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PRINCIPAL INVESTIGATOR: Mac Gabhann, Dr. Feilim

CONTRACTING ORGANIZATION: Johns Hopkins University, The
Baltimore MD 21218-2680

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6. AUTHOR(S)
Mac Gabhann, Dr. Feilim

E-Mail: feilim@jhu.edu

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3400 N Charles St W400 Wyman Park Bldg
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14. ABSTRACT

The vascular endothelial growth factor (VEGF) family of cytokines promotes vascularization, tumorigenesis and metastasis in many cancers. Our goal is to develop computational models that combine mechanistic topological data on the VEGF protein interaction network with gene expression datasets for a large population of prostate cancers. We have assembled databases of prostate cancer gene expression data, and analyzed the data using bioinformatic techniques, identifying key VEGF-based subgroups of prostate cancer plus biomarkers that identify these groups. We have also created new computational models to simulate prostate cancer, based on the individualized gene expression data. These models will be used to simulate therapies that target the pathway. The therapies to be tested include anti-ligands such as bevacizumab but also anti-receptors and small molecules such as tyrosine kinase inhibitors. In this way, we can build on both the successes and the failures of anti-VEGF trials to date in order to develop more effective therapies for prostate cancer. This progress will continue, and we will be able to develop models of therapies including bevacizumab and other drugs, in order to design improved therapeutic approaches (both for individuals and for the population).

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Introduction

The vascular endothelial growth factor (VEGF) family of cytokines promotes vascularization, tumorigenesis and metastasis in many cancers (*1*). Although prostate cancer is vasculature- and VEGF-dependent, Phase III clinical trials have failed to show benefit for the anti-VEGF bevacizumab (*2*). Some recent Phase II clinical trials for castration-resistant prostate cancer have revived hope in this treatment (*3, 4*) and underscored the need to better understand these drugs and their targets. In order to improve our understanding, we will create prostate-cancer-specific computational models of VEGF and its receptors. These models will be used to simulate therapies that target the pathway. The therapies to be tested include anti-ligands such as bevacizumab but also anti-receptors and small molecules such as tyrosine kinase inhibitors (*5*). In this way, we can build on both the successes and the failures of anti-VEGF trials to date in order to develop more effective therapies for prostate cancer.

Body

In this section, we will go through the five tasks outlined in the statement of work and describe the progress made towards accomplishing these tasks.

Task 1. Collate the publicly available prostate cancer gene expression datasets. (months 1-36)
1a. Collate the currently available datasets (months 1-3)
1b. Develop a monitoring policy for new datasets and (months 1-3)
1c. Incorporate new datasets as they become available (months 4-36)

Progress to date for Task 1: We have identified the publicly available datasets for prostate cancer gene expression, and we have assembled these into working databases for our needs. Because these high-throughput studies use different platforms, we keep those separate and only perform comparative analyses on datasets with the same platform. For example, we do not mix RNASeq datasets with microarray datasets. However, we do compare the outcomes of these analyses, to determine whether there the qualitative insights are consistent from set to set. An example of this methodology is available in our published breast cancer study (*6*). Specifically, for the prostate cancer study and for the results presented here, we are working primarily with 176 samples from the TCGA study, quantified using RNASeq. As defined in the statement of work, we will continue to collect and analyze datasets as the project progresses.

Task 2. Analyze the gene expression datasets (months 1-12)
2a. Bioinformatic analysis of angiogenesis genes for prostate cancer (months 1-9)
2b. Write manuscript describing findings (months 9-12)

Progress to date for Task 2: We have analyzed the collated prostate cancer data.

First, we performed principal component analysis (PCA) of 39 VEGF and semaphorin ligands and receptors in prostate tumor samples to find patterns of co-regulation among these genes (**Fig. 1**), with comparisons to breast and kidney cancer. Four out of five VEGF receptors (FLT1, KDR, FLT4, and NRP2) had large negative loadings on the first prostate principal component, indicating that these genes had high correlations in the prostate cancer dataset. This pattern was also noted in the breast and kidney cancer datasets, suggesting that correlation of the expression of multiple VEGF receptors may be common to multiple types of tumors.

The second principal component in the prostate dataset was notable due to the large negative loadings for SEMA3A, SEMA3C, SEMA3D, and SEMA3E, with a large positive loading for NRP1 (**Fig. 1**). NRP1 promotes VEGF signaling, while class 3 semaphorins typically inhibit angiogenesis; thus, samples with high positive second principal components likely possess a pro-angiogenic signature.

