Proposing Ligands and an Active Site in NgBR for Cancer Treatment

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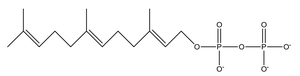
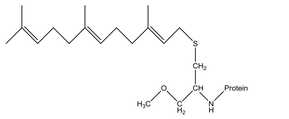
Abstract

Nogo-B, the primary Nogo isoform , is expressed in blood vessels and binds to the Nogo-B Receptor (NgBR).[1] The NgBR protein increases endothelial cell (EC) migration to wherever Nogo-B (soluble protein) is present while decreasing the migrating ability of vascular cells.[2,3] Since blood vessels are essential to life, Nogo-B is found in most tissues.[4,5] The intracellular domain of NgBR can bind to the Farnesyl group in Ras, an oncogene which is an important mediator in the RTK pathway.[1] Increased interactions between the NgBR and its ligand may cause cancerous cell growth. Experiments in Dr. Robert Miao's lab at the Medical College of Wisconsin have shown that in zebrafish with their NgBR genetically knocked out, blood vessels were more localized in the central regions of the fish and did not expand outwards as much as in fish with the receptor.[3] The same result was seen in mice, in which mice deficient in Nogo-B had fewer vessel formations.[6,7] The next experiment that Miao's lab is interested in is directed to finding the essential domains (active site) of NgBR. They will determine this by developing mutants in the NgBR, then placing the gene back into the zebrafish to observe the changes. Though this is the next step, the active site, structure, and essential domains in NgBR are still unknown. Therefore we used the homologous structure Farnesyl diphosphate synthetase, whose PDB code is 2VG1, as a template. 2VG1 is bounded by a ligand, E,E-farnesyl diphosphate (EE-FPP) and helps make the cell wall for *Mycobacterium tuberculosis*.[8] It also helps us to envision how NgBR can bind to a Farnesyl group on Ras. Using a Blast search, the percent sequence homology was found to be 40%.[9] We found key amino acids in the active site by defining various distances around the ligand and observing which amino acids were present. Within 6 Å of this chain are seven hydrophobic amino acids-- Ile105, Glu107, Gln110, Ser113, Phe114, Ile117, Tyr135, His137, Gln138. These create a pocket for the hydrophobic tail of Farnesyl through Van de Waal interactions. The other end of Farnesyl contains a nitrogen and an oxygen. Within 6 Å of these groups are Ser249 and Leu251. Ser249 is polar and thus is likely attracted to the polar head of Farnesyl. Leu251 is non polar, but is shown to be 4.58 Å from the polar head of the ligand. It is possible this non-polar amino acid helps form a pocket for the ligand and stabilizes the attraction.



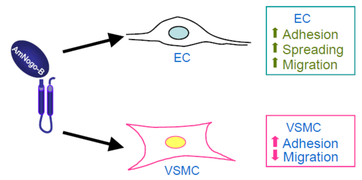
E,E-Farnesyl diphosphate (EE-FPP)



S-Farnesyl

NgBR in the body

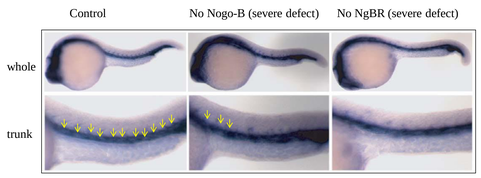
NgBR plays a very important cardiovascular role in the body. It is found in the endothelial cells (EC), which are the thin layer of cells that line the inner surface of blood vessels. These cells are vital because they facilitate the movement of nutrients from the blood and its surrounding tissues. NgBR is also important in vascular endothelial growth factor (VEGF) migration .[3] The protein VEGF helps repair tissue that has been injured by restoring oxygen to the area when blood circulation is insufficient. Finally, NgBR also hinders the movement of vascular smooth muscle cells (VSMC).[6] These processes are all essential for the remodeling of vascular tissue. NgBR is also believed to bind to the Farnesyl group of Ras. Interactions between NgBR and the Farnesyl ligand of Ras may promote the growth of cancerous cells. Finding a way to block the binding of NgBR and Ras could potentially inhibit the growth of these cells.



L. Acevedo, et al, *Nat. Med.* **2004**, *10*, 382

Got NgBR?

If NgBR is not present, an injury to vascular cells can lead to a rapid increase of smooth muscle cells in the blood vessel, which restricts blood flow.[6] A cell which lacks NgBR also experiences no endothelial migration, which helps improve blood flow. Finally, when NgBR is knocked out, the function of VEGF is reduced.



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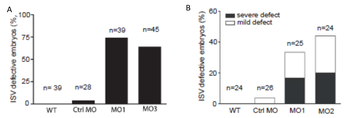
These images show that zebrafish who had either Nogo-B or the NgBR knocked out had severe defects in the formation of intersomitic vessels (ISV).[3] The zebrafish that lacked the receptor showed no ISV formation, while the zebrafish that lacked Nogo-B had limited ISV formation. ISV is noted by the yellow arrows.

