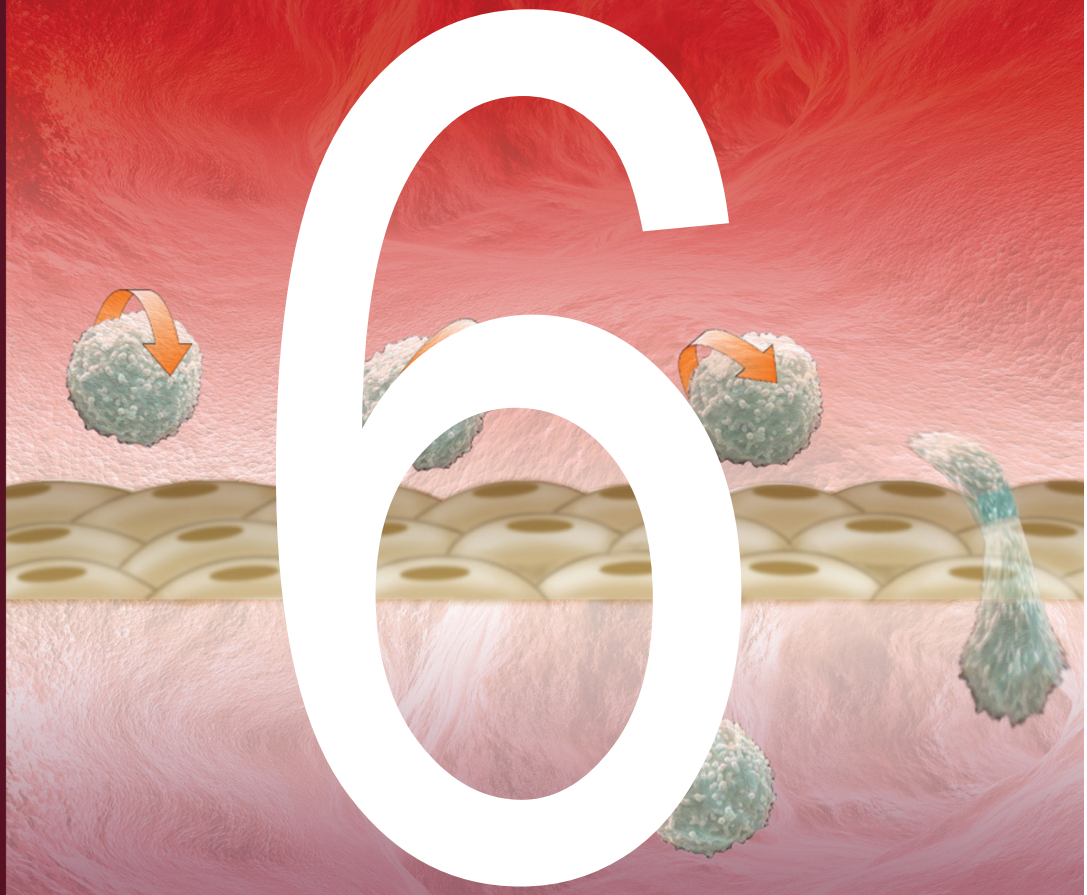
Mechanisms of Vascular Disease:

A Reference Book for Vascular Specialists



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Mechanisms of Vascular Disease

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A Reference Book for Vascular Specialists

Robert Fitridge
The University of Adelaide, The Queen Elizabeth Hospital, Woodville, Australia
Matthew Thompson
St George's Hospital Medical School, London, UK

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List of Contributors

David G Armstrong
The University of Arizona
Southern Arizona Limb Salvage Alliance
Tucson, AZ
USA

Vishwanath Biradar
Intensive Care Unit
The Queen Elizabeth Hospital
Woodville, SA
Australia

Matthew Bown
Department of Vascular Surgery
University of Leicester
Leicester
UK

Andrew W Bradbury
University Department of Vascular Surgery
Birmingham Heartlands Hospital
Birmingham
UK

Edward Choke
Department of Vascular Surgery
University of Leicester
Leicester
UK

Gillian Cockerill
Department of Clinical Sciences
St George's Hospital Medical School
London
UK

Prue Cowled
Department of Surgery
University of Adelaide
The Queen Elizabeth Hospital
Woodville, SA
Australia

Helen Daly
Royal Perth Hospital
Perth, WA
Australia

Mital Desai
University Department of Vascular Surgery
Royal Free Hospital
University College
London
UK

Robert F Diegelmann
Department of Biochemistry
Medical College of Virginia
Richmond, VA
USA

Timothy K Fisher
Rashid Centre for Diabetes and Research
Sheikh Khalifa Hospital
Ajmon
UAE

Robert A Fitridge
Department of Surgery
University of Adelaide
The Queen Elizabeth Hospital
Woodville, SA
Australia

Gail Gillespie
Royal Perth Hospital
Perth, WA
Australia

Jonathan Golledge
Vascular Biology Unit
School of Medicine & Dentistry
James Cook University
Townsville, QLD
Australia

George Hamilton
University Department of Vascular Surgery
Royal Free Hospital
University College
London
UK