We used K-means clustering to determine groups of prostate tumor samples with distinct patterns of VEGF and semaphorin expression. Consensus methods, where clustering was performed on random subsets of the data multiple times, showed that 3 clusters gave the best clustering (**Fig. 2**). These 3 clusters were primarily distinguished by scores of principal component 1 (PC1), as shown in the clustering heatmap (**Fig. 3**). Thus, one cluster has high expression of the FLT1/KDR/FLT4/NRP2 signature identified by principal component analysis, while another cluster has low expression of this signature.

We expanded the list of genes of interest by augementing the VEGF and Semaphorin families with other angiogenesis-related growth factors and receptors. Examining this expanded dataset, we wanted to identify whether a gene expression-based indicator could be found in prostate cancer that correlated with significant gene expression changes across the whole angiogenesis network. Thus, we screened all the genes in the RNA-Seq dataset for genes whose expression had a bimodal distribution. Of the 11 genes found to have bimodal distributions, one of them, ERG, resulted in high classification accuracy (93%) when a partial least squares discriminant analysis (PLS-DA) model was built using the 85-gene dataset (Fig. 4). ERG+ samples had higher expression of PDGFA and PDGFC, and lower expression of SEMA3E and SEMA3F. High expression of ERG is correlated with fusion of the TMPRSS2 and ERG genes, and is associated with poorer prognosis in prostate cancer patients (*7*).

We are preparing a manuscript based on these findings and continuing to analyze the data to identify additional insights.

Task 3. Build canonical prostate cancer VEGF transport model (months 1-15)
3a. Collate anatomical and other prostate-specific parameters (months 1-12)
3b. Build computational model of prostate cancer within human body (months 3-6)
3c. Simulate and analyze prostate cancer with representative (average) gene expression (months 6-12)
3d. Write manuscript based on canonical (average) prostate cancer VEGF model (months 12-15)
3e. Post code for model on public model database sites (after manuscript acceptance)

Progress to date for Task 3: We have built the transport model for prostate cancer. The efficacy of drugs that target proteins is dependent on the protein interaction network of the target. There are several VEGF isoforms and multiple VEGF receptors on endothelial cells (*8*). The overall vascular response to the drug is not obvious because of the multiple competing ligands and receptors. Only by including all of these in a computational simulation can we find the impact of the drug, and how that impact changes depending on the variable expression of the competing ligands and receptors.

To show the utility of this method, and to show preliminary data for Task 4, we simulated the transport and receptor binding of VEGFA in compartmental models representing a *population* of prostate cancer patients. The simulation results show multiple different metrics that the model can output to relate to angiogenesis signaling. Each dot on the graphs represents a different individual's tumor. **Fig. 5** shows the effect of each of 4 gene expression inputs on various simulated model features. VEGFA expression (left column) has the strongest association with the features related to VEGF concentrations and receptor binding. Surprisingly, the correlations between VEGFA expression and total VEGFR1/VEGFR2 binding are higher than the correlations between VEGFA expression and plasma total VEGF/tumor free VEGF. This kind of insight is very relevant when it comes to assessing plasma VEGF as a potential biomarker for diagnosis, prognosis, and evaluation of treatment response.

Task 4. Build virtual patient bank (months 12-36)
4a. Incorporate available patient-specific parameters into computational models (months 12-18)
4b. Monitor new datasets (see #1c above) and add virtual patients as appropriate (months 18-36)

Progress on Task 4: As per the statement of work, the substantial work on Task 4 will take place in Years 2 and 3 of the grant. Some preliminary work for Task 4 was described in Task 3 above.

Task 5. Test anti-VEGF pathway therapies against the virtual patient population (months 18-36)
5a. Incorporate anti-VEGF pathway therapies into the canonical code (months 18-21)
5b. Test the anti-VEGF pathway therapies against the population of virtual patient models (months 21-36)
5c. Analyze the data from these results, in particular a bioinfomatic analysis to reduce the number of possible therapies and identify contiguous therapy-responsive subgroups of patients with accompanying biomarkers (months 21-36)
5d. Write manuscript based on virtual patient-specific predictions (months 33-36)
5e. Post code for model on public model database sites (after manuscript acceptance)

Progress on Task 5: As per the statement of work, work on Task 5 will take place in Years 2 and 3 of the grant.

