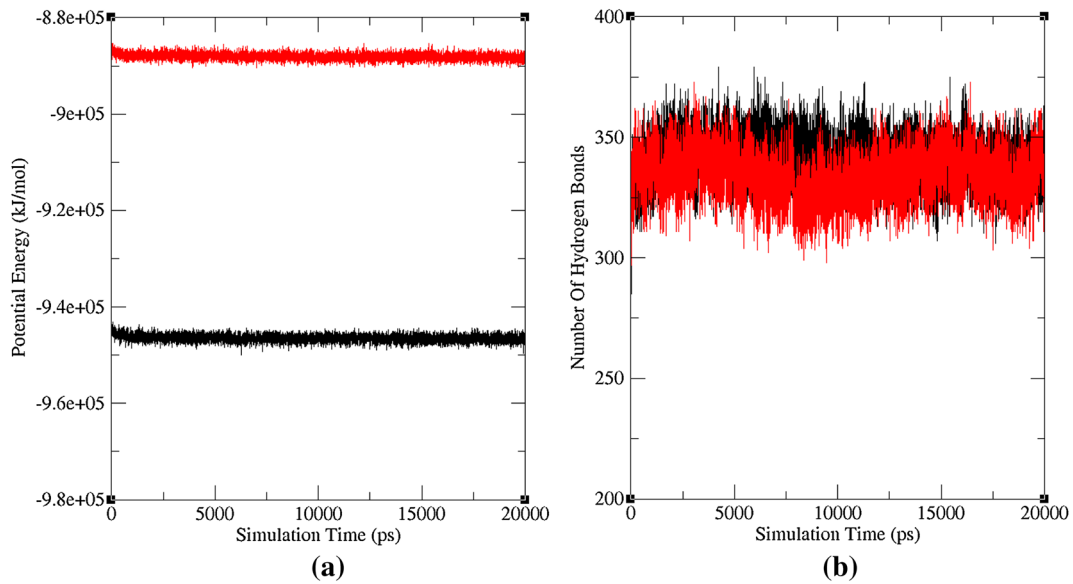
Fig. 3 Molecular dynamics simulation study: **a** Potential energy analysis, **b** Intramolecular hydrogen bond analysis. [Coloring scheme—native (*black*) and mutant (*red*)]
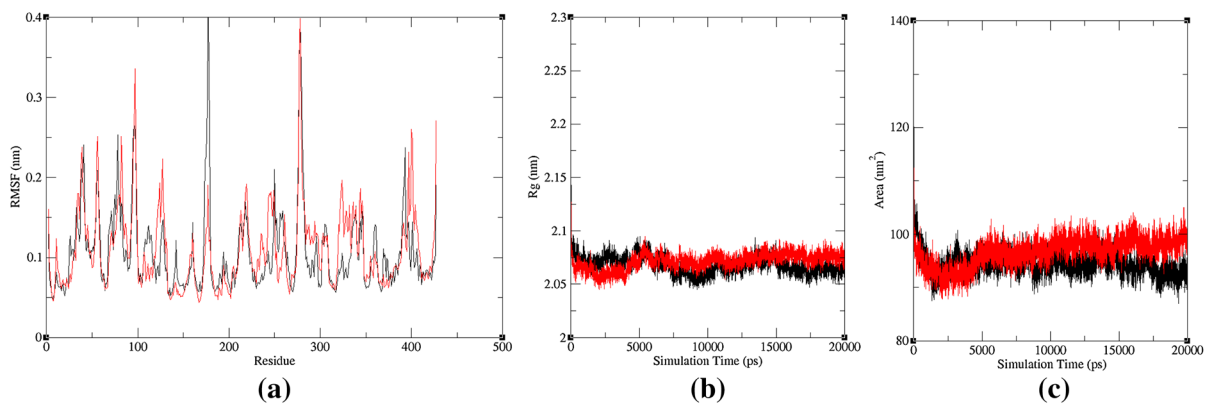
Fig. 4 Molecular dynamics simulation study: **a** RMSF analysis, **b** radius of gyration analysis and **c** solvent-accessible surface area analysis. [Coloring scheme—native (*black*) and mutant (*red*)]

was significantly higher for the native type β-tubulin than mutant type β tubulin throughout simulation time. These evidence undoubtedly indicates that mutation at position 270 significantly alters the conformations of the amino acid, and thus confers resistance for paclitaxel binding. Furthermore, RMSF analysis was initiated not only to gain insights into the existence of intramolecular interactions but also to understand the cause of inefficient binding of paclitaxel with mutant protein. The result is shown in Fig. 4a. It is evident from figure that flexibility of the residues of mutant type β-tubulin is higher than that of native type

residues. The higher flexibility in mutant type residues is the evidence of lesser involvement of these residues in hydrogen bonding formation in the complex structures. We believe that this inherent flexibility of amino acid residues is likely to play an important role in the ligand binding. Finally, the Rg and SASA for the native and mutant structure of β-tubulin was analyzed. Rg is indicative of level of compaction in the structure i.e. how folded or unfolded the polypeptide chain is. The results are shown in Fig. 4b, c indicate that Rg and SASA were significantly higher for the mutant type of β-tubulin compared to the native type. This shows that

after mutation the overall structure becomes more unfolded during the simulation. This results in the loss of its structural activity and leads to improper binding of paclitaxel.