Mark Hamilton
Department of Surgery
University of Adelaide
The Queen Elizabeth Hospital
Woodville, SA
Australia

Robert J Hinchliffe
St George's Vascular Institiute
St George's Hospital
London
UK

Richard D Kenagy
Department of Surgery
University of Washington
Seattle, WA
USA

Paul Kerr
Department of Pharmacology
University of Alberta
Alberta
Canada

Michael MD Lawrence-Brown
Curtin Health Innovation Research Institute
Curtin University
Perth, WA
Australia

Brian Lepow
The University of Arizona
Department of Surgery
Southern Arizona Limb Salvage Alliance
Tucson, AZ
USA

Kurt Liffman
CSIRO Material Science & Engineering and School of Mathematical Sciences
Monash University
Melbourne, Vic
Australia

Ian Loftus
Department of Vascular Surgery
St George's Hospital
London
UK

Mark J McCarthy
Department of Surgery and Cardiovascular Sciences
University of Leicester
Leicester
UK

Greg S McMahon
Department of Surgery and Cardiovascular Sciences
University of Leicester
Leicester
UK

Simon McRae
Adult Haemophilia Treatment Centre
SA Pathology
Adelaide, SA
Australia

Joseph L Mills
The University of Arizona
Southern Arizona Limb Salvage Alliance
Tucson, AZ
USA

Lyle Moldawer
Department of Surgery
University of Florida
Gainesville, FL
USA

John L Moran
Faculty of Health Sciences
University of Adelaide
The Queen Elizabeth Hospital
Woodville, SA
Australia

Stephen Nicholls
The Heart and Vascular Institute
Cleveland Clinic
Cleveland, OH
USA

Ian M Nordon
St George's Vascular Institiute
St George's Hospital
London
UK

Paul E Norman
School of Surgery
University of WA
Fremantle, WA
Australia

Karlheinz Peter
Baker IDI Heart & Diabetes Institute
Melbourne, Vic
Australia

Frances Plane
Department of Pharmacology
University of Alberta
Alberta
Canada

Janet T Powell
Imperial College
London
UK

Sandeep Prabhu
Baker IDI Heart & Diabetes Institute
Alfred Hospital
Melbourne, Vic
Australia

Rishi Puri
The Heart and Vascular Institute
Cleveland Clinic
Cleveland, OH
USA

Stephan A Schug
Royal Perth Hospital
Perth, WA
Australia

Gregory S Schultz
Department of Obstetrics and Gynaecology
University of Florida
Gainesville, FL
USA

Rahul Sharma
Baker IDI Heart & Diabetes Institute
Alfred Hospital
Melbourne, Vic
Australia

Guo-Ping Shi
Department of Cardiovascular Medicine
Brigham & Women's Hospital
Harvard Medical School
Boston, MA
USA

Michael Stacey
University Department of Surgery
Fremantle Hospital
Fremantle, WA
Australia

Ilija D Sutalo
CSIRO Material Science & Engineering
and Curtin Health Innovation
Research Instutute
Curtin University
Highett, Vic

Raymond Tam
Department of Pharmacology
University of Alberta
Alberta
Canada

Matthew Thompson
St Georges Hospital Medical School
London
UK

Martin Veller
Department of Surgery
University of Witwatersrand
Johannesburg
South Africa

Mauro Vicaretti
Department of Vascular Surgery
Westmead Hospital
Westmead, NSW
Australia

Matt Waltham
Academic Department of Surgery
St Thomas' Hospital
London
UK

Matthew L White
Vascular and Endovascular Surgery
University of Arizona
Tucson, AZ
USA

David P Wilson
School of Medical Sciences
Discipline of Physiology
University of Adelaide
Adelaide SA
Australia

Qingbo Xu
Department of Cardiology
Kings College
University of London
UK

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Abbreviation List

a1-PI	a1-protease inhibitor
5-HT	5-Hydroxytryptamine/Serotonin
AAA	Abdominal aortic aneurysm
AAS	Acute aortic syndrome
AAV	Adeno-associated viruses
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ACS	Abdominal compartment syndrome
ACTH	Adrenocorticotropic hormone
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADP	Adenosine diphosphate
AIDS	Acquired immune deficiency syndrome
ALI	Acute lung injury
AMP	Adenosine monophosphate
AMPA	α-amino-3 hydroxy-5-methylisoxazole
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
AOD	Aortic occlusive disease
AP1	Activated protein 1
APC	Activated protein C
APC	Antigen presenting cell
APLAS	Antiphospholipid antibody syndrome
ApoAI	Apolipoprotein AI
ApoE	Apolipoprotein E
APS	Antiphospholipid antibody syndrome
APTT	Activated partial thromboplastin time

