Drug Design for Inhibition of Extracellular Signal-regulated Kinase 2

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ABSTRACT

ERK-2, an extracellular signal-regulated kinase, also known as MAPK, mitogen activated protein kinase, is an important protein in the angiogenesis pathway. ERK-2 is vital in controlling the proliferation of vascular endothelial cells. A vascular endothelial growth factor (VEGF) binds its receptor, a tyrosine kinase receptor, starting a signal cascade. In humans, the VEGF receptor recruits phospholipase C gamma (PLC-γ) which is activated by protein kinase C (PKC) via phosphorylation. An active PLC-γ phosphorylates ERK-2, bypassing the normal Ras-Raf-MEK-MAPK pathway.[1] The phosphorylated ERK-2 enters the nucleus and phosphorylates, or activates, transcription factors such as Elk-1 and TFIIIB, resulting in cell proliferation.[2]

ERK2 is highly regulated in order to prevent excess endothelial cell proliferation. ERK-2 activity is regulated by the phosphorylation state, typically by using MAPK phosphatases (MKPs) to deactivate ERK-2 and PLC-γ to active it. DUSP5, a MAPK, contains a phosphatase domain and an ERK2 binding domain. When DUSP5 binds to ERK-2, it dephosphorylates, thus deactivates, ERK-2 using the phosphatase domain. If ERK-2 is not dephosphorylated it remains active leading to excessive cell proliferation. Inhibitors for ERK-2 are a current research topic; Alex M. Aronov et al. at Vertex Pharmaceuticals have developed a drug that inhibits ERK-2, which is currently in Phase 1 clinical trials.[3]

INTRODUCTION

Extracellular signal-regulated kinase 2 (ERK-2, also known as MAPK) is part of a protein signaling cascade (**Figure 1**) occurring when an extracellular growth factor binds its respective receptor on the surface of the cell. In a vascular endothelial cell, the growth factor is VEGF, which binds VEGFR, a transmembrane tyrosine kinase. In humans, upon VEGF-A binding, phosphorylation of VEGFR2 on Tyr1175 leads to recruitment of PLC-γ which, via activation of PKC, phosphorylates ERK-2[4] on Thr185 and Tyr187[5].

Activated ERK has a multitude of effects on the cell, including neuronal differentiation, mitogenesis, oncogenic transformation, and apoptotic cell death[6]. In addition to a host of transcription factors such as c-Jun, c-Fos, Elk-1, and c-Myc, MAPK substrates include membrane-associated and cytoplasmic proteins such as kinases, cytoskeletal elements, phospholipase A2, and stathmin[7]. In vascular endothelial cells, ERK activation typically leads to angiogenic sprouting, the growth of blood vessels.[8]

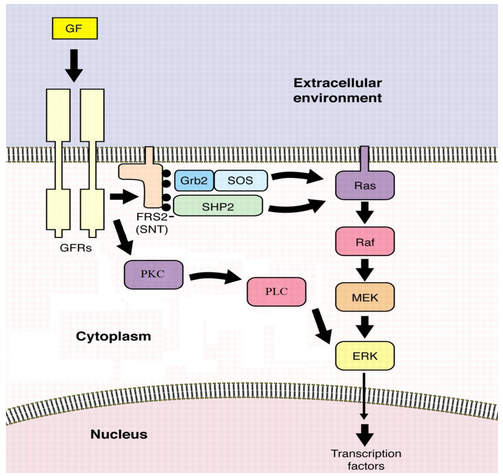

Figure 1. The upper pathway represents a typical example of a signaling cascade in which ERK-2 is involved. The signaling cascade utilized by vascular endothelial cells is depicted by the bottom pathway. [12]

ERK-2 is regulated through control of the phosphorylation states of Thr185 and Tyr187. Phosphorylation of both these residues is required to maintain high activity.[9] This phosphorylation is achieved through the aforementioned signaling cascade. DUSP5, a mitogen-activated protein kinase (MKP), dephosphorylates ERK-2. DUSP5 has both a catalytic domain and an ERK binding domain that it uses to bind to ERK2. DUSP5 dephosphorylates Thr185 and Tyr187 and prevents ERK-2 from leaving the nucleus, effectively deactivating the kinase.[1]

ERK-2 inhibition is a key target in anticancer drug design because constitutive action of the ERK-2 pathway has been reported in lung, colon, pancreatic, renal, and ovarian cancers.[10] An inhibitor of ERK-2 could potentially stop cell proliferation in these tumors, but could also prevent angiogenesis, and the resulting blood supply, by blocking the VEGF pathway. A search is currently underway for a potent, selective and orally bioavailable inhibitor of ERK.[11] Our project was to design such an inhibitor.

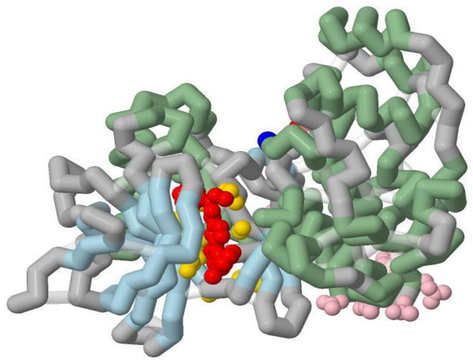

Figure 2. 3D representation of ERK-2 with bound inhibitor (red). Green segments represent β sheets, blue segments represent α helices and gray segments represent nonspecific secondary structure. Important residues in the active site are depicted in yellow.

