Oscillation of Angiogenesis and Vascular Dropout in Progressive Human Vascular Disease

Vascular Pattern as Useful Read-Out of Complex Molecular Signaling

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Oscillation of Angiogenesis and Vascular Dropout in Progressive Human Vascular Disease

When analyzed by VESsel GENeration Analysis (VESGEN) software, vascular patterns provide useful integrative read-outs of complex, interacting molecular signaling pathways. Using VESGEN, we recently discovered and published our innovative, surprising findings that angiogenesis oscillated with vascular dropout throughout progression of diabetic retinopathy, a blinding vascular disease. Our findings provide a potential paradigm shift in the current prevailing view on progression and treatment of this disease, and a new early-stage window of regenerative therapeutic opportunities. The findings also suggest that angiogenesis may oscillate with vascular disease in a homeostatic-like manner during early stages of other inflammatory progressive diseases such as cancer and coronary vascular disease.
Cardiovascular Alterations, Immunosuppression & Bone Loss: NASA-defined risk categories for human space exploration
Recent results with Peter Kaiser MD, Cole Eye Institute
Oscillation of vessel density with progression of diabetic retinopathy

"Eye as a Window to the Body"
True of other vascular-dependent progressive diseases such as solid tumors?

Vascular pattern as integrative read-out of complex signaling
VESGEN software for mapping and quantification of progressive angiogenesis and microvascular remodeling

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VESGEN
APPLICATIONS

Vascular Trees
Human Retina
Avian CAM, Yolksac and Murine/Avian Coronary Vessels
*(Solid Tumors?)*

Vascular Networks
Mouse Postnatal Retina
CAM Lymphatic Vessels

Vascular Tree-Network Composites
Normal and Abnormal Embryonic Coronary Vessels

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Mapping and Quantification of Microvascular Remodeling and Angiogenesis by VESGEN
Panel to specify vessel type

Main panel
- Image specification
- Algorithm selection
- Process initiation
Clinical Images of Human Retina

Extracted Vascular Pattern

Region-Based Fractal Analysis of Linearized Pattern

Normal

Early Diabetic Retinopathy

Late Diabetic Retinopathy

Mapping of Progressive Diabetic Retinopathy by VESGEN

Mild NPDR  Moderate NPDR  Severe NPDR  PDR

Vascular Trees

Arteries

Veins

Generations (G)

Grouping by Vascular Remodeling Status (VRS)

Angiogenesis Oscillates with Vascular Dropout during Progression of Diabetic Retinopathy

Slight Trend toward Increasing Diameter of Larger Vessels during Progression of Diabetic Retinopathy

Oscillation of Angiogenesis with Vascular Dropout: Systems Biology Analysis

Space-Filling Capacity of Arterial and Venous Trees by VESGEN Analysis of Branching Generations ($G_1,...,G_8$ or $G_9$) as $f(D_f, N_v, L_v, Br_v+E_v, D_v, T_v, \theta ...)$

Vessel Number Density, $N_v$
Vessel Length Density, $L_v$
Vessel Diameter, $D_v$

Fractal Dimension, $D_f$
$Br_v+E_v$ from Branch Point Density, $Br_v$ and Endpoint Density, $E_v$

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Basic Research to Innovative Translational Medicine
Dynamic Balance Hypothesis

VESGEN Hypothesis: ‘Signature’ Vascular Patterns

FGF-2 as a Simple Stimulator
(Fibroblast Growth Factor-2)

VEGF as a Complexity Factor
(Vascular Endothelial Growth Factor-2)

TGF-β1 as a Simple Inhibitor
But Complex Potentiator
(Transforming Growth Factor-β1)

The form of an object is a 'diagram of forces'
- D'Arcy Thompson
Fig. 7 Parsons-Wingerter et al.
CAM LYMPHATIC NETWORK

Confocal Microscopy

VESGEN Network Output

Distance Mapping

Colorized Skeleton

VASCULAR NETWORKS IN TRANSGENIC MOUSE RETINA

Fluorescence Microscopy

VESGEN Network Output
Distance Mapping
Colorized Skeleton

with J Sears & Q Ebrahem (Cole Eye Institute), from Vickerman et al, Anatomical Record A 292(3), 2009
CORONARY VESSEL NETWORK-TO-TREE TRANSITIONS

Vickerman et al, VESGEN Review, Anatomical Record A 292(3), 2009
TA Treatment in CAM Vascular Tree

A. Quail CAM Control

B. TA (8 ng/CAM)


D. 1 mm

E. TA (8 ng/CAM)

F. Generations

(0)
Vascular Targeting of Tumor Vessels In RIP-Tag Transgenic Mice by iv Injection

Vascular Targeting: $\alpha_5\beta_1$ Integrin Antibody iv in Transgenic RIP-Tag2 Pancreatic Tumors

Integrin antibody iv

Vascular Targeting: Increasing Expression of α5β1 Integrin in Blood Vessels with RIP-Tag2Tumor Progression

