Bioinformatics Analysis of Protein-Inhibitor Interactions for BRAF

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Abstract

In this study, Vemurafenib, Encorafenib, and Dabrafenib's binding affinities were tested. These are all drugs used to inhibit mutated BRAF proteins, which is known to cause several types of cancer. Vemurafenib is usually used to inhibit BRAF V600E (the mutated form of the protein) when presented as melanoma while the other two drugs are used in addition to MEK inhibitors. The computational methods used were primarily docking because molecular docking allows comparisons between the different binding interactions between the drugs and to see which drugs have a higher binding affinity (Journal of Chemical Information and Modeling. (n.d.)). It also identifies which compounds have strong interactions with each other. Both the wild type and the mutant form of BRAF were tested against the three drugs above. Dabrafenib was used as a control against the other two because of how (compared to the other two drugs) it is a well-established and effective targeted therapy. Encorafenib had the lowest free energy for both wild type and mutant BRAF compared to any of the other drugs, and it was also the sole drug where the mutant form had a lower free energy compared to the wild type). Vemurafenib had the highest free energy for both its mutant and its wild type form . The genetic sequence pulled from ClusterOmega is an area of BRAF that is close to the binding site, possibly meaning that BRAF may adapt to different environments or have to interact with different partners in different species, or possibly that its specificity is affected by the environment and other regulations that are different for many species.

Intro

Like all cancers, melanoma originates from major mutations, specifically from mutations that affect the cell cycle. The BRAF gene plays a central role in the signaling pathway, specifically the MAPK/ERK signaling pathway. This pathway regulates cell proliferation, differentiation, and survival. The V600E mutation is a substitution that is particularly significant because it leads to the activation of BRAF kinase and drives uncontrollable cell growth. This results in multiple cancers, including melanoma, colorectal, and non-small cell lung cancer. This mutation is very common and has become a key target in precision oncology. More particularly, it is quite common in cancers (such as colorectal) that have poor prognosis (Barras, D.,et.al. (2017)). However, BRAF mutant cancers can be divided into two biologically and functionally distinct types based on gene expression profiles, which has major implications for targeted therapy. They can be classified into Class 1, Class 2, and Class 3 mutations, as well as BM1 subtypes and BM2 subtypes.

Dabrafenib is a selective BRAF inhibitor that was developed to specifically bind and inhibit the mutant BRAF V600E protein. It is a major advancement in targeted therapy, especially due to its FDA approval for use in mutant cancers. However, even though it has shown clinical efficiency, its binding selectivity compared to other drugs is less known. Additionally, more and more cancers including melanoma have started to become drug resistant, meaning new inhibitors need to be developed(Chang, X.,et.al. (2020)). Binding affinity is made up of multiple different aspects such as free energy, hydrogen bonds, ionic interactions, and hydrophobic contacts. Binding affinity and selectivity is important when it comes to inhibitors and drugs because that is how drugs effectively work. Better binding affinity means that the drug is more likely to inhibit the mutated protein in such a way that the protein does not remain being

activated. For BRAF V600E, Dabrafenib is approved, but other drugs such as Vemurafenib and Encorafenib may have potential as well (Chapman, P. B.et.al. (2011)).

Multiple computational methods like molecular docking and dynamic simulation can allow researchers to compare these affinities in silico, and, by comparing the binding interactions between different drugs, next generation candidates with higher binding affinity or reduced off target effects can pave the way to more effective and durable therapies.(Journal of Chemical Information and Modeling. (n.d.)) Docking simulations can identify which certain compounds maintain strong interactions with the forms of the molecule, suggesting potential for developing the next generator inhibitor targeting those mutations. It can also observe changes in finding affinity due to mutations, which is needed for designing drugs that can effectively target both the normal and mutated version of the protein (Bugnon, et.al. (2024)). If the molecular structure of the protein is not known, docking can be used to find it and can perform using models to predict binding interaction.

By analyzing the binding affinities of different drugs to both wild-type (unmutated version) and mutant BRAF (specifically BRAF V600E), drug specificity and identifying candidates with improved targeting can be specified, and the design of more effective next-generation inhibitors can be created. The mutant and wild- type forms must be compared in order to see how the drugs work when the protein has no mutation and how it works when it does: essentially, serving as a control. The mutation leads to a change of function of the protein, so the drug must be tested against both wild type and mutated to see how this difference will affect the binding affinity between the two and the drugs (Smallridge, R. C., et.al. (2014)). Additionally, this can further be translated to studying and finding inhibitors for Class 2 or Class 3 mutations for BRAF. However, an important caveat is that it is difficult to predict clinical effectiveness through binding affinity as binding affinity does not account for pharmacokinetics, toxicity, or cellular signaling dynamics.

