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Original Article

Evaluation of the Structural Impact of the Missense Mutation *JAK2* V617F in Janus Kinase 2 by Docking Study

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Abstract: Myeloproliferative neoplasm is a group of blood cancers, including three main diseases: essential thrombocythemia, primary myelofibrosis, and polycythemia vera. Several molecular signaling pathways such as JAK/STAT, PI3K or SHP have been demonstrated that play a crucial role in controlling the signaling-mediated immune response related to Myeloproliferative neoplasms. The mutation JAK2 V617F substitutes phenylalanine for valine at position 617 in the JH2 domain of exon 14, leading to constitutive activation of the JAK-STAT and other pathways resulting in uncontrolled cell growth. This mutation has been found in a large proportion of myeloproliferative neoplasm patients. In this study, we performed a docking simulation test by Yasara, Pyrx, and Pymol to evaluate the effect of this variant on the model structure of the JH2 domain compared to wild-type allele, thus verifying the impact of mutation V617F on the function of Janus Kinase. The result showed that there was a significant effect that this mutation can cause on the interaction between the JH2 model and ATP. In detail, after redocking simulation, the wild type structure showed 16 hydrogen bonds binding between ATP and amino acids including T555, T557, K581, Q626, E627, N673, K677, P700, and R715 with the binding energy of -10 (kcal/mol), while the mutant model expressed 15 hydrogen bonds linked with the amino acids of T555, T557, K581, Q626, E627, N673, K677, and N678, with the binding energy of -9.6 (kcal/mol). This result may provide a better understanding of the critical role of Janus kinase in the pathogenesis of myeloproliferative disorders.

Keywords: Janus kinase, JAK2 V617F, Molecular Docking, Myeloproliferative neoplasms.

1. Introduction

Myeloproliferative neoplasms (MPNs) are a group of hematological malignancies diseases that have an extraordinary growth of mature peripheral blood elements, promoted by abnormal hematopoiesis of erythrocytes, white blood cells and platelets in the bone marrow [1]. According to an estimation by the Leukemia & Lymphoma Society Organisation, only in the United States alone, the number of people suffering from one of the malignant

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myeloproliferative diseases is nearly 300,000 patients, with nearly 20,000 new cases each year [2]. *JAK2* is a gene encoding for the non-receptor protein tyrosine kinases, which plays an important role in the genetic network that contributes to myeloproliferative neoplasms outcomes. JAK2 protein is composed of 4 domains: an N- terminal FERM (band 4.1, ezrin, radixin, moesin) domain, an SH2-like (SH2L) domain, a kinase-like or pseudokinase domain [JH2 (Janus homology-2)], and a C-terminal tyrosine kinase domain (JH1) [3]. The FERM and SH2L domains were supposed to be necessary for intermediating interaction between JAK2 and receptor, however, the specific JAK2-receptors binding remains not fully understood [4]. Besides, the JH1 domain keeps a crucial role in JAK2 structure. The function of this domain was catalyzing trans-phosphorylation of two specific tyrosine residues, stabilizing the active state, and recruiting the transcription proteins (STAT) [3]. JH2 is a pseudokinase domain, which was predicted to negatively regulate the JH1 domain phosphoryl activation. Although JH2 does not possess the phosphorylation site but it can bind to Adenosine triphosphate-associated Mg^{2+} ion (ATP-Mg). This mutation can cause a loss of the normal inhibitory function provided by the pseudokinase (JH2) domain upon the active (JH1) kinase domain-in this model. However, it is unclear whether the disrupted JH1/JH2 interface occurs within an individual JAK2 molecule or between JAK2 dimers. The mutation may also result in direct activation of the JH1 domain via an SH2-JH2 linker [5]. Therefore, this can be the critical point to fully penetrating the JH2 function. In this study, the interaction of ATP-JH2 is applied to test different variants' effects.

