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
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

Molecular Regulation

Avian CAM

Transgenic Mouse

4. Humans

Cells in Vitro

Blood Vessels

Blood Vessels
Main panel
- Image specification
- Algorithm selection
- Process initiation

Panel to specify vessel type
Clinical Images of Human Retina

Early Diabetic Retinopathy

Late Diabetic Retinopathy

Extracted Vascular Pattern

Region - Based Fractal Analysis of Linearized Pattern

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)

**Arterial**

- **VRS 1**
- **VRS 2**
- **VRS 3**
- **VRS 4**

**Venous**

- **Progressive Ranking by Clinical Diagnosis**

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 \((G_1 \ldots G_8 \text{ or } G_9)\) as \(f(D_f, N_v, L_v, Br_v+E_v, D_v, T_v, \theta \ldots)\)

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

VESGEN

VESGEN

The form of an object is a 'diagram of forces'
- D'Arcy Thompson

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)
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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

Mouse Retina
Control (P15)

TA-Treated

CORONARY VESSEL NETWORK-TO-TREE TRANSITIONS

TA Treatment in CAM Vascular Tree

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

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

Vascular Targeting: $\alpha_5\beta_1$ 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

**VESGEN**

*for Vascular Mapping & Quantification*

**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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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)

frame

e5_a3

e5_a2

e5_a1

e5_a0

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

Control

FGF-2

Increased Density

Increased Diameter

24 h

48 h


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

A
GRAYSCALE
25 μg HKa/CAM

B
BINARY

C
BRANCHING GENERATION

D
Medium Control

E

F

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

Box width (pixels)

\[ 1024 = 2^{10} \]
\[ 512 = 2^9 \]
\[ 256 = 2^8 \]
\[ 128 = 2^7 \]

Fractal dimension, \( D_f \)

\[ \text{Slope} = -1.297 \]
\[ r^2 = 0.970 \]

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

Clinical Fluorescein Angiography of Human Retina
Mild NPDR

50° FA

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