

Computational Analysis of Structural and Dynamic Changes in HER2 L869R/D769Y Double Mutation

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ABSTRACT

HER2 mutations play a role in the molecular heterogeneity of breast cancer and influence the kinase activity and therapy response. Within these mutations, the L869R and D769Y mutations belong to functionally important parts of the HER2 kinase domain; however, the structural effect of co-occurrence is not well-characterized. In this study, we explored the conformational changes of the L869R/D769Y mutation in HER2 kinase domain using the comparative molecular dynamics simulation method. Wild-type and mutant structures of HER2 kinase domain were used in 200 ns long molecular dynamics simulation, and further structural analyses included the RMSD, RMSF, Rg, SASA, PCA, and Gibbs free-energy landscape analysis. The mutant structure showed larger backbone and residue level fluctuations, especially in the structurally important parts of the kinase domain. However, despite these changes, the similar Rg and SASA values suggested no significant structural disruption. PCA analysis showed the increased sampling range of the conformational space, whereas free-energy landscape analysis revealed several available conformations in the mutant. Overall, these results show that the L869R/D769Y mutation causes structural remodeling of the conformational dynamics and energy landscape in the HER2 kinase domain without the global structural disruption.

Keywords: HER2; L869R/D769Y; Breast cancer; Molecular dynamics simulation; Conformational dynamics

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Introduction

Breast cancer is a heterogeneous disease where molecular changes involving receptor tyrosine kinase signaling contribute to tumor initiation, development, and treatment responses [1]. Human epidermal growth factor receptor 2 (HER2), which is encoded by the ERBB2 gene, is one of the key drivers of oncogenicity in a subgroup of breast cancers [2]. While HER2 amplification and overexpression have been the two main molecular markers associated with HER2-positive disease, HER2 somatic activating mutations may cause abnormal HER2 activity in breast cancers, even those without HER2 amplification. Systematic analysis of over 12,000

breast cancer patients found that about 3% had HER2 mutations, and these usually occurred within the intracellular part of the receptor [3]. Studies have further shown that some kinase domain mutations, such as D769Y, D769H, V777L, V842I, and R896C, cause HER2 signaling activation, indicating that HER2 mutation is another mechanism of oncogenicity [4]. HER2 belongs to the ErbB family of receptor tyrosine kinases and features a kinase domain that goes through conformational changes necessary for catalysis. Thus, the structure and dynamics of this kinase domain play an essential role in the understanding of the effects of particular mutations on the HER2 protein activity. A number of

recurrent mutations occur in key functional parts of the kinase domain, namely the β 3- α C segment, α C helix, activation loop, and ATP-binding site. Among these, the residue D769 is of special significance in the α C helix. Mutation of this residue has been known to enhance the kinase activity of HER2. Initial functional studies found that D769Y and D769H were activating mutations, and further structural and biochemical studies have provided evidence that these mutations may affect the structure of the activation loop and the HER2 kinase domain itself [5,6]. The L869R mutation can be described as yet another biologically significant change within the HER2 kinase domain. This mutation takes place in the activation loop and is considered to be a gain-of-function mutation. In terms of its structure, it has been shown that a substitution of leucine with an arginine at the 869 site results in a beneficial contact between R869 and D769, which might stabilize the active conformation of the kinase. It might also impair contacts that maintain the inactive form, which explains increased signaling activity related to L869R. Hence, it can be said that positions 769 and 869 form structurally and functionally related sites in the HER2 kinase domain [7,8].