Key Research Accomplishments

- Development of prostate-specific computational model of VEGF transport
- Creation and simulation of first-draft patient-specific computational models
- Identification of possible biomarkers of multi-gene growth factor networks in prostate cancer

Reportable Outcomes

None yet. We anticipate publications to report by the end of Year 2.

Conclusion

As described above, we have collated prostate cancer gene expression data, analyzed the data using bioinformatic techniques, and created new computational models to simulate prostate cancer, based on the individualized gene expression data. This progress will continue, and we will be able to develop models of therapies including bevacizumab and other drugs, in order to design improved therapeutic approaches (both for individuals and for the population).

So what: Without the computational model, the extensive knowledge of mechanisms that have resulted from many researchers studying these pathways could not be used in combination with the high-throughput data. In short, our models allow us to place the individualized data in its correct context – the complex molecular interaction networks inside tumors.

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Appendices

None

Supporting Data

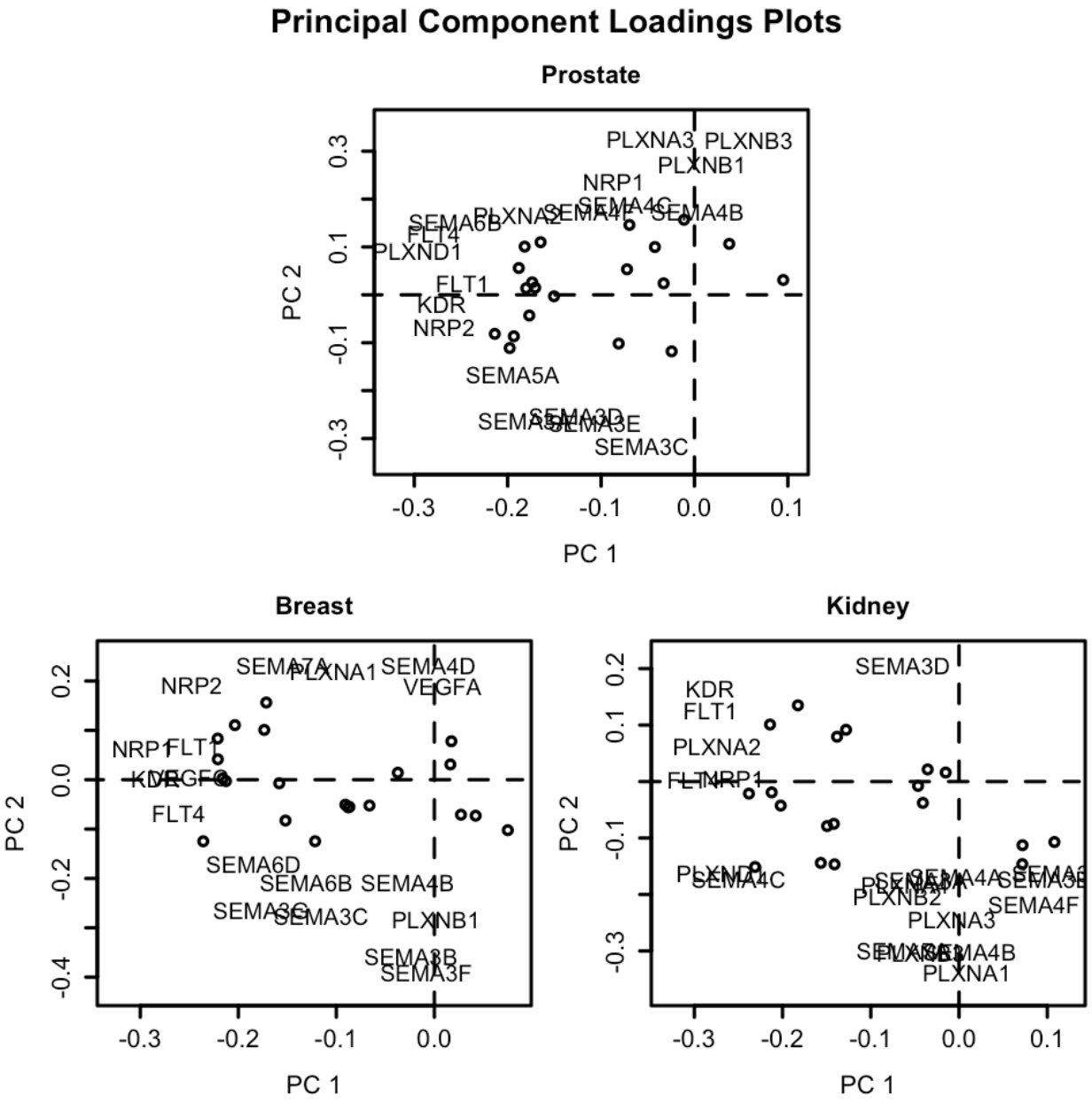

Figure 1. Principal Component analysis for Prostate Cancer. These loading plots show the key genes that make up the first and second principal components (PCs) of these data sets. The breast and kidney cancer plots are included for comparison; it is important to place prostate cancer in context with other cancers, in order to be able to interpret how the success or failure of VEGF-targeting therapeutics in those cancers can provide guideposts for prostate cancer therapeutics.

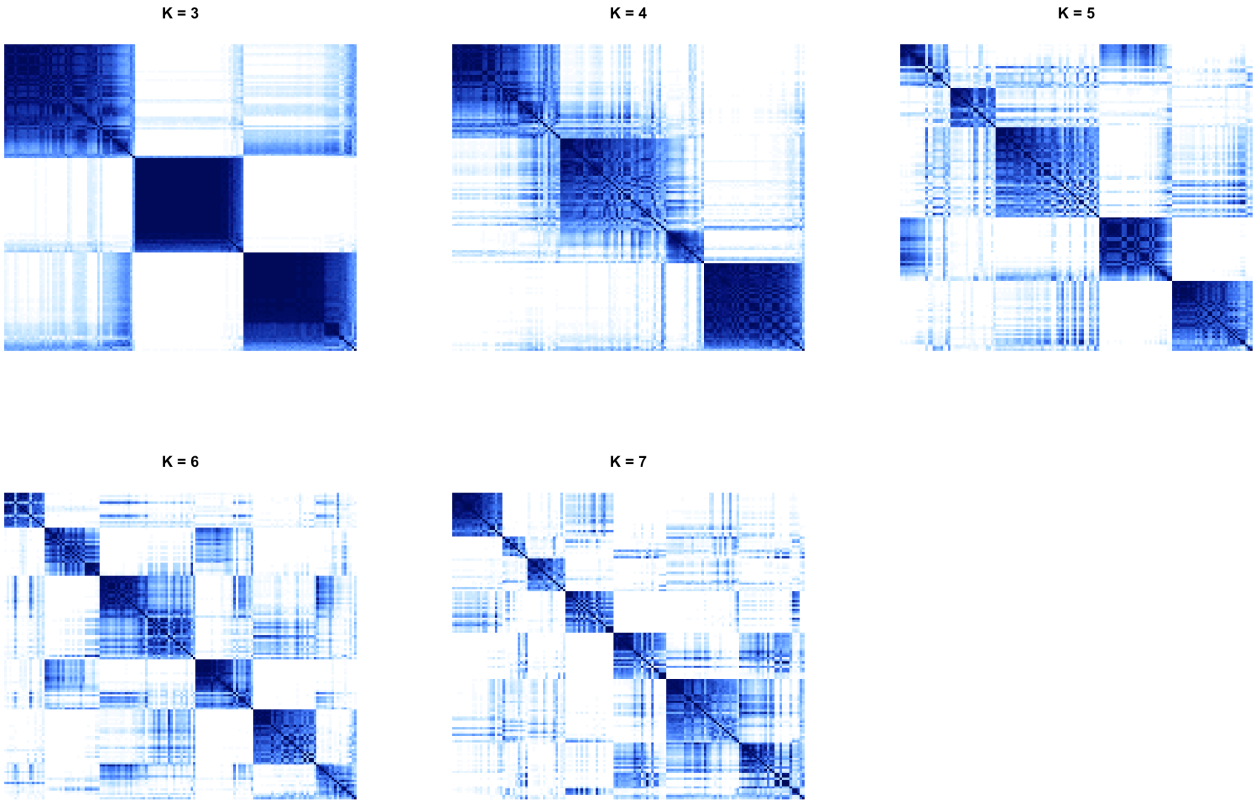

Figure 2. K-means clustering of prostate cancer data shows that creating three subgroups provides the cleanest and most consistent separation of data.

