Rule-based Modeling of VEGF Signaling

Nilgun Yilmaz 1, 2, James R. Faeder 3

1 Bioengineering & Bioinformatics Summer Institute, Dept. of Computational Biology, University of Pittsburgh, 15260
2 Molecular Biology and Genetics Department, Bilkent University, Ankara/ TURKEY
3 Department of Computational Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA USA
Introduction

- VEGF (Vascular Endothelial Growth Factor) is crucial for angiogenesis, the growth of new blood vessels from pre-existing microvessels, which has an important role in diseases such as cancer, arthritis and diabetic retinopathy.

- There are several published models for VEGF binding to their receptors but no model contains downstream events of receptor aggregation such as kinase activation or phosphorylation of receptor tyrosines. Development of such models may provide comprehensive understanding of the pathway dynamics and assist in developing new anti-tumor drugs and other therapies.
Introduction

We are developing a model of this VEGF pathway, including VEGFR sites that are known to couple directly to angiogenesis, vasculogenesis, and vascular permeability. This model is an extension of previously published models for the ligand-induced aggregation of VEGF receptors and co-receptors with the addition of kinase activation, receptor phosphorylation, and recruitment of downstream effectors.

- The members of VEGF family that are used in this study:
  - VEGF121, VEGF165 isomers
  - VEGFR1, VEGFR2 (signalling receptors)
  - Neuropilin-1 (non-signalling co-receptor)
Background
Background

- VEGF165 isomer is important because it is the only identified VEGF isomer which induces pathological angiogenesis.
  (Since Neuropilin directly interacts and has an influence in direction of its binding to VEGFRs, it is targeted to control the effects of VEGF165 experimentally.)

- **3 ways for targeting Neuropilin-1:**

  1-) Blocking Neuropilin expression

  2-) Blocking VEGF165-Neuropilin binding

  3-) Blocking Neuropilin coupling with VEGFR2
Methods

Because of having large complexes and many number of phosphorylation sites, BioNetGen software platform is used for development. In this platform objects and their components represent signaling molecules and their functional elements, and rules describe biochemical interactions.
Methods

How this BioNetGen language is used?

begin seed species
R2: VEGFR2(y951~u.d|0,lg).VEGFR2(y951~u.d|0,lg) 0
R1: VEGFR1(y1309~u.d|0,lg,nrp_b).VEGFR1(y1309~u.d|0,lg,nrp_b) 0
NR1: NRPI(R_b.Ig165) 0
121: VEGF121(b1,b2) 0
165: VEGF165(n,r,r) 0
TGF: TGF(Tgl,Tgl) RNLeither

begin molecule types
  1 VEGFR2(y951~u.p.d.lg)
  2 VEGFR1(y1309~u.p.d.lg,nrp_b)
  3 NRPI(R_b.Ig165)
  4 VEGF121(b1,b2)

begin reaction rules
# VEGF11 binding to the VEGFR2 dimers :kn9,kn13f
Binding(VEGF11_to_prelinerizedVEGFR2, VEGFR2(y951~u lg.d|0).VEGFR2(y951~u lg.d|0 lg)+VEGFR2(b1 b2))→VEGFR2(b1 b2 lg lg),VEGFR2(d l lg lg p)
# VEGF11 binding to the VEGFR2 dimers :kn9,kn13f
Binding(VEGF11_to_prelinerizedVEGFR2, VEGFR2(y951~u lg.d|0).VEGFR2(y951~u lg.d|0 lg)+VEGFR2(b1 b2))→VEGFR2(b1 b2 lg lg),VEGFR2(d l lg lg p)
# VEGF11 binding to the neuroglin :kn9,kn13f,kn9,kn13f
Binding(VEGF11_to_nRGPI, VEGF11(b1 r)+NRPI(R_b.Ig165))→NRPI(R_b.Ig165),VEGF11(b1 r lg lg) kn9,kn13f
# VEGF11 binding Neuroglin coupling with VEGFR2 dimers :kn9,kn13f
Identification of 3 cases of Neuropilin targeting, while developing model:

1-) Blocking Neuropilin expression
(Trash symbolizes absence of molecule)

2-) Blocking VEGF165-
Neuropilin binding
(PIGF is a molecule that competes with VEGF165)

3-) Blocking of VEGFR2-
Neuropilin Coupling
(Antibody competes with VEGFR2 by binding to the same or overlapping site on the Neuropilin but not affects VEGF165 binding)
Results - key notes

• Neuropilin is non-signalling co-receptor but has an important role in directing VEGF165 isomer and binding it to VEGF165 isomer and binding it to VEGFR2 signalling receptor which in turn induces angiogenesis

• Reaction Rates, structural information and environmental data are provided from literature (see references)
Results

Figure 1: Concentrations of VEGF165-Neuropilin and VEGF165-VEGFR2 complexes depending on the Neuropilin expression levels versus time. Concentration units are pmol/cm^3 and time unit is hours.

- Less Neuropilin expression results in decrease in the concentration of VEGF165-Neuropilin complex directly proportional to the blockage of the expression level.
- Transient decrease of yellow line (A) might be rooted from not having accurate kinetics for some of the reaction rates.
- Transient decrease in the VEGF165-VEGFR2 complex conc. is resulted from Neuropilin-independent VEGF165 binding to VEGFR2 because of increased concentrated free VEGF165 in the environment.
Results

Figure 2: Concentrations of VEGF165-Neuropilin and VEGF165-VEGFR2 complexes depending on the PIGF conc. levels which blocks VEGF165-Neuropilin binding. Complexes’ conc. units are pmol/cm^3, PIGF conc. unit is uM and time unit is hours.

• Initial decrease in the concentration of VEGF165-Neuropilin complex (A) is because of PIGF competition with VEGF165 and directly proportional to the concentration of PIGF.
• The reason of this transient decrease is internalization of PIGFs that are bound to Neuropilin which in turn decreases the concentration level of PIGF in the environment. Thus it cannot compete with VEGF165 anymore.
• Since there is less initial concentrated PIGF is in the environment (A), it is internalized suddenly and this results sudden VEGF165-NRP complex formation over steady state.
• Initial decrease in the concentration of VEGF165-VEGFR2 complex (B) is because of PIGF blocking of VEGF165-Neuropilin binding. Thus the VEGF165, that are induced to bind VEGFR2 by Neuropilin binding, can not form VEGF165-VEGFR2 complexes anymore.
Results

Figure 3: Concentrations of VEGF165-Neuropilin and VEGF165-VEGFR2 complexes depending on the Antibody conc. levels which in turn blocks Neuropilin-VEGFR2 coupling versus time. Complexes’ conc. units are pmol/cm^3, Antibody conc. unit is uM and time unit is hours.

- Initial decrease in the concentration of VEGF165-Neuropilin complex is (A) might be because of less initial concentrated free VEGF165 in the environment.
- Initial decrease in the concentration of VEGF165-VEGFR2 complex (B) is because of antibody blocking of VEGFR2-Neuropilin coupling. Thus the VEGF165, that are induced to bind VEGFR2 by Neuropilin binding, can not form VEGF165-VEGFR2 complexes anymore.
- The reason that makes this decrease (B) transient is, internalization of Antibodies and so decreasing in the concentration of antibody levels in the environment.
Acknowledgements

The national BBSI program (http://bbsi.eeicom.com) is a joint initiative of the NIH-NIBIB and NSF-EEC, and the BBSI @ Pitt is supported by the National Science Foundation under Grant EEC-0234002.

• James R. Faeder
• Department of Computational Biology, University of Pittsburgh
• Leonard R. Harris (post-doc, Faeder’s Lab)
Thanks!