This biological significance of these residues is especially apparent in terms of HER2 targeted treatment. Neratinib is a reversible pan-HER tyrosine kinase inhibitor, which shows efficacy in targeting HER2 mutant breast cancers. Nevertheless, HER2 mutations have been observed in patients with breast cancers in connection with the course of neratinib treatment, illustrating the possibility of the genetic and structural changes in the kinase domain [9]. In particular, the HER2 T798I gatekeeper mutation was found in a patient with L869R-mutant breast cancer in connection with neratinib progression and was experimentally proven to be resistant to neratinib [7]. Further studies showed the existence of other secondary HER2 mutations at L755, D769, T862 and other critical sites, underscoring the heterogeneity in response of HER2 mutations to treatment. It is also important to note that not all the secondary HER2 mutations exhibit same level of functional significance or response to drugs. In an experimental study on acquired HER2 mutations, the mutants D769Y, D769H, L755S individually retained sensitivity to neratinib, but certain combination mutants like L869R, especially L869R/L755S and

L869R/T862A were found to show greater resistance to neratinib than the single mutants, while the combination L869R/D769Y did not show any marked difference in neratinib sensitivity [8]. The implications of the above observations are significant because it indicates that the functional outcome of HER2 mutations is not always predictable by simply combining the properties of the individual substitutions. Rather, the structural environment and dynamic interactions among the co-existing mutations may influence the conformational dynamics of the kinase. Therefore, the L869R/D769Y double mutant pair is structurally significant since both individual mutations target regions that affect HER2 kinase activation, whereas together they change the structural surroundings of D769–L869 to two very different side chain chemistry: the acidic aspartic acid in position 769 is replaced by aromatic tyrosine, whereas leucine in position 869 is replaced by positive charge arginine. Molecular dynamics and structure studies have shown that the interactions of D769 and amino acids in the activation loop stabilize the active HER2 structure [8,10]. It was proposed from the molecular dynamics simulation study that the native D769 residue interacts with the amide part of L869, whereas replacement of D769 with tyrosine or histidine affects the physical and chemical surroundings of the activation loop. Thus, the presence of L869R and D769Y may affect the local flexibility, amino acid coupling, and the conformational space of the kinase domain [7].

Even though there exists a lot of experimental information on individual HER2 mutations and certain resistance-associated combinations of mutations, the impact of the specific L869R/D769Y combination has not been well studied at the structural level yet. In particular, it is unknown how the change of two amino acid residues in the α C-helix and activation loop affects the structural dynamics, flexibility, overall motion, and conformational landscape of the HER2 kinase domain. In this study, the structural and dynamic impact of L869R/D769Y double mutations on HER2 was studied through 200 ns of molecular dynamics simulations and comparative analysis between wild-type HER2 kinase domain and mutant HER2. Structural deviation, residue mobility, compactness, and solvent accessibility were characterized by

means of RMSD, RMSF, and radius of gyration (Rg), and SASA, respectively. Principal component analysis was conducted to investigate the changes in collective dominant motions and sampling. In addition, Gibbs free energy landscape analysis was conducted to understand the energetically accessible conformations. Through the use of such combination, the current research intends to explore the effects of L869R/D769Y double mutations on the dynamical behavior and conformational space of HER2 kinase domain.

2. Materials and Methods

2.1 Structural model preparation of HER2 L869R/D769Y

The 3D structure of the HER2 kinase domain was modeled using SWISSMODEL [11]. In this regard, the wild-type HER2 kinase domain structure was chosen as the template structure while introducing the double mutation L869R/D769Y at the corresponding residues using SwissPDBViewer [12]. The structural models were then checked and prepared for further simulations through energy minimization. In this regard, the models were prepared through appropriate protonation, addition of missing atoms, and removal of any structural inconsistencies as needed. Energy minimization was done to the structures generated before running molecular dynamics simulation.

2.2 Molecular dynamics simulation

The molecular dynamics (MD) simulations were carried out employing GROMACS [13] utilizing the CHARMM36 force field [14]. The processed structures of the proteins were placed in a simulation box and solvated using a TIP3P [15] water model. The systems were neutralized using appropriate counterions followed by energy minimization in order to avoid unfavorable steric interactions. After the completion of the energy minimization process, the simulations were carried out using NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensembles. The simulated system was then subjected to production MD simulation for 200 nanoseconds without restraints. The simulations were saved at specified time intervals in order to conduct analysis on them. The same simulation protocol was used in both the wild type and L869R/D769Y systems.

