The Importance of Understanding DUSP5 for Angiogenesis Prevention

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Abstract

The study of DUSP5 bears a high relevance in the study of disease found in blood vessels.[10,11] The particulars of the study include tumors/growths, angiogenesis that occur within the blood vessels.[4] If we discover how blood vessels are built, we should be able to accordingly know how to destroy them or prevent their growth in the particular cases of disease. Dr. Ramchandran and his team have taken a special interest in researching into battling the development of growths and tumors in the skin of children. Studies are being conducted on zebra fish as a model organism, due to the availability of the fish in addition to the ability to easily view a living vascular system (after 48 hours or so, the fish's vascular system will be fully developed and fully visible through its clear skin).[8,9] The DUSP5 and Snrk-1 proteins are regulators of cells involved in the vascular mutations of the DUSP5 protein that result in the tumors.[4] A mutation in the DUSP5 protein (e.g. S147P) makes it unable to dephosphorylate the pERK 1/2 protein to ERK 1/2.[1,6] This inability to carry out its function is believed to be the cause of the tumor proliferation in the body. Why does this occur? Phosphorylated ERK ½ (pERK) activates cell surface tyrosine kinases within the nucleus, which include the epidermal growth factors.[5,6] When the ERK cannot be dephosphorylated, it cannot be told to stop activating the growth factors which leads to these tumors. Experiments have shown that the mutated DUSP5 protein is unable to dephosphorylate, since the presence of a mutant DUSP5 shows a constant amount of pERK in the cell with very little ERK.[1]

DUSP5 Active Site Structure and Function

Introducing the DUSP5 Protein

Figure 1 shows the entire structure of the DUSP5 protein. The protein contains two domains. The circled domain serves two purposes – It not only binds our ligand, but it also dephosphorylates the ERK protein. This domain was prepared from the DUSP5 phosphatase domain which contains the active site. The other domain of the protein was created using homology modeling. This particular domain is responsible for binding to ERK itself. The Pro-Ser connection between the two domains then flexes to place the active site at the proper position to dephosphorylate ERK. The process can be described as a "clamping" action, in which both domains are vital to the binding of the ERK by essentially clamping down on it. If you think of it as a hand grabbing something, it is comparable to having the advantage of an opposable thumb for use to get a better grip on something; in this case, a better grip on the ERK protein.

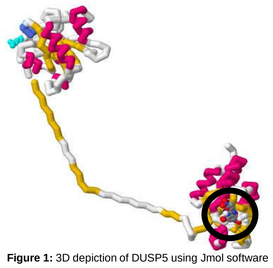

Figure 1: 3D depiction of DUSP5 using Jmol software

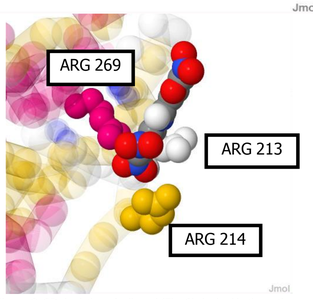

Figure 2: 3D depiction of a ligand (Fig. 3) docked in the active site of the DUSP5 protein using Jmol software.

Figure 2 is a Jmol rendering of the active site of the DUSP5 phosphatase domain. The main interactions of the ligand come from ionic interactions between negatively charged oxygens on the ligand with positively charged arginines on the protein. The active side chains are indicated by the atoms that are not transparent. Arg 269 is represented by the dark pink-red side chain, Arg 213 is represented by the white side chain, partially hidden by the ligand in this orientation, and Arg 214 is represented by the yellow-orange side chain.

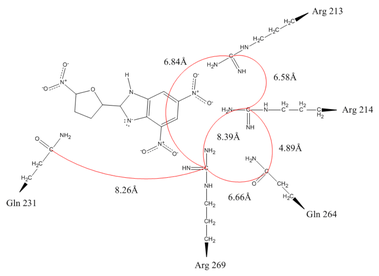

Figure 3: 2D depiction of a ligand bound in the active site of the DUSP5 protein using the ChemDraw software program.

These interactions can be seen much more clearly on the two dimensional rendering of the active site (Fig. 3). The ligand prepared by Dr. Sem and Dr. Ramchandran is currently bound in the active site. The bound ligand takes full advantage of the regions of positively charged sidechains by positioning negatively charged oxygens inside the active site pocket. However, there are other potentially interactive sidechains that could be utilized for tighter binding or spatial orientation. In the 2D depiction, we see that side chains like Gln 231 and Gln 264 may be contributors in the active site binding, not only contributing to the main positive-negative charge interactions, but to interactions with other side chains as well as hydrophobic/hydrophilic side chain and ligand interactions. We attempted to design ligands that take advantage of the other side chains while retaining the ionic interactions that make up the bulk of the binding process.