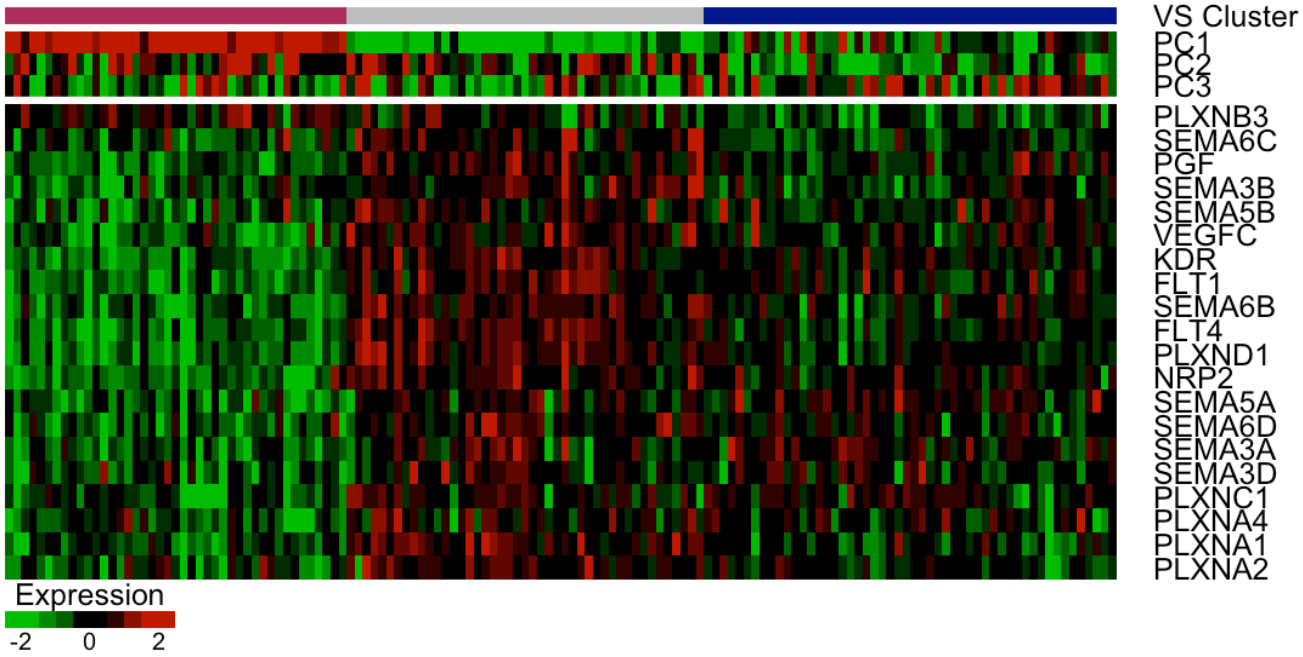

Figure 3. Clustering of prostate cancer data shows the three clusters that emerge from the gene expression of the VEGF family of growth factors and their receptors, and of the Semaphorin family (which competes with VEGFs for binding the key Neuropilin receptors). Red indicates higher expression, green indicates lower expression. Values for three principal components (PC) are also shown.

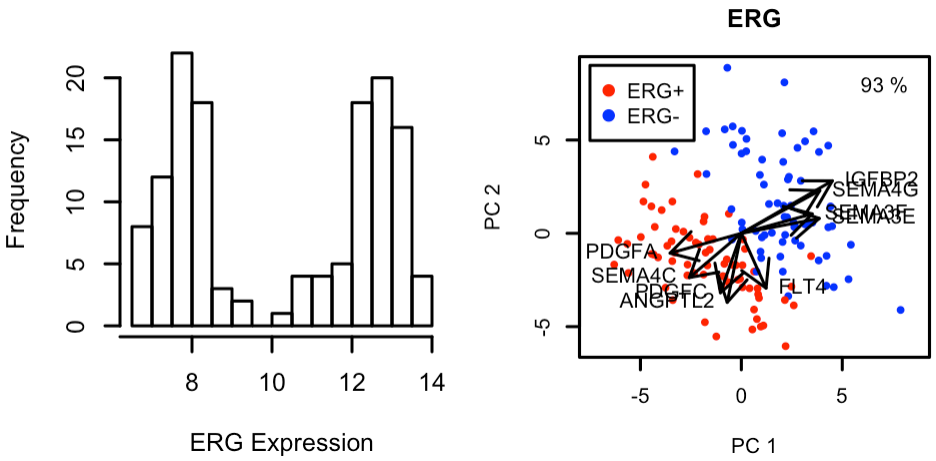

Figure 4. ERG expression is associated with differences in expression of key angiogenesis-related proteins. Left, ERG expression is bimodal within the prostate cancer population. Right, the principal components of angiogenesis-related gene expression are highly predictive of ERG expression.