Conclusions

Despite recent advances in treatment modalities, cancer remains a major source of morbidity and mortality throughout the world. The reasons for this failure are certainly diverse but the key unresolved issue in anticancer chemotherapy is resistance against anticancer drugs. In particular, the involvement of β-tubulin mutations as a cause of paclitaxel resistance is the major concern in cancer chemotherapy. In the present study, we have investigated the mechanism of palictaxel resistance with the aid of computational techniques. The result of the stability analysis signifies that F270V mutation alters the structural stability of the β-tubulin protein. The binding affinity data obtained from the docking study explains the ineffectual binding of paclitaxel with mutant (F270V) type β-tubulin. The results of RMSF analysis undoubtedly indicate the stable binding of paclitaxel with native type protein rather than mutant type. The results from our analysis also indicate that mutation significantly alters the flexibility of the binding pocket residues which results in the marked decrease in the existence of hydrogen bonding network in the mutant (F270V) protein. Finally, based on all our results, we conclude that Arg282 is needed for the effective binding of paclitaxel with β-tubulin protein. Hopefully, the results obtained from this study may be of valuable guidance for the management of paclitaxel resistance in the near future.

References

Berrieman HK, Lind MJ, Cawkwell L (2004) Do beta-tubulin mutations have a role in resistance to chemotherapy? Lancet Oncol 5:158–164

Choi CH (2005) ABC transporters as multidrug resistance mechanisms and the development of chemosensitizers for their reversal. Cancer Cell Int 5:30

Choudhury D, Ganguli A, Dastidar DG, Acharya BR, Das A, Chakrabarti G (2013) Apigenin shows synergistic anti-cancer activity with curcumin by binding at different sites of tubulin. Biochimie 5(6):1297–1309

Contini A, Cappelletti G, Cartelli D, Fontana G, Gelmi ML (2012) Molecular dynamics and tubulin polymerization kinetics study on 1,14-heterofused taxanes: evidence of stabilization of the tubulin head-to-tail dimer-dimer interaction. Mol BioSyst 8(12):3254–3261

Darden T, Perera L, Li L, Pedersen L (1999) New tricks for modelers from the crystallography toolkit: the particle mesh Ewald algorithm and its use in nucleic acid simulations. Structure 7(3):55–60

Drukman S, Kavallaris M (2002) Microtubule alterations and resistance to tubulin-binding agents (review). Int J Oncol 21:621–628

Dumontet C, Sikic BI (1999) Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. J Clin Oncol 17:1061–1070

Durrant JD, McCammon JA (2011) Molecular dynamics simulations and drug discovery. BMC Biol 9:71–79

Elengoe A, Naser MA, Hamdan S (2014) Modeling and docking studies on novel mutants (K71L and T204V) of the ATPase domain of human heat shock 70 kDa protein 1. Int J Mol Sci 15(4):6797–6814

Entwistle RA, Winefield RD, Foland TB, Lushington GH, Himes RH (2008) The paclitaxel site in tubulin probed by site-directed mutagenesis of *Saccharomyces cerevisiae* beta-tubulin. FEBS Lett 582(16):2467–2470

Feldman HJ, Snyder KA, Ticoll A, Pintilie G, Hogue CW (2006) A complete small molecule dataset from the protein data bank. FEBS Lett 580(6):1649–1653

Ganesh T, Guza RC, Bane S, Ravindra R, Shanker N, Lakdawala AS, Snyder JP, Kingston DG (2004) The bioactive Taxol conformation on beta-tubulin: experimental evidence from highly active constrained analogs. Proc Natl Acad Sci USA 101(27):10006–10011

Gascoigne KE, Taylor SS (2009) How do anti-mitotic drugs kill cancer cells? J Cell Sci 122:2579–2585

Gasteiger J, Rudolph C, Sadowski J (1990) Automatic generation of 3D-atomic coordinates for organic molecules. Tetrahedron Comput Methodol 3:537–547

Guex N, Peitsch MC (1997) SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18(15):2714–2723