METHODS AND RESULTS

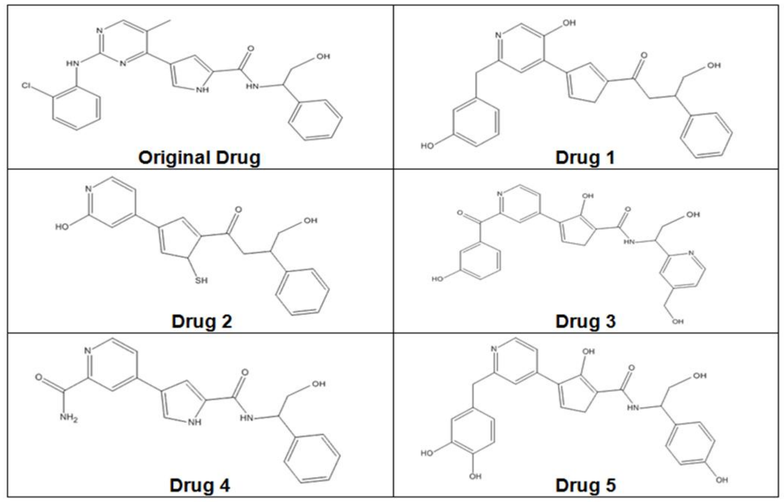

Figure 3. Inhibitors (Drug 1-5) designed using DS Visualizer and Spartan. Original Drug was designed by Aronov et al. and X-ray Crystallographic data was collected from ERK-2 with Original Drug bound in ERK-2 active site. [11]

The dephosphorylation of ERK2- by MKPs, specifically DUSP1 and DUSP5, in endothelial cells (ECs) has been characterized using fluorescence cross-correlation spectroscopy (FCCS). FCCS has described the strength of MKP-ERK-2 interactions in ECs. ERK2-mCherry, DUSP1-eGFP, and DUSP5-eGFP constructs were used to achieve fluorescence emissions. As the MKPs and ERK-2 interacted, the observed emissions diminished, which were dependent on the concentrations of the MKPs and ERK-2. This allowed for the effective interaction strength, K_D^{eff}, to be determined. The interaction strength between DUSP5-ERK2 was observed to be significantly stronger than that of DUSP1-ERK-2, suggesting that DUSP5 may be an appropriate inhibitor of ERK-2.

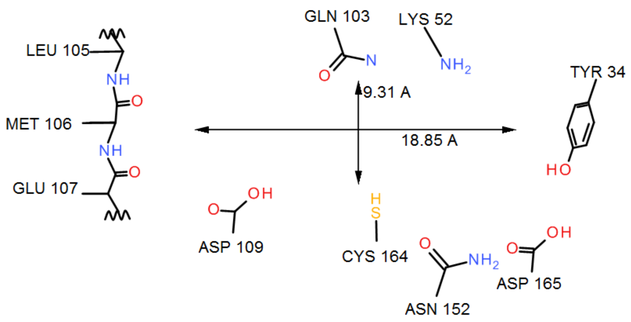

Figure 4. 2D representation of ERK-2 active site, showing important amino acids that can interact with a bound molecule.

A plausible inhibitor of ERK-2 was developed by employing molecular modeling techniques. Five molecules, shown in Figure 3, were initially designed based on desired interactions, such as hydrogen bonding and Van der Waals interactions, with residues in the active site, Figure 4. The energy of each molecule was minimized in Spartan using a 3-21G basis set. The molecules were fit into the active site of ERK-2 using Discovery Studio 3.0 Visualizer. Drug 3 was determined to be the molecule with the highest compatibility in the active site.

The high compatibility of Drug 3 with the active site is due to the number of interactions it has with ERK-2's active site amino acids. Drug 3 can form a number of hydrogen bonds with ERK-2, specifically with Lys52, Gln103, Asp165, and the backbone of Met106 and Leu105. There are also van der Waals interactions between Drug 3 and the aromatic ring of Tyr34.

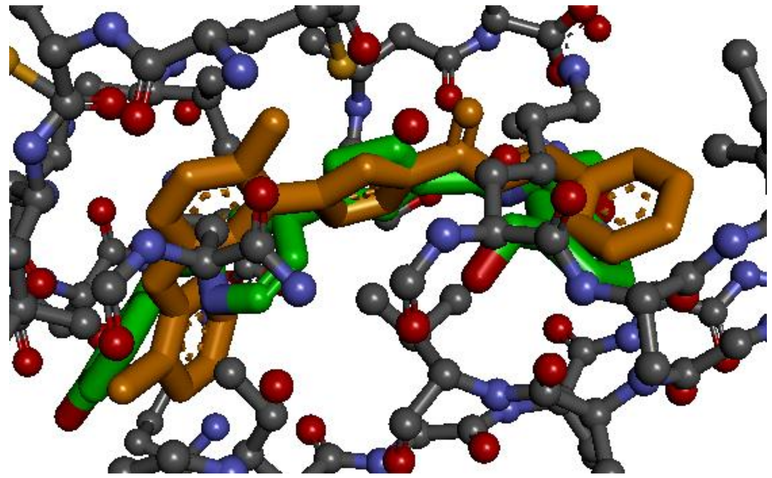

Figure 5. 3D depiction of ERK-2's active site with both the original drug (orange) and Drug 3 bound (green). Created using DS Visualizer.

SUMMARY

ERK-2 is an extremely important protein involved in a variety of cellular processes, specifically cell proliferation. We have designed a compound that effectively binds the active site of ERK-2, inhibiting its function. The next step in this process would be to assay the binding of Drug 3 and determine its K_D^{Eff}. If all goes well, Drug 3 would enter into clinical testing in order to access its ability to be an effective inhibitor of ERK-2 in test subjects.

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