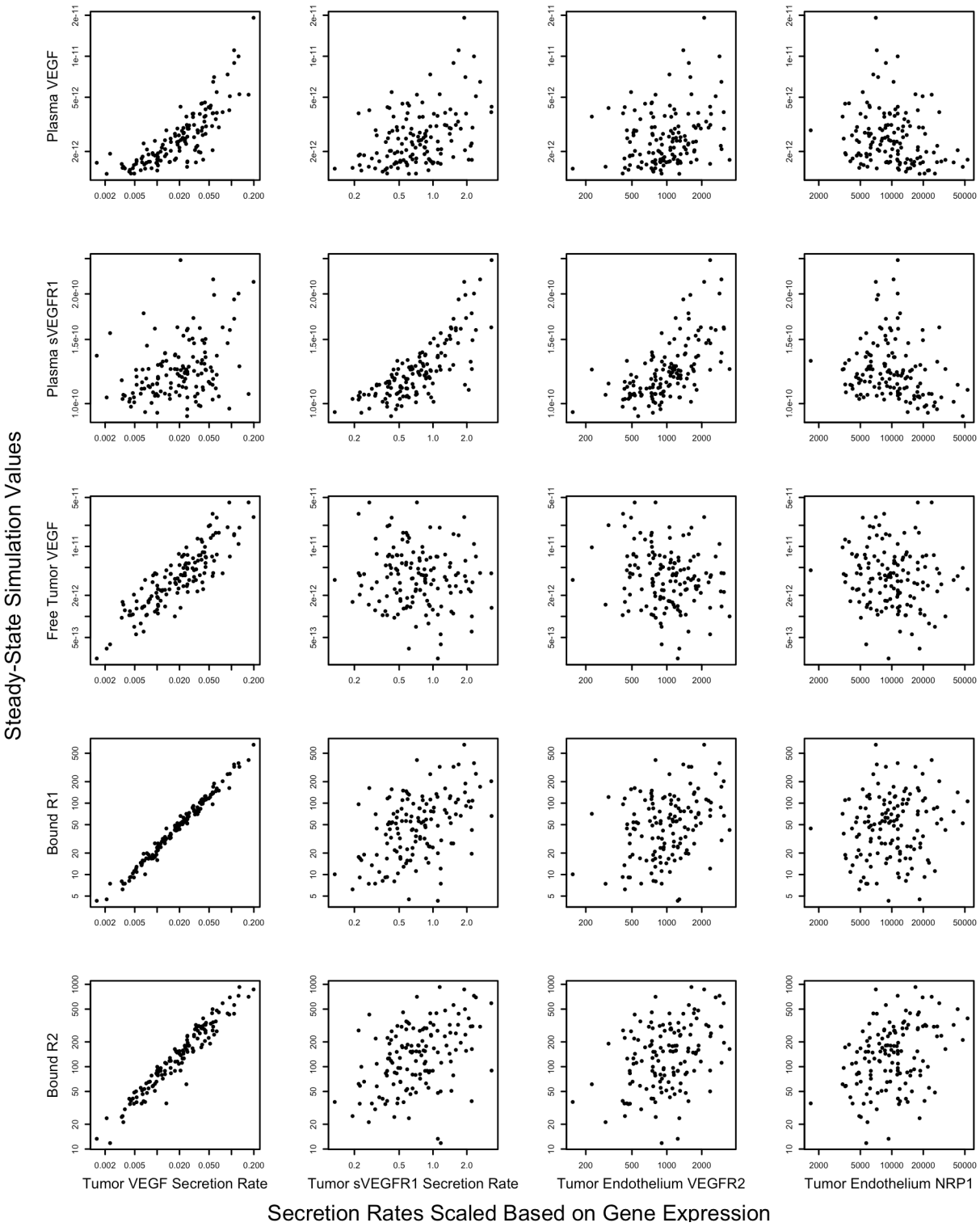

Figure 5. Whole-body simulations of VEGF distribution in prostate cancer. Each dot represents one individual tumor, each with a unique signature of gene expression across the multiple growth factors and receptors of the VEGF family. These simulations predicts multiple outputs related to VEGF signaling, such as ligated receptors.