NgBR Experiments Done at MCW

Dr. Robert Miao of the Medical College of Wisconsin has been researching Nogo-B and its functions. One important experiment he has performed involved genetically knocking out either the Nogo-B protein or its receptor in embryonic zebrafish. Imaging showed that when Nogo-B and NgBR are not paired, ISV formation is hindered. It was also shown that when either the protein or its receptor is injected into the zebrafish, the protein can partially "rescue" the fish and reverse some of the defects.[3]



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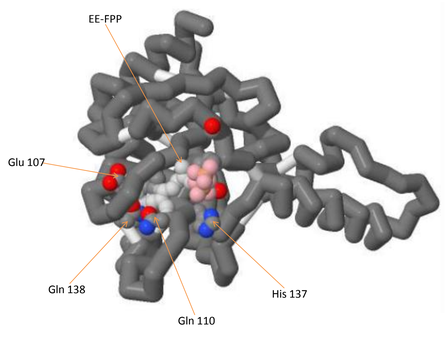
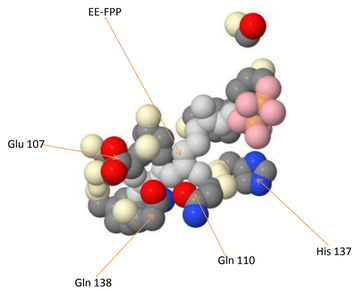
Graph A indicates how many embryos were defective (missing 5 or more ISV). How many embryos were in each experimental group is represented by 'n'. MO1 indicates the embryo had Nogo-B knocked out while MO3 means NgBR is missing from the fish. Graph B indicates the severity of the defect in the fish. A severe defect indicates 3 or more ISV were missing, while a mild defect indicates an irregularity in the orientation of the ISV was observed.[3]

Our model



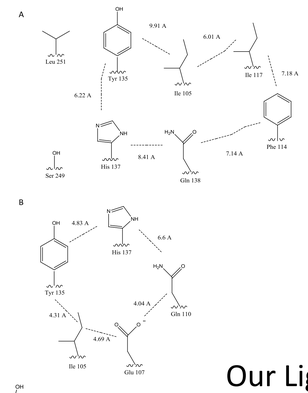
Our model of NgBR is a homology model based on the protein 2VG1. In the active site a E,E-farnesyl diphosphate (EE-FPP) has been positioned. The protein our model has been developed from binds to the EE-FPP ligand. This is important because we are proposing that NgBR binds to a similar Farnesyl group in Ras. We have created ligands to bind in this active site which would inhibit the binding of NgBR to the Farnesylated Ras, thus preventing the oncogene from functioning. In this model, only the amino acid side chains in the proposed active site are shown. Dark grey indicates the carbon backbone, red indicates a hydrogen atom, blue indicates an oxygen, light grey is the carbon tail of the EE-FPP ligand, while pink and orange are oxygen and phosphorous, respectively.

Active Site



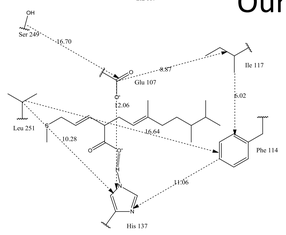
This is a close up of the proposed active site of NgBR. Light yellow indicates carbons from the backbone, while dark grey indicates carbons on the side chain. The active site contains eleven amino acids. These are Ile105, Glu107, Gln110, Ser113, Phe114, Ile117, Tyr135, His137, Gln138, Ser249, and Leu251. Ras' Farnesyl group has a 12 carbon hydrophobic tail. Within 6 Å of this chain are nine amino acids-- Ile105, Glu107, Gln110, Ser113, Phe114, Ile117, Tyr135, His137 and Gln138. These amino acids create a pocket for the hydrophobic tail of Farnesyl. This pocket measures 10.19 Å across at its widest point and 7.99 Å at its shortest. It is 15.92 Å long. The polar end of the ligand contains oxygen and phosphorous. It is important to note that this polar head is where the Farnesyl group would bind to Ras. Thus, the phosphates on EE-FPP can be ignored. Within 6 Å of the polar group are Ser249 and Leu251. Ser249 is polar and thus likely attracted to the polar oxygen on the ligand. Leu251 is non polar, but is 4.58 Å from the ligand. It is possible Leu251 helps form a pocket for the ligand and stabilize the attraction.

Pharmacophore Model

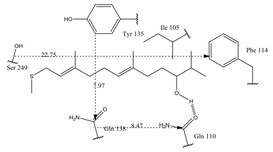


This 2D depiction of NgBR's active site includes the distances between each amino acid, as found using Swiss PDB. Image A shows the distances between the amino acids in a side view of the hydrophobic pocket. These amino acids point their hydrophobic features inwards to bind with the tail of the ligand while their hydrophilic features are pointed away from the tail. Image B depicts the distances between the amino acids at the opening of the ring that the tail inserts into. This opening contains amino acids which have polar groups attached. The polar atoms point away from the chain and help stabilize the head of Farnesyl.