There are several studies demonstrating that the mutation *JAK2* V617F is the most common somatic mutation leading to MPNs (70%). This mutation is responsible for 95% of polycythemia vera (PV) cases, 50% of essential thrombocythemia (ET) cases, and approximately 50%-60% of cases of primary myelofibrosis (PMF) cases worldwide [6]. Accumulating evidence indicated that the mutation *JAK2* V617F plays a crucial role in the JAK/STAT signaling pathway, which is involved in a variety of immune diseases and many types of cancer [7]. However, the mechanism of how this mutation disturbs the molecular signaling network has not been fully understood. In this present study, we examined the deleterious effect of this mutation by molecular docking study to demonstrate the effect of this variant compared to wild type allele.

2. Experimental

2.1. Structure Validation

The sequence of Tyrosine Janus kinase 2 protein was obtained from UniProt database (https://www.uniprot.org/uniprot/O60674). The three dimensions structure of the JH2 domain was homology modeled using SWISS-MODEL [8]. The crystal 3D structure of the JAK2 pseudokinase domain (4FVQ and 4FVR) was obtained from the research of R.M. Bandaranayake *et al* [9]. The process is automated running from alignment, and model building to get the complete 3D protein based on the available X-ray crystallography and nuclear magnetic resonance spectroscopy structure which is already demonstrated. The *JAK2* V617F model was built with the template of 4FVR (JH2- mutant V617F, Mg-ATP-bound form). Meanwhile, the ATP-Mg 3D structure which is ligand binding was taken from 3DChem.com - Chemistry, Structures & 3D Molecules website (https://www.3dchem.com).

2.2. Molecular Docking

The docking simulation was performed using Yasara (http://www.yasara.org/md.htm), Pymol (https://pymol.org/2/), Pyrx computer program (https://pyrx.sourceforge.io/), and SWISS-MODEL online tool (https://swissmodel.expasy.org/) in this study. The receptors and ligand were loaded into Yasara to implement an energy minimization process to find the arrangement of configuration space for stable conformation. After that, the

docking simulation was performed by Pyrx to get the binding affinity of complex formation. Pymol software took a role in chemical bonds in detail observation of ATP-Mg and JH2 domain of JAK2 protein.

3. Results and Discussion

The *JAK2* V617F mutation which demonstrated a significant effect on MPNs disease was also checked to see whether it causes any modification in JH2 and ATP binding. The docking was started after inputting receptors, ligands, and choosing ATP binding pocket (T555, T557, K581, Q626, E627, V629, S633, K677, and N678). Among all of these models, the JH2-ATP binding complex of wild-type and *JAK2* V617F mutation was demonstrated by the X-ray diffraction method. The redocking simulation method was performed in this study (Figure 1).