2.3 Molecular dynamics trajectory analysis

The structural and dynamical behavior of the wild-type and L869R/D769Y HER2 kinase domain structures were analyzed from the 200 ns molecular dynamics production simulations. First, the trajectory files were processed to remove the periodic boundary effects and fitted onto the protein backbone structure to remove translation and rotation movements before analysis. Root-mean square deviation (RMSD) analysis of the protein backbone was done to examine conformational differences during the simulation runs, while root-mean square fluctuation (RMSF) was performed for the C α atoms to assess flexibility at the residue level. Radius of gyration (Rg) was calculated to examine the compactness behavior of the kinase domain, while solvent accessible surface area (SASA) calculation was done to examine solvent exposure changes during the simulation time course. To study the most important motions in the conformational ensemble of the kinase domain, the analysis of the dominant modes of motion was carried out using the principal component analysis (PCA) applied to the covariance matrix of the protein fluctuations. Diagonalization of the covariance matrix was performed in order to obtain the principal eigenvectors and eigenvalues, the first two principal components (PC1 and PC2) were extracted, and the trajectories were projected onto the PC1-PC2 space. The PC1-PC2 distributions obtained for the wild type and mutant L869R/D769Y kinase domains were analyzed to assess the difference in the motions and conformational sampling in both cases. Additionally, the Gibbs free-energy landscape (FEL) was calculated using the first two principal components obtained by means of PCA. The free energies of the conformational states were calculated using the probability distributions of the sampled conformational states to find the favorable states of the system and their positions in the accessible conformational space. The FEL of the wild type and L869R/D769Y systems were compared in order to assess the differences caused by the mutations. All trajectory analyses were conducted under uniform conditions for the wild type and L869R/D769Y systems so that it would be easier to compare the changes caused by mutations in HER2 kinase dynamics.

Result

Root Mean Square Deviation (RMSD)

The backbone RMSD analysis was done to study the structural stability and conformational variations of the apo and the L869R/D769Y mutants of HER2 kinase domain. The apo simulation showed an initial increase in RMSD and a comparatively stable trajectory; however, backbone RMSD was predominantly within the range of about 0.16–0.21 nm towards the end of the simulation period. On the other hand, the RMSD value of the mutant showed greater variation in the course of the trajectory. Although RMSD of the mutant was predominantly within the range of about 0.18–0.25 nm for a large fraction of the simulation period, there were transient increases in RMSD at 120 ns and a considerable increase at approximately 160–190 ns with RMSD being in the range of 0.30–0.45 nm. This indicates that the L869R/D769Y mutation favors more conformational variations in comparison with the apo simulation. However, the decrease in RMSD at the end of the trajectory implies that the conformational variations could be due to switching between conformations (Fig 1).

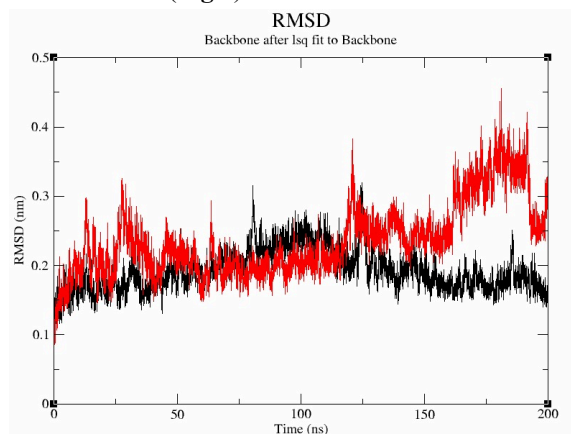


Figure 1. Backbone RMSD profiles of HER2-WT and L869R/D769Y systems during 200 ns MD simulation.