ARDS	Acute respiratory distress syndrome
AT	Antithrombin
ATP	Adenosine triphosphate
AVP	Ambulatory venous thrombosis
β2-GPI	β2-glycoprotein Ib
bFGF	Basic fibroblast growth factor
BKCa	Large conductance calcium activated potassium channel
BMPs	Bone morphogenetic proteins
BMS	Bare metal stent
CAD	Coronary artery disease
CaM	Calmodulin
CAM	Cell adhesion molecule
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
cGMP	Cyclic guanine monophosphate
CD	Cluster of differentiation
CD40L	Cluster of differentiation 40 ligand
CEA	Carotid endarterectomy
CETP	Cholesteryl ester transfer protein
CFD	Computational fluid dynamics
CG	Cationized gelatin
CGRP	Calcitonic gene regulated peptide
CHD	Coronary heart disease
CI	Confidence interval
CIMT	Carotid intimal-media thickness
c-JNK	c-Jun N-terminal kinase
CK-MB	Creatinine kinase (Myocardial specific)
CNCP	Chronic noncancer pain
cNOS	Constitutive nitric oxygen synthase enzyme
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CROW	Charcot restraint orthotic walker
CRRT	Continuous renal replacement therapy

CRP	C-reactive protein
CRPS	Complex regional pain syndromes
CT	Computational tomography
CTA	Computed tomographic angiography
CTD	Connective tissue disorders
CTGF	Connective tissue growth factor
CYP	Cytochrome P450
CVD	Cardiovascular disease
CVI	Chronic venous insufficiency
DAG	Diacylglycerol
DES	Drug-eluting stent
DRG	Dorsal root ganglion
DNA	Deoxyribonucleic acid
DSA	Digital subtraction arteriography
DTS	Dense tubular system
DVT	Deep vein thrombosis
EC	Endothelial cell
ECM	Extracellular matrix
EDCF	Endothelium-derived contracting factor
EDH	Endothelium-dependent hyperpolarisation
EDS	Ehlers-Danlos syndrome
EET	Epoxyeicosatrienoic acids
ELAM-1	Endothelial-leukocyte adhesion molecule-1
ELG	Endoluminal grafts
ELISA	Enzyme linked immunosorbent assay
E_K	Equilibrium potential
E_M	Membrane potential
eNOS	Endothelial nitric oxide synthase enzyme
EPC	Endothelial progenitor cells
EPCR	Endothelial protein C receptor
ePTFE	Expanded polytetrafluoroethylene
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate

ET	Essential thrombocytosis
ET-1	Endothelin 1
EVAR	Endovascular aortic aneurysm repair
EVLA	Endovenous LASER ablation
FDA	Food and drug administration
FDPs	Fibrin degradation products (soluble)
FGF	Fibroblast growth factor
FGF-2	Fibroblast growth factor 2
FMN	Flavin mononucleotide
FVL	Factor V Leiden
GABA	Gamma-aminobutyric acid
GABA B	Gamma-aminobutyric acid subtype B
G-CSF	Granulocyte colony stimulating factor
GMCSF	Granulocyte-macrophage colony stimulating factor
GP	Glycoprotein
GPCR	G-protein coupled receptor
GSV	Great saphenous vein
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HIF	Hypoxia inducible factor
HIT	Heparin induced thrombocytopenia
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMG Co-A	Hydroxymethylglutaryl coenzyme-A
HMW	High molecular weight
HPETE	Hydroperoxyeicosatetraenoic acid
HETE	Hydroxyeicosatetraenoic acids
HR	Hazard ratio
hsCRP	High-sensitive C-reactive protein
HSP	Heat shock protein
HUV	Human umbilical vein
IAH	Intra-abdominal hypertension

IAP	Intra-abdominal pressure
IAPP	Intra-abdominal perfusion pressure
ICAM-1	Inter-cellular adhesion molecule-1
ICAM-2	Inter-cellular adhesion molecule-2
ICP	Intra-compartmental pressure
ICU	Intensive care unit
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IHD	Ischemic heart disease
IL	Interleukin
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL1-β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ILT	Intraluminal thrombus
IKCa	Intermediate conductance calcium-activated potassium channels
IMH	Intramural haematoma
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase enzyme
IP(3)	1,4,5-inositol triphosphate
IRI	Ischemia reperfusion injury
IVIG	Intravenous pooled immunoglobulin
IVUS	Intravascular ultrasound
KGF	Keratinocyte growth factor
KGF-2	Keratinocyte growth factor-2
LAP	Latency associated peptide
LCS	Limb compartment syndrome
LDL	Low density lipoprotein
LDS	Loeys-Dietz syndrome
LLC	Large latent complex
LEC	Lymphatic endothelial cells

LFA-1	Lymphocyte function-associated antigen-1
LO	Lipoxygenase
LOX	Lysyl oxidase
LOPS	Loss of protective sensation
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
LTGFBP	Latent TGF binding protein
MAC-1	Macrophage-1 antigen
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor
MFS	Marfan syndrome
MHC	Major histocompatibility
MI	Myocardial infarction
MIP-1	Macrophage inflammatory protein-1
MLC_{20}	Myosin light $chain_{20}$
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MMP	Matrix metalloproteinase
MODS	Multiple organ dysfunction syndrome
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MRTA	Magnetic resonance tomographic angiography
MTHFR	Methylenetetrahydrofolate reductase
MT-MMP	Membrane-type