Hari M, Loganzo F, Annable T, Tan X, Musto S, Morilla DB, Nettles JH, Snyder JP, Greenberger LM (2006) Paclitaxel-resistant cells have a mutation in the paclitaxel-binding region of beta-tubulin (Asp26Glu) and less stable microtubules. Mol Cancer Ther 5(2):270–278

He L, Jagtap PG, Kingston DG, Shen HJ, Orr GA, Horwitz SB (2000) A common pharmacophore for Taxol and the epothilones based on the biological activity of a taxane molecule lacking a C-13 side chain. Biochemistry 9(14):3972–3978

He L, Yang CP, Horwitz SB (2001) Mutations in beta-tubulin map to domains involved in regulation of microtubule stability in epothilone-resistant cell lines. Mol Cancer Ther 1:3–10

Iman M, Davood A, Nematollahi AR, Dehpoor AR, Shafiee A (2011) Design and synthesis of new 1,4-dihydropyridines containing 4(5)-chloro-5(4)-imidazolyl substituent as a novel calcium channel blocker. Arch Pharm Res 34(9):1417–1426

Iman M, Saadabadi A, Davood A, Iran J (2013) Docking studies of phthalimide pharmacophore as a sodium channel blocker. Basic Med Sci 16(9):1016–1021

Jordan A, Hadfield JA, Lawrence NJ, McGown AT (1998) Tubulin as a target for anticancer drugs: agents which interact with the mitotic spindle. Med Res Rev 18:259–296

Khan S, Vihinen M (2010) Performance of protein stability predictors. Hum Mutat 31(6):675–684

Lin Y, Yoo S, Sanchez R (2012) SiteComp: a server for ligand binding site analysis in protein structures. Bioinformatics 28(8):1172–1173

Lindahl E, Hess B, Van der Spoel D (2001) GROMACS 3.0: a package for molecular simulation and trajectory analysis. J Mol Model 7:306–317

Meagher KL, Carlson HA (2005) Solvation influences flap collapse in HIV-1 protease. Proteins 58(1):119–125

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and Auto-DockTools4: automated docking with selective receptor flexibility. J Comput Chem 30(16):2785–2791

Orr GA, Verdier-Pinard P, McDaid H, Horwitz SB (2003) Mechanisms of Taxol resistance related to microtubules. Oncogene 22(47):7280–7295

Parthiban V, Gromiha MM, Schomburg D (2006) CUPSAT: prediction of protein stability upon point mutations. Nucleic Acids Res 34:239–242

Raghav D, Sharma V (2013) An in silico evaluation of deleterious nonsynonymous single nucleotide polymorphisms in the ErbB3 oncogene. Biores Open Access 2(3):206–211

Rathinasamy K, Jindal B, Asthana J, Singh P, Balaji P, Panda D (2010) Griseofulvin stabilizes microtubule dynamics, activates p53 and inhibits the proliferation of MCF-7 cells synergistically with vinblastine. BMC Cancer 10:213

Schuttelkopf AW, Van Aalten DM (2004) PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. Acta Crystallogr D Biol Crystallogr 60(Pt 8): 1355–1363

Shing JC, Choi JW, Chapman R, Schroeder MA, Sarkaria JN, Fauq A, Bram RJ (2014) A novel synthetic 1,3-phenyl bis-urea compound targets microtubule polymerization to cause cancer cell death. Cancer Biol Ther 15(7)

Stewart ZA, Westfall MD, Pietenpol JA (2003) Cell-cycle dysregulation and anticancer therapy. Trends Pharmacol Sci 24:139–145

Teng S, Srivastava AK, Wang L (2010) Sequence feature-based prediction of protein stability changes upon amino acid substitutions. BMC Genomics 11(2):S5

Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ (2005) GROMACS: fast, flexible, and free. J Comput Chem 26(16):1701–1718

Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G (2010) Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC Bioinform 11:548

Vydra N, Toma A, Glowala-Kosinska M, Gogler-Piglowska A, Widlak W (2013) Overexpression of heat Shock transcription factor 1 enhances the resistance of melanoma cells to doxorubicin and paclitaxel. BMC Cancer 13:504

Wang HW, Nogales E (2005) Nucleotide-dependent bending flexibility of tubulin regulates microtubule assembly. Nature 435:911–915

Xu S, Chi S, Jin Y, Shi Q, Ge M, Wang S, Zhang X (2012) Molecular dynamics simulation and density functional theory studies on the active pocket for the binding of paclitaxel to tubulin. J Mol Model 18(1):377–391

Yin S, Bhattacharya R, Cabral F (2010) Human mutations that confer paclitaxel resistance. Mol Cancer Ther 9(2):327