Furthermore, from an evolutionary standpoint, many common portions of genes are conserved in order to make sure proteins with critical roles in core cellular processes do not change (Magliery, T. J.,et.al (2005)). Core critical roles are involved with replication, transcription, translation, metabolism (like the glycolytic pathway), cell wall/membrane/envelope biogenesis, and intracellular trafficking and transport (Magliery, T. J.,et.al (2005)). Basically, these are essential processes that are indispensable to an organisms' survival, and mutations or changes usually are harmful rather than helpful. Understanding this, BRAF is a protein that has a critical role and therefore it should be mostly conserved across species, with the only mutations being from individuals, not entire species themselves (Magliery, T. J.,et.al (2005)). Comparing species' amino acid sequences for the BRAF protein allows a glimpse into which sections of the BRAF sequences and which sections of the protein are highly variable and which sections are conserved.

Methods

For this research project, I use the BRAF V600E protein as my target. The known inhibitor for this drug is Dabrafenib, so this is my known control. I compare other specific drugs to the wild and mutant type protein, which confirms the drug specificity and new molecules with higher affinity or modifications. This can lead to designing next generation inhibitors and also expand drug categories based on inhibitors.

I use ChimeraX to visualize protein structures and trim them by removing solvents, the lipid, and any duplicate chains. For the docking aspect, I use Swissdock, specifically the

Attracting Cavities feature. For the molecules themselves, I use PDB 3q4c for the wild type BRAF protein. For the binding analysis itself, all the wild type BRAF have a box size of 20-30-20 and a box center of -18-17-2. I use PDB 6v34 for the mutant BRAF and for the analysis itself, all the mutant type have a box size of 20-30-20 and a box center of 0- -10-15.

The drugs that I tested, against both wild type and mutant BRAF, were Dabrafenib, Vemurafenib, and Encorafenib. For the Silico drug design I'd be using PubChem or Zinc Database. Dabrafenib is FDA approved, Vemurafenib is as well but Dabrafenib has been tested with a reputation of consistantly being effective. Encorafenib is FDA approved only alongside etuximab and mFOLFOX6 (FDA (2024)).

For each of the tests, I found the hydrogen bonds, ionic interactions, cation-π interactions, hydrophobic contacts, and π-stacking within each binding and included those in the figures. I also pulled the lowest amount of free energy (best binding) possible using the cluster options given. The clusters are different binding arrangements possible.

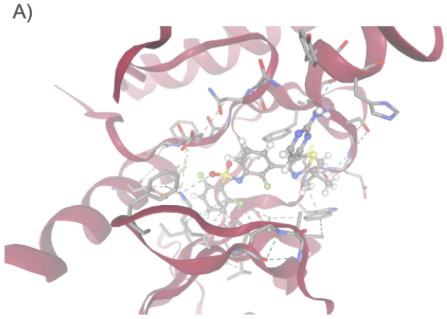
To compare the BRAF sequence across different species, I used the National Center of Biotechnology to gather the protein sequences for different species. The species used were *Homo sapiens, Mus musculus, Bos taurus, Gulo gulo luscus,* and *Xenopus laevis* (common names are humans, house mice, taurine cattle, North American wolverines, and African clawed frogs respectively). I then put them in Clustal Omega to align the species and find where the most variety in sequences were. Then, using ChimeraX, those sequences were found on a human BRAF protein and the structures of protein from the sequences that were the least conserved across species were found.

Results

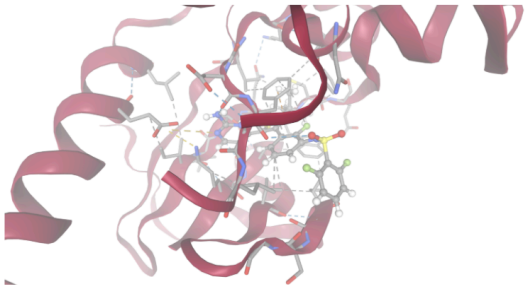
In order to test the three drugs' binding affinity, BRAF wild type and BRAF V6000E were both set to dock with each of the three drugs: Dabrafenib, Vemurafenib, and Encorafenib.