Root Mean Square Fluctuation (RMSF)

Residue level flexibility was evaluated using root mean square fluctuation (RMSF). Compared to the apo form, the L869R/D769Y mutant has higher fluctuations in some parts of the HER2 kinase domain. The greatest difference in RMSF between the mutant and apo forms is found in the N-terminal region of the kinase domain, which has RMSF higher than 0.4 nm for some residues in the mutant but not for the corresponding residues of the apo form. Some areas in the vicinity of mutation sites also had greater

fluctuations for the mutant than the apo form. Greater fluctuations were also seen near the residues from 875 to 885 and 920 to 940. However, some regions in the middle of the kinase domain are flexible for both forms. Thus, the increase of RMSF in the mutant form shows the effect of combined substitution on the local flexibility of the kinase domain near mutation sites (Fig 2).

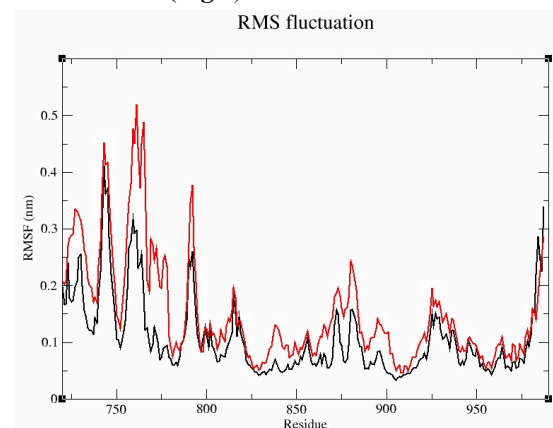


Figure 2. Residue-wise RMSF profiles of HER2-WT and L869R/D769Y systems during 200 ns MD simulation.

Radius of gyration (Rg)

Rg was calculated as an indicator of the variations in global conformational dynamics and global compactness of the HER2 kinase domain along the 200 ns molecular dynamics simulation. While the apo form showed Rg values within approximately 1.89–1.92 nm, with moderate fluctuations in the entire process of the simulation, the L869R/D769Y mutant showed an increased Rg range with values distributed within about 1.90–1.94 nm and with significant fluctuations at certain stages of the simulation. A transient decrease in the Rg values was seen for the mutant during approximately 120 to 160 ns of the simulation, while an increased value and fluctuation of Rg were seen towards the last 40 ns of the simulation. The increased Rg distribution range for the L869R/D769Y mutant compared to that of the apo system is indicative of an altered global conformational dynamics by the mutation, although without a persistent increase in Rg values (Fig 3).

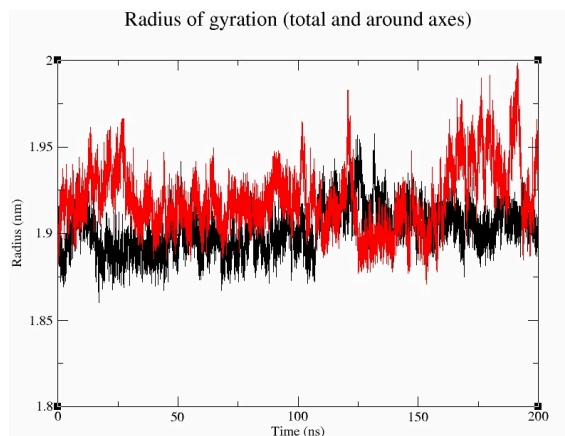


Figure 3. Radius of gyration (Rg) profiles of HER2-WT and L869R/D769Y systems during 200 ns MD simulation.

Solvent-accessible surface area (SASA)

SASA calculation has been performed to quantify the change in the solvent accessibility of HER2 kinase domain during 200 ns MD simulation time. The both the apo and L869R/D769Y mutant systems show significant temporal variation in SASA, where most of the SASA values were found between 140–148 nm². Within the initial 45 ns period, the mutant shows a few peaks in SASA values, which are close to 150–153 nm². On the other hand, within the time period from approximately 50 ns to 115 ns, the mutant often had SASA values close to 133–136 nm². Later on, the SASA plots for the two systems overlap each other in most of the cases, especially later part of the simulation. Thus, the lack of consistent difference in SASA implies that the L869R/D769Y mutation does not induce any persistent global change in solvent accessibility of the kinase domain (Fig 4).