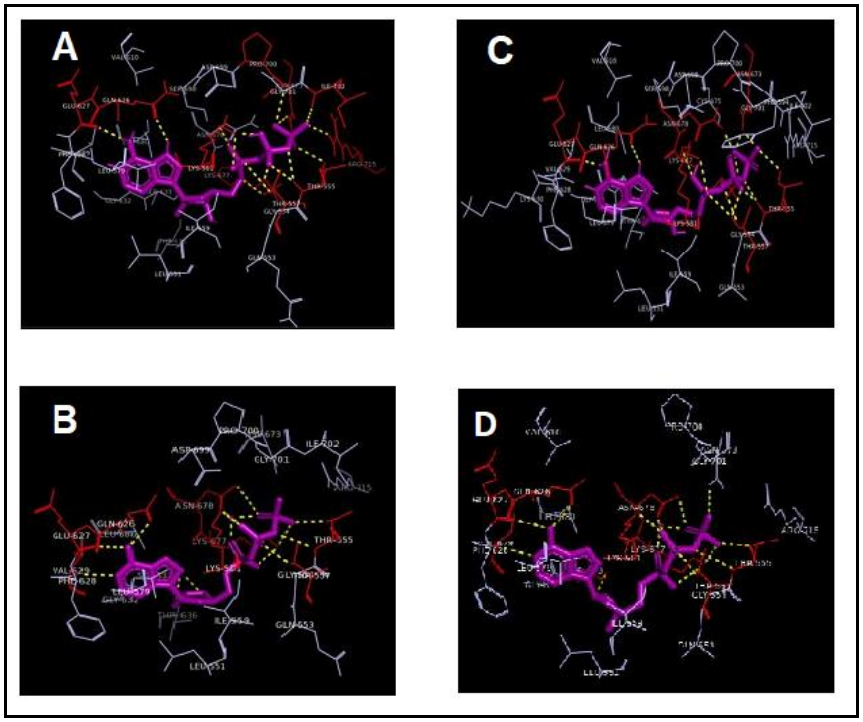

Figure 1. JH2 model and ATP-Mg interaction, the red residues exposed forming polar interaction, light blue one forming nonpolar interaction, hydrogen bonds were showed with yellow color, the ATP-Mg was expressed in purple. (A) Wild-type in-silico docking, (B) Wild-type X-RAY diffraction, (C) V617F mutation in silico docking, (D) V617F mutation X-RAY diffraction.

The results showed that the redocking simulation of wild-type (WT) bound to the residues and demonstrated model have 6 over 9 matched residues observed including T555, T557, K581, Q626, E627, and K677 which is known as an active binding pocket (Table 1). The number of hydrogen bonds in the 4FVQ complex was 13, and in the redocking complex was 16. Meanwhile, a high resemblance of 8 coincident residues over 10 (T555, T557, K581, Q626, E627, N673, K677, and N678) and the number of hydrogen bonds (15 hydrogen bonds in 4FVR and 16 *in silico* simulation) were explored between V617F redocking complex and the demonstrated one. It can be seen that *in silico* redocking simulation exposed a high

similarity compared with the determined one. Therefore, the redocking model can partly represent the initial prediction of the effect of mutations on intracellular interaction.

Table 1. Docking Simulation Results between models 4FVQ, 4FVR, redocked in silico of wild-type and V617F

Protein models	Amino acids interact with ligand
4FVQ	T555(2.7 Å; 3.2 Å), T557(2.7 Å), K581(2.6 Å), Q626(2.8 Å), E627(3.0 Å), V629(2.9 Å), S633(3.1 Å; 3.2 Å), K677(2.7 Å; 2.7 Å), N678(2.1 Å; 3.4 Å)
in silico wild-type	T555 (2.5 Å; 2.5 Å; 2.7 Å), T557 (2.0 Å; 2.3 Å), K581 (2.0 Å; 2.7 Å; 2.8 Å), Q626 (2.2 Å), E627 (2.2 Å), N673 (2.0 Å; 2.2 Å; 2.2 Å) , K677 (2.5 Å), P700 (3.4 Å), R715 (2.1 Å)
4FVR	T555(2.8 Å; 3.4 Å), T557(2.6 Å), K581(2.7 Å), Q626(2.7 Å), E627(3.1 Å), V629(2.9 Å), S633(2.8 Å), N673(3.1 Å), K677(2.7 Å; 3.2 Å), N678(2.0 Å; 3.2 Å)
in silico V617F	T555(2.2 Å; 2.6 Å; 2.6 Å), T557(2.4 Å; 2.7 Å; 3.3 Å), K581(2.5 Å; 2.6 Å), Q626(2.3 Å), E627(2.0 Å), N673(1.8 Å; 2.0 Å), K677(2.2 Å, 2.7 Å); N678 (2.1 Å)

In general, the three-dimensional conformation of all models was undistinguished (Figure 1). The WT complex showed 16 hydrogen bonds binding with amino acids including T555, T557, K581, Q626, E627, N673, K677, P700, and R715 with the binding energy of -10 (kcal/mol) (Table 2). Meanwhile, the V617F model expressed 15 hydrogen bonds linked between ATP and amino acids involving T555, T557, K581, Q626, E627, N673, K677, and N678, with a binding energy of -9.6 (kcal/mol). Moreover, the binding energy and the number of hydrogen bonds of the mutated domain slightly reduced compared to the wild-type with a small difference among their linked amino acids. Therefore, V617F can cause an effect on the binding between the JH2 domain and ATP.