Figure 1

A)



B)



Comparison between Wild type BRAF and Braf V600E with Dabrafenib. A: BRAF wild type binding with Dabrafenib using Attracting cavities on Swissdock. B: BRAF V600E binding with Dabrafenib using Attracting cavities on Swissdock

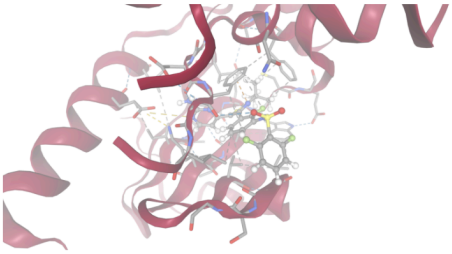
Type of Protein	Free Energy	Hydrogen Bonds	Ionic Interactions	Cation-π Interactions	Hydrophobic Contacts	π-Stacking
BRAF wild type	-69.980622	12.000	2.000	1.000	10.000	1.000
BRAF V600E	-68.640448	10.000	2.000	1.000	14.000	0.000

Table 1: Binding Data Between BRAF protein forms and Dabrafenib using Attracting Cavities on Swissdock

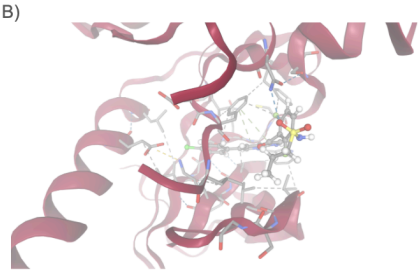
Between BRAF wild type and BRAF V600E, BRAF wild type had a better binding affinity with Dabrafenib since that binding had a free energy of -69.980622 (Table 1). Compared to BRAF V600E's -68.640448, BRAF wild type had a lower free energy and therefore a better bonding (Table 1). BRAF wild type has more hydrogen bonds, less hydrophobic contact, and more π-stacking compared to its mutant form (Table 1). The difference in free energy is minimal, and it may be that it is dependent on π-stacking because hydrogen bonding and hydrophobic contacts are near equal in importance when it comes to binding affinity, just in different ways (Pace, C. N., et.al. (2014)).

Figure 2

A)



B)



Comparison between Wild type BRAF and Braf V600E with Vemurafenib. A: BRAF wild type binding with Vemurafenib using Attracting cavities on Swissdock. B: BRAF V600E binding with Vemurafenib using Attracting cavities on Swissdock

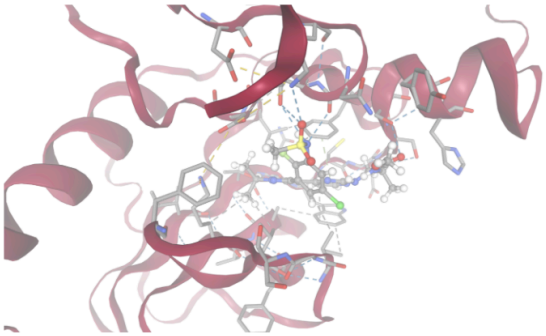
Type of Protein	Free Energy	Hydrogen Bonds	Ionic Interactions	Cation-π Interactions	Hydrophobic Contacts	π-Stacking
BRAF wild type	-11.531363	8.000	2.000	1.000	9.000	2.000
BRAF V600E	-11.189369	10.000	0.000	0.000	11.000	1.000

Table 2: Binding Data Between BRAF protein forms and Vemurafenib using Attracting Cavities on Swissdock

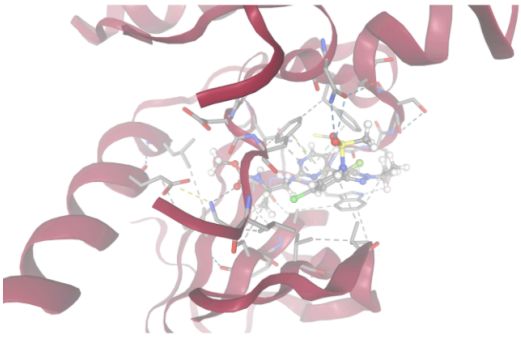
Similar to what occurred to Dabrafenib, BRAF wild type had a greater binding affinity compared to BRAF V600E with Vemurafenib. BRAF wild type had a free energy of -11.531363 while BRAF V600E had a free energy of -11.189369 (Table 2). Something that is interesting is that both the free-energies for binding with Vemurafenib are much lower compared to binding with Dabrafenib. Vemurafenib has been tested to work better than Dabrafenib, so the fact that it has a higher free energy and therefore does not bind as well raises some interesting points (Chapman, P. B., et.al. (2011)).

Figure 3

A)



B)