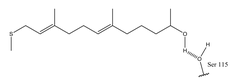
Our Ligands



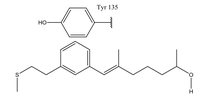
In this ligand a carboxylate group was added to the chain, which is stabilized by an H-bond with His137.



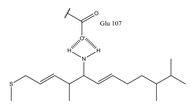
In this image a sulfur atom at the head of the ligand can be stabilized by Ser249 in our active site. We added an OH group in the chain which is stabilized by an H-bond with Glu110.



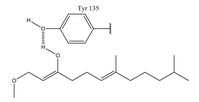
For this ligand, an OH group was added to the terminal carbon of the hydrophobic chain which creates an H-bond with Ser115.



In this ligand, a benzene was added to the hydrophobic chain. Benzene is 2.82 Å across, so we knew it would fit inside our pocket. The ring is stabilized by π-π interactions with Tyr135.

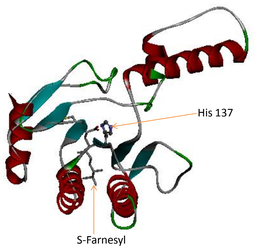


In this ligand, an NH_2 group was added near Glu107 so that it could be stabilized by creating two H-bonds with the negatively charged oxygen.

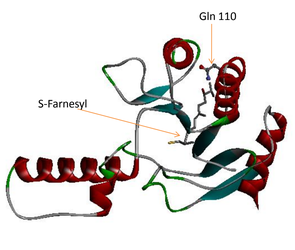


An OH group was added in this ligand near Tyr135 which is stabilized by an H-bond with the oxygen on Tyrosine.

3D Rendering of Ligands



NgBR with carboxylate mutation H-bonded to His137



NgBR with hydroxide mutation H-bonded to Gln110

Discussion

The best ligand we created to bind with NgBR is the one which incorporates a carboxylate and forms an H-bond with His137. His137 is oriented close to the opening of the hydrophobic tunnel. It is only 10.28 Å from Leu251, which is located directly at the opening. This close proximity strengthens the interactions between His137 and the carboxylate attached to the hydrophobic chain. We feel the H-bonding in this ligand is stronger than the H-bonds in the other ligands we have created because of the proximity. The tunnel of our active site contains hydrophobic and polar amino acids. The polar amino acids are Glu107, Gln110, Ser113, Tyr135, His137, Gln138, and Ser249. Gln110 points away from the tunnel and thus has no interaction with the ligand while Tyr135 is parallel with the tunnel and forms a π –π interaction. The other polar amino acids can form H-bonds with the ligand. The hydrophobic, non-polar amino acids stabilize the ligand with Van der Waal interactions. The pocket of our active site was measured to be 16.92 Å long. Our ligand measures 16 Å long. The height of our hydrophobic pocket opening is 10.17 Å tall at the highest point. The tallest point of our ligand is 6 Å tall, which indicates to us the ligand would have no difficulty entering the active site and would have a secure fit.

Future Work

In the future, Dr. Maio would like to focus his research on trying to determine the active site of NgBR. To do this, he proposes to remove the NgBR in embryonic zebrafish, mutate the protein, and then place it back into the fish. The observed changes in the zebrafish should help clarify the domain of the protein.

Methods

To create the 3D models of NgBR and the active site, Jmol 12.0.14 was used. Spartan Student was used to minimize the energy of the ligands we created using Hartree-Fock 3-21G. We mimicked the interactions between the ligand and the protein using Discovery Studio 3.0 Client. All the distances between amino acids were measured using SwissPDB Viewer. Chemdraw was used to draw the pharmacophore model and the 2D representations of our ligands.

Summary

NgBR plays an important role in cancer by binding to Farnesylated Ras which, through the RTK pathway, leads to tumorous cell growth. It is also shown to increase the formation of blood vessels. This makes NgBR a candidate for further research. Researchers hope that by developing inhibitors to the receptor, interactions such as that between Ras and NgBR could be stopped. Though the crystallized structure of NgBR is not known, a homology protein using Farnesyl diphosphate synthetase as a template and the amino acid sequence from NgBR was made. The active site of this homologous protein was found to have a hydrophobic tunnel that houses the carbon tail of the Farnesyl ligand. The key amino acids in the active site were found to be Ile105, Glu107, Gln110, Ser113, Phe114, Ile117, Tyr135, His137, Gln138, Ser249, and Leu251. Using the hydrophobic and polar properties of these amino acids as a guide, ligands mutated from Ras' Farnesyl group were created. It is the hope these newly mutated ligands may bind to NgBR and be useful as an inhibitor to help stop cancer growth.

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Dr. Margaret Franzen, Milwaukee School of Engineering
Center for BioMolecular Modeling, Milwaukee School of Engineering

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