Table 2. Docking simulation results between JH2 wild-type, V617F and ATP-Mg

Protein models	Binding energy (kcal/mol)	Amino acids interact with ligand
Wild-type	-10	T555 (2.5 Å; 2.5 Å; 2.7 Å), T557 (2.0 Å; 2.3 Å), K581 (2.0 Å; 2.7 Å; 2.8 Å), Q626 (2.2 Å), E627 (2.2 Å), N673 (2.0 Å; 2.2 Å; 2.2 Å) , K677 (2.5 Å), P700 (3.4 Å), R715 (2.1 Å)
V617F	-9.6	T555 (2.2 Å; 2.6 Å; 2.6 Å), T557 (2.4 Å; 2.7 Å; 3.3 Å), K581 (2.5 Å; 2.6 Å), Q626 (2.3 Å), E627 (2.0 Å), N673 (1.8 Å; 2.0 Å), K677 (2.2 Å, 2.7 Å); N678 (2.1 Å)

Myeloproliferative neoplasms, which are blood cancer containing two main groups of Ph-positive and Ph-negative, cause a high disease burden, reduce life quality and shorten survival time. In this study, the ET, PV, PMF belonging to Ph-negative are concentrated on due to limited understanding of the causes of these diseases. This study is the pioneering step focusing on the JAK2 gene, especially the *JAK2* V617F variant which is known as the predominant contribution to MPNs in the world with the extremely high presentation in Ph-negative subgroup both worldwide [10] and among the Vietnamese population [11, 12]. The *JAK2* V617F, which is the most common mutation leading to MPNs, was also taken to find the binding affinity in JH2-ATP interaction. Following the *JAK2* V617F

breakthrough detection in MNPs, scientists paid attention to the mechanism and different factors related to this mutation [13].

Our result showed that the binding affinity between *JAK2* V617F mutation and ATP was slightly reduced, and the number of hydrogen bonds with different amino acids showed a small difference compared to wild-type. Therefore, this mutation can affect the function of JH2 binding with ATP, which reduced the stability of JH2 and increase the inhibitory of kinase domain [5]. *JAK2* V617F was demonstrated to enhance kinase activity but seemed to have no effect on substrate preference [14]. This variant was demonstrated to rigidify the α-helix C in the N lobe of JH2 after the crystal structures of both JAK2 JH2 wildtype and mutant were analyzed. *JAK2* V617F was indicated to stabilize the αC helix of the JH2 structure through several mechanisms by molecular dynamics simulations [9]. In comparison with this study, the main novel finding of our research was the variant can also change the binding energy, change the number of hydrogen bonds between ATP-Mg complex and different amino acid residues compared to wildtype. Moreover, the JH2 model is all in the form of natural complex interaction with different ligands which were built up by using X-ray diffraction or Nuclear Magnetic Resonance (NMR) method. These interaction formations were performed in the intracellular environment, catalyzed by multiple agents in the most suitable condition. Therefore, the results could change when analyzing the actual experiment observation. The *in silico* docking simulation may not be accurate in JH2-ATP interaction. Further in silico and experiment study about docking simulation will be accomplished with other different docking and visualized software.

4. Conclusion

In conclusion, our docking simulation of *JAK2* V617F mutation models and ATP-Mg showed that this variant may influence on the interaction structure between the JH2 region of JAK2 protein and ATP-Mg. The results of the *in-silico* study are consistent with the published X-ray experimental structures, demonstrating certain reliability of the method according to bioinformatics software.

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