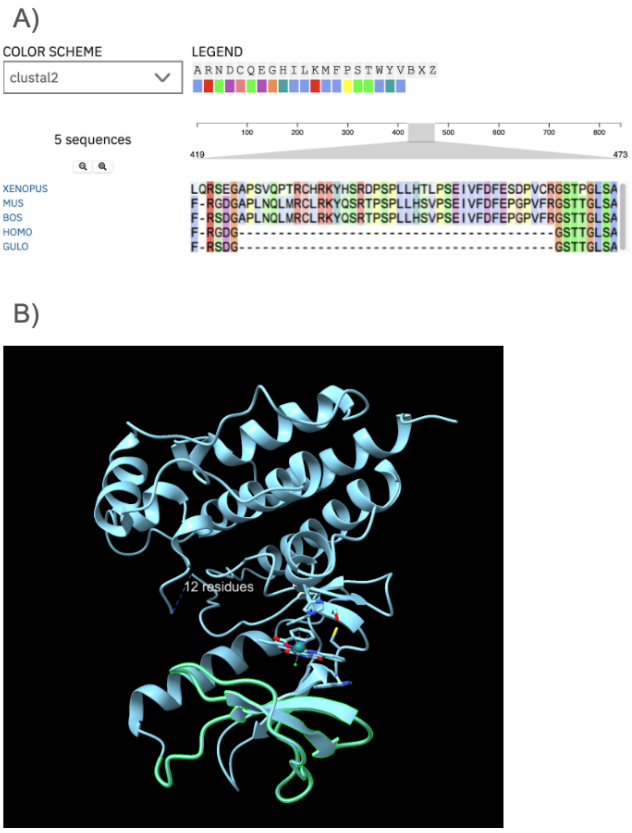
Comparison between Wild type BRAF and Braf V600E with Encorafenib. A: BRAF wild type binding with Encorafenib using Attracting cavities on Swissdock. B: BRAF V600E binding with Encorafenib using Attracting cavities on Swissdock

Type of Protein	Free Energy	Hydrogen Bonds	Ionic Interactions	Cation-π Interactions	Hydrophobic Contacts	π-Stacking
BRAF wild type	-131.231568	14.000	3.000	1.000	8.000	1.000
BRAF V600E	-144.914000	12.000	0.000	0.000	11.000	3.000

Table 3: Binding Data Between BRAF protein forms and Encorafenib using Attracting Cavities on Swissdock

For Encorafenib, BRAF V600E had a greater binding affinity compared to its wild type since BRAFV600E has a free energy of -144.914000 while BRAF wild type has a free energy of -131.231568 (Table 3). Similar to both Vemurafenib and Dabrafenib, BRAF V600E had less hydrogen bonds and more hydrophobic content compared to its wild type (Table 1, Table 2). The difference here is that there are more π-stacking interactions with BRAF V600E compared to BRAF wild type.

Figure 4



BRAF amino acid variability and protein structure. A: Comparison/ alignment chart between species of BRAF amino acid sequence. 419 to 473 is captured. B: Actual structure of the sequence derived from A found on human BRAF protein chain A.

When the five species are compared with one another, one of the main sequences where there was difference was the sequence 419 to 473. This was then put into ChimeraX to see where the structure was on human BRAF protein. The structure highlighted in green. This is the same structure that has been present in the past figures (Figure 1, Figure 2, Figure 3) and is present right under the ligand.

Discussion

Currently, Dabrafenib is the drug of choice when it comes to treating melanoma and other cancers caused by BRAF mutations. However, the findings from the study show that Encorafenib is a better choice. Its free energy for both when combined with a wild type and mutant BRAF are significantly higher compared to the other two drugs (Table 3). Additionally, this was the only drug in this study that had a lower free energy when binding with the mutant form compared to unbinding with the wild type. This is significant because it means that this drug has better affinity with the mutant form, the form that causes the cancer.

An interesting thing to note is that for Dabrafenib, the wild type BRAF had more hydrogen bonds and π-stacking while BRAF V600E had more hydrophobic contact, yet the wild type had

a lower free energy (Table 1). This similarly happens with the Vemurafenib (Table 2). However, with Encorafenib, even though BRAF V600E had less hydrogen bonds and greater hydrophobic contact, it still had lower free energy. The only difference between Encorafenib and the other two drugs is that with Encorafenib, BRAF V600E had greater π-stacking (Table 3). This means that π-stacking could possibly have an impact on binding affinity and free energy availability. Additionally, as stated before, Encorafenib was more spread out through the proteins (Figure 3). It was a larger molecule compared to Dabrafenib and Vemurafenib and this could possibly have also had an impact in the huge difference between the free energy for both wild type and the BRAF V600E between the three drugs. Encorafenib could possibly be used on its own instead on in correlation with another medication like it was previously (FDA (2024)).

For what was analyzed with Figure 4, the fact that the sequence pulled is in an area of BRAF that is so close to the binding site in human BRAF could possibly mean that BRAF may adapt to different environments or have to interact with different partners in different species (Luo, H.,et.al. (2015)). If its specificity is affected by the environment or other regulations that are dependent and ever changing, it could explain why such an important and critical protein is variable so close to the binding site (Luo, H.,et.al. (2015)). There are not as many strict regulations on the area compared to the actual binding site itself, but again the fact that it is so close could mean that BRAF has or will in the future evolve for more diverse functions (Magliery, T. J.,et.al (2005)).

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