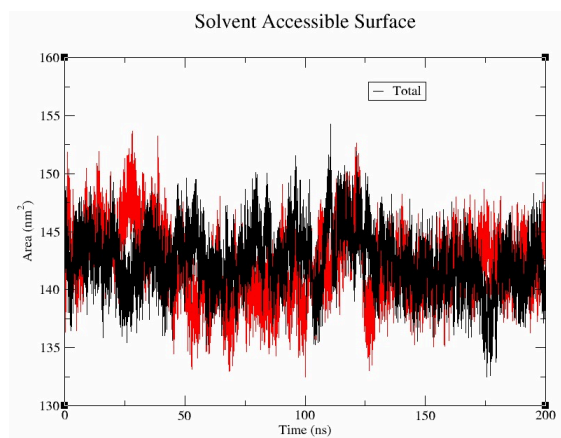


Figure 4. Solvent-accessible surface area (SASA) profiles of HER2-WT and L869R/D769Y systems during 200 ns MD simulation.

Principle Compound Analysis (PCA)

PCA was carried out to study the dominant collective motions and conformational space that were explored in the 200 ns simulations. The WT trajectory covered a PC1-PC2 space that ranged from −6 to +4 nm along PC1 and from −4 to +4 nm along PC2, where the density of conformations was most prominent in the center of the projected space. On the other hand, a second set of conformations was also observed in the negative side of PC1. For the L869R/D769Y mutant, a larger PC1-PC2 space was covered, which ranged from −7 to +8 nm along PC1 and from −6 to +7 nm along PC2, and contained more than one conformational space. The larger space and multiple sets of populated conformational spaces observed for the mutant indicated that the mutant showed increased conformational heterogeneity and different collective motions compared with the WT. The increased conformational heterogeneity is therefore compatible with the PC1-PC2 distribution found through PCA and also the higher structural fluctuations revealed through RMSD and RMSF (Fig 5).

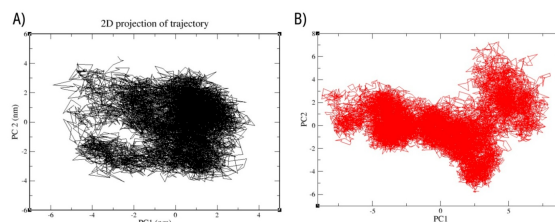


Figure 5. Principal component analysis (PCA) of HER2-WT and L869R/D769Y systems based on the first two principal components.

Gibbs Free Energy Landscape (FEL)

The Gibbs free energy landscape (FEL) analysis was carried out based on the two most significant principal components (PC1 and PC2) for the characterization of the conformational states sampled during the molecular dynamics simulations for 200 ns. The wild-type showed a relatively wide and interconnected area with low energy, where most of the favorable conformations were found in the middle and right part of the PC1 and PC2 space. The energy landscape of the wild type had the relative energy range of approximately 0–12.2 kJ/mol. In contrast,

the landscape of the L869R/D769Y mutant was heterogeneous, with multiple low-energy areas, which were separated by some intermediate areas, having the energy range of approximately 0–14.3 kJ/mol. It means that there were several energetically favorable basins, which imply sampling of multiple accessible states by the mutant. Overall, the results of the FEL analysis show that the mutation L869R/D769Y leads to the alteration of the energy landscape of HER2 kinase domain without a dominant conformational state (Fig 6).

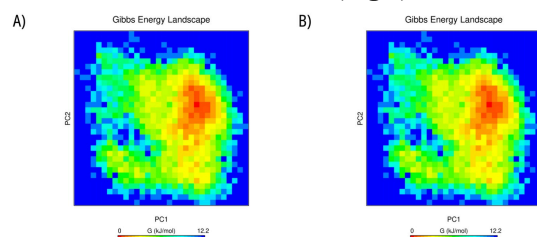


Figure 6. Free energy landscape (FEL) profiles of HER2-WT and L869R/D769Y systems based on principal components.

Table 1: Comparative analysis of molecular dynamics simulation parameters for HER2 wild-type and L869R/D769Y double-mutant systems, including RMSD, RMSF, radius of gyration (Rg), and solvent-accessible surface area (SASA).

Mutation s	RMSD(nm)	RMSF(nm)	Rg(n m)	SASA(n m ²)
HER2WT -Apo	0.192 ± 0.029	0.107 ± 0.068	1.902 ± 0.013	142.417 ± 2.560
HER2- L869R/D7 69Y	0.236 ± 0.053	0.152 ± 0.094	1.920 ± 0.019	142.185 ± 3.025

Discussion

This study has explored the structure and dynamics of the effect of the double mutation (L869R/D769Y) of HER2 through 200 ns molecular dynamics simulations. The two mutations occur in functionally important parts of the HER2 kinase domain with D769 residing in the α C-helix and L869 situated in the activation loop [8,16]. As the regions are involved in the dynamic regulation of kinase activity, changes in both the regions at once might have some effect on the dynamic balance of the kinase domain. These dynamic results have shown that double

mutations L869R/D769Y mainly affect the conformational dynamics of HER2 while maintaining the general structural arrangement of the kinase domain. The RMSD results also revealed a greater degree of conformational fluctuation for the mutant L869R/D769Y complex than WT, especially towards the latter part of the simulation. In particular, the mutant exhibited transient spikes in backbone deviations with notable fluctuations occurring between approximately 160 and 190 ns, followed by a decrease towards the end of the simulation period. This finding implies that the mutant explores alternative conformations and not constant structural destabilization. In addition, the RMSF results provided additional proof to this conclusion as shown in the figure, which indicates increased fluctuations at a residue level for various parts of the mutant. In particular, increased flexibility is noted for the part where D769 resides and the part close to the activation loop where L869 resides. Therefore, this suggests that the dual substitution of D769 and L869 alters the dynamics of the kinase domain structure. Therefore, both the RMSD and RMSF values show that the mutation affects both the general conformational dynamics as well as local residue dynamics. Notably, the enhanced dynamic behaviour was not associated with any indication of global expansion of the structure. The Rg values of the mutant were generally comparable to those of WT, although the mutant experienced slightly wider variations in Rg over time and some temporary rises at certain points in the trajectory. This indicates that the L869R/D769Y mutation is not associated with the global loss of compactness or disorganization of the kinase domain. Likewise, the profiles of SASA for the WT and mutant also exhibited considerable overlap during the course of the simulation. While there were some temporary differences in SASA between the two at different points in the trajectory, there were no persistent global changes in SASA associated with the mutant protein. The above results based on the Rg and SASA calculations show that the mutation-induced modifications involve mainly conformational and local structural dynamics. Increased flexibility within the regions adjacent to the mutation sites is also structurally significant, since both D769 and L869 belong to different, yet functionally related parts of the kinase domain. Substitution of an acidic residue by a tyrosine residue

will add an additional large hydrophobic group to the side chain and modify the local environment. In contrast, the replacement of leucine by arginine residue will bring positively charged side chain close to the activation loop region [7,17]. Thus, modification of two residues will possibly affect the local environment and interaction between α C-helix and activation loop. Increased flexibility in the regions close to the mutations is in accordance with this possibility of reorganization of the local environment. However, the current analysis does not provide direct evidence of any interaction between D769 and R869. Thus, these changes should be interpreted as indicative of changing dynamics in the vicinity rather than as proof that there is disruption or formation of a certain residue-residue interaction. PCA further confirmed earlier results, which revealed differences in the concerted conformational dynamics of both proteins. The trajectory of the WT occupied a relatively well-defined area of the PC1 vs PC2 plot, while the L869R/D769Y trajectory covered a larger and more heterogeneous conformational area with several populated sub regions. This indicates that the mutation does not affect only the increase in the mobility of particular amino acids but affects their coordinated movements. In protein kinases, such coordinated dynamics of structural regions are especially important since the functional transitions between different states involve movements of particular structural regions, such as the α C helix and the activation loop [18]. Thus, the larger conformational space sampled in the mutant can be attributed to the larger backbone and residue flexibility indicated by the RMSD and RMSF analyses. The FEL plot analysis gave an energy view of the above-mentioned conformational changes. The WT system had a rather interconnected low-energy region while the L869R/D769Y system showed the existence of several distinct low-energy basins with intermediate-energy regions between them. The existence of more than one favorable basin suggests that the mutant system adopts several conformations that are energetically allowed during the course of the simulation. The above observation cannot be considered as an indication of the instability of the system but rather the rearrangement of the conformational ensemble with increased numbers of structurally accessible states [8]. These findings from FEL are thus complementary to the PCA analysis in

demonstrating that the increased conformational flexibility of the mutant is linked with the modification of its energy landscape. As can be observed from all parameters, there is a common trend associated with the dynamic rearrangement of the HER2 kinase domain in response to the L869R/D769Y double mutation. As follows from the higher RMSD and RMSF values, the presence of greater conformational and residue-level dynamics is evident. However, the similar Rg and SASA values show that the integrity and solvent exposure of the kinase domain remain unchanged. In addition, the wider distribution of the PCA and the existence of multiple FEL basins imply that the mutant samples more diverse conformations than the WT. These results indicate that the L869R/D769Y mutation changes the dynamic balance of HER2 without causing any significant damage to its overall structure.

Such dynamical modifications are especially important when discussing the behavior of double HER2 mutants. Several experimental works have shown that various combinations of HER2 mutations can yield different phenotypes and response to drugs even if the combination contains one common mutation. Therefore, the effects of L869R/D769Y cannot be predicted based on the known behavior of L869R or D769Y separately. In addition, it would be wrong to consider the double mutation as a mutation responsible for developing resistance to any drugs without additional experimental support [8]. Based on the obtained results, one can assume that the modifications affect the dynamical properties of the kinase domain and its conformations. In summary, the results of the current investigation can be interpreted in the framework of a model, in which L869R/D769Y causes remodeling of the conformational space and dynamics, but not destabilization of the global structure. This mutant-related change in dynamics, increased flexibility, more diverse collective motions, and heterogeneous free energy landscape may indicate an altered conformational equilibrium of the HER2 kinase domain. This work provides the insight into the structural mechanism of action of this particular co-existing mutation, and highlights the need for further structural analysis of the HER2 mutations, depending on the context in which they occur. Additional experimental work and residue specific interaction

studies would be beneficial to determine if these conformational dynamics have an impact on kinase function, ligand binding or drug sensitivity.

Conclusion

The structural and dynamical impact of the double HER2 mutations L869R/D769Y was investigated through 200ns molecular dynamics simulation. In addition to the enhanced conformational mobility of the mutant as evidenced by high RMSD and RMSF values, the mutant showed high residue level flexibility as seen in the backbone deviations of the residues of interest. Although there is a higher flexibility in the mutant, the similarity in the radius of gyration and SASA values indicate that the packing and solvent accessibility of the kinase domain was relatively conserved in the mutant. PCA analysis also highlighted the greater diversity in the conformations sampled by the mutant while the free energy profile of the mutant showed a diverse population of possible states. Overall, these results show that L869R/D769Y mutation restructures the conformational dynamics and free energy landscape of the HER2 kinase domain without significant changes in the global structure. This data provides a structural foundation to analyze the ramifications of the aforementioned HER2 mutation and serves as a starting point for experimental studies of its impact on kinase activity.

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Author's contribution:

VS-Conceptualization, original draft writing and data analysis

JA-Editing and Reviewing

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