Vascular Targeting: α5β1 Integrin Antibody iv in Transgenic apc Intestinal Tumors

Increased Expression of $\alpha_5\beta_1$ Integrin Antibody iv in Transgenic apc Intestinal and MCa-IV Mammary Tumors

FRACTAL SCALING of Vascular Network and Integrin Expression with Tumor Progression

Time 0.000 sec

velocity profile

Spatial Avg of Velocity vs. Time

average velocity

velocity

1774.02
1419.21
1064.41
709.61
354.80
0.00

microns/sec
Integrative Systems Biology:
‘Fingerprint’ or ‘Signature’ Mapping of Dominant Vascular Patterns Induced by VEGF and Other Angiogenesis Regulators
Fractal-Based VESGEN for Multi-Parametric Pattern Analysis according to Branching Generation

Clinical Research Application: Oscillation of Angiogenesis with Vascular Dropout in Diabetic Retinopathy
General paradigm for progression of other diseases such as cancer?

Integrated Scaling of Vascular Anatomy with Signaling Regulators such as $\alpha 5\beta 1$ Integrin during Tumor Progression

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Acknowledgements

**NASA Glenn Research Center**
Mary Vickerman MS, Patricia Keith MS, Mark Wernet PhD, Terri McKay BS, Dan Gedeon, Alan Hylton MS, Daniela Ribita BS, Harry Olar BS, Camille Everhart, Dedra Whitfield

**Cleveland Clinic Foundation**
*Cole Eye Institute*
Peter Kaiser MD, Jonathan Sears MD, Quteba Ebrahem MD

*Lerner Research Institute*
Paul DiCorleto PhD, Unni Chandrasekharan PhD, Ron Midura PhD

**University of New Mexico School of Medicine/Ohio Aerospace Institute**
Krishnan Radhakrishnan MD PhD

**University Hospitals, Case Western Reserve University**
Steven Fisher MD, Hong-Bin Liu PhD
Michiko Watanabe PhD, Ganga Karunamuni BS, Monica Montano PhD

**NASA, NIH, NSF (University of Washington)**

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Particle Imaging Velocimetry (PIV) for Analysis of Blood Flow *in Vivo*
spatial average of velocity magnitude by frame

11/18/05

velocity magnitude (microns/sec)

avg velocity magnitude per frame (masked)
7-frame average
### Stimulation of Angiogenesis

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>FGF-2</th>
<th>Increased Density</th>
<th>Increased Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td><img src="image" alt="control_24h" /></td>
<td><img src="image" alt="fgf-2_24h" /></td>
<td><img src="image" alt="increased_density_24h" /></td>
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<td><img src="image" alt="increased_density_48h" /></td>
<td><img src="image" alt="increased_diameter_48h" /></td>
</tr>
</tbody>
</table>


*Arteriosclerosis Thrombosis Vascular Biology 20:1250-1256 (2000)*
Angiogenesis in the Developing Quail CAM

E6  E7  E8

E9  E10  E11
The Rate of Angiogenesis in the Quail CAM is a Linear Process

HMW Kininogen Treatment in CAM Vascular Tree

Keith McCrae, Hematology & Oncology
Engineering Testbed for VESGEN Innovations:
Quail Chorioallantoic Membrane (CAM)

Embryo

CAM Specimen
Inhibition of Angiogenesis

Control  TGF-β1  Angiostatin  Triamcinolone Acetonide

24 h

48 h

Unique ‘Signature’ Patterns:

Vasculature as Integrative Read-Out System of Complex Molecular Signaling

Observation

Dominant molecular regulators of vascular remodeling and angiogenesis induce vascular patterns that are spatio-temporally unique

Hypothesis as Consequence

Dominant regulators can be deduced from alterations in vascular pattern as integrative read-out of complex molecular and systems signaling
Box-Counting Algorithm for Fractal Dimension

a 1024 = 2^{10}
  512 = 2^{9}
  256 = 2^{8}
  128 = 2^{7}

Box width (pixels)

b Fractal dimension, \( D_f \)

Slope = -1.297
\( r^2 = 0.970 \)

K Radhakrishnan, P Parsons-Wingerter, M B Vickerman, P K Kaiser
Vascular Pattern is Altered in Early-Stage Diabetic Retinopathy

Normal

Nonproliferative

Fractal Dimension

- Normal
- NPDR

Center  Periphery  Center  Periphery  Paramacular
Clinical Fluorescein Angiography of Human Retina
Mild NPDR

50° FA

Vascular Trees

Arteries
Veins

Krishnan Radhakrishnan, Peter Kaiser (Cole Eye Institute)
Steroid Triamcinolone Acetonide (TA): Inhibition of Angiogenesis in the CAM

TA Inhibition Selectively Targets Small Vessels

and Thins Vessels throughout the Vascular Tree

VEGF TRAP Expression in Developing Coronary Tree

Steven Fisher, Hong-Bin Liu (Cardiology), Krishnan Radhakrishnan