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Method (Students)

The ligands were manually created and docked using the Spartan, ChemDraw and DS Visualizer programs. The main program that we worked with, to better understand the spatial orientation of the protein and any potential ligands was Jmol (examples pictured in left column). In combination with using 3D software, as well as ChemDraw, we were able to better understand the purpose and proposed mechanisms of DUSP5 activities, and the simple cartoon depiction of the DUSP5 in Fig. 4 shows its role in the cell. The result of the mutation (S147P) creates a geometric change (imagine the chain connecting the two domains becoming tangled) in the dumbbell formation making it unable to dephosphorylate the ERK. Using the cartoon to the right, you can see the importance of the orientation of the two domains in the reaction. The hope is that docking a ligand here would fix the geometric change that is created by the mutation and correct its inability to dephosphorylate the ERK.

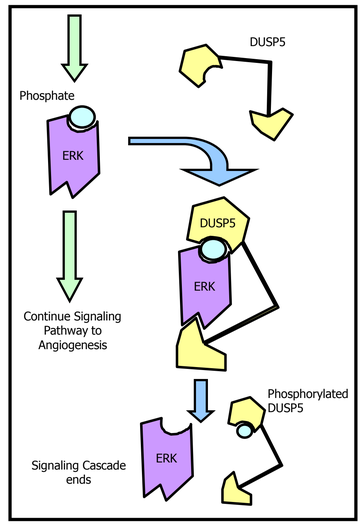

Figure 4: Cartoon representation of how the DUSP5 protein dephosphorylates the pERK 1/2 protein.

Methods (Corresponding Lab)

Fluorescent data was taken (FLIM method) in cultures containing human umbilical vein epidermal cells (HUVEC). The fluorescent protein used for the imaging was enhanced cyan fluorescent protein (eCFP). A 440-nm diode was used as the excitation source for the eCFP. The light that was emitted entered a 440/530 dichromic mirror and then goes through a fiber in the detector containing an emission filter and a detector that collects photons. SymPhoTime software was used to process the resulting images for analysis. All fluorescence was collected, whether the eCFP was active or not (visible v. non-visible) and was used to construct the data you see in Fig. 5 and 6.

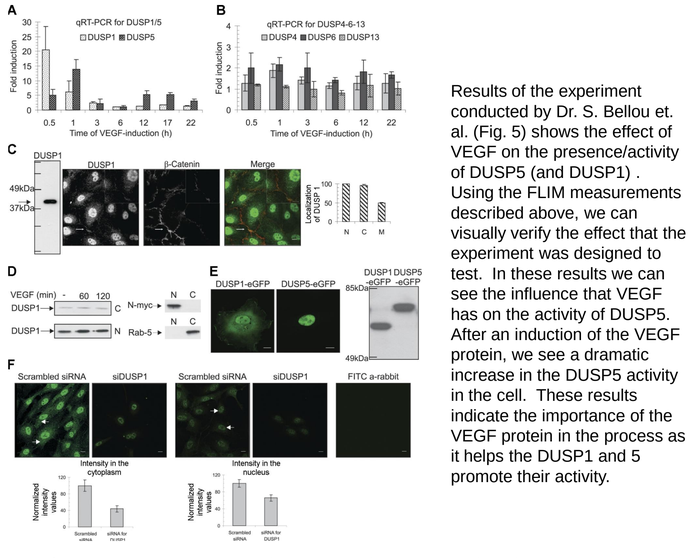

Figure 5[1]: Fluorescent data corresponding to the DUSP1/5 concentration found in the nucleus of HUVEC's controlled by the induction of VEGF.

Results of the experiment conducted by Dr. S. Bellou et. al. (Fig. 5) shows the effect of VEGF on the presence/activity of DUSP5 (and DUSP1) . Using the FLIM measurements described above, we can visually verify the effect that the experiment was designed to test. In these results we can see the influence that VEGF has on the activity of DUSP5. After an induction of the VEGF protein, we see a dramatic increase in the DUSP5 activity in the cell. These results indicate the importance of the VEGF protein in the process as it helps the DUSP1 and 5 promote their activity.

Figure 6 shows experimental results of controlling expression of DUSP5/DUSP1 and VEGF to show the [de]phosphorylation correlation of these two protein along with the presence of ERK1/2 in the cells. The results are important as they show both the location of the protein interactions as well as the activity of the three of them based on their relative concentrations in the cell. These results are compatible with the previous figure as we already know the correlation between DUSP5 and VEGF. Now these results further our understanding of the relations between VEGF, DUSP5, and ERK1/2. The duration of ERK phosphorylation activity decreases with the increase of the DUSP5, which then impacts the VEGF activity. The intensity of the fluorescence in the figure shows the correlation between the three proteins when taking into account their respective concentrations.

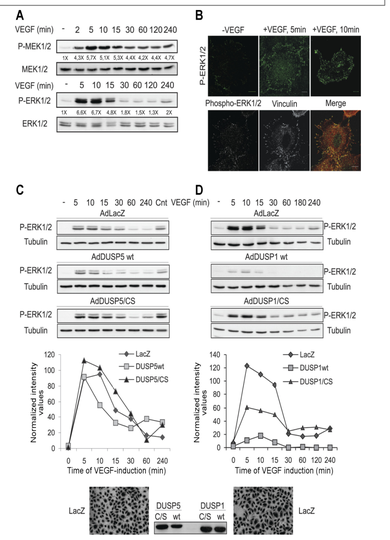

Figure 6[1]: Fluorescent data showing the 'bigger picture' results of ERK ½ concentration in the cell as a result of varying the VEGF (and therefore DUSP 1/5) concentration.

Additional Ligands (Proposed by the Students)

Figure 7 shows ligands that our group manually drew using the Jmol and ChemDraw programs. Energy minimization was done through the Spartan program under the Hartree-Fock/3-21G basis set. Here we see four alternate ligands with one docked into the active site (interacting with Asp 232, Arg 213, and Arg 214). The interactions that we see are the hydrophobic interactions of the carbon rings, but more importantly, the charged interactions of positive and negative between the amino acid sidechains and the charged chains of the ligands are the most significant. The ligand that we see docked in the active site is also portrayed in a 3D orientation using Jmol (Fig. 8) with Asp 232 as the orange side chain, the Arg 213 as the pink, and the Arg 214 as green.

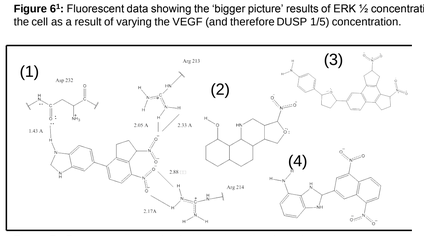

Figure 7: Four manually constructed ligands by the students.

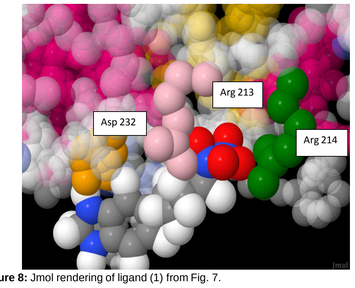

Figure 8: Jmol rendering of ligand (1) from Fig. 7.

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•All references were used, but not necessary cited. If an article was read in the process of understanding the DUSP5 protein, it was credited as a source, even if information was not drawn from it into the abstract.

Summary

In conclusion, we see that in the presence of HUVEC's, VEGF has the ability to control creation (transcription) of the DUSP5 protein (as well and DUSP1). The FLIM, or fluorescent data, also points to that fact that DUSP5 is a nuclear protein, in that it is only found in the nucleus, in contrast to the other proteins in the experiment which are found in both the nucleus and the cytoplasm. The data also shows the correlation and trends that reveal when the ERK 1/2 is in its pERK 1/2 form, (phosphrylated by the VEGF protein) it is up to the DUSP5 protein to dephosphorylate the pERK ½ which results in the inhibition of proliferation of EC's (used HUVEC in Dr. Ramchandran's lab). The data also reveals that DUSP5 has some coordinating influence: it can anchor in the nucleus which prevents ERK ½ from exiting into the cell's cytoplasm which is where it needs to go to get phosphorylated. In other words, DUSP5 can control the activity of ERK ½ by keeping it in the nucleus. All in all, we find that VEGF influences the transcription of DUSP5 which regulates the activity of the ERK ½ protein, which in turn, prevents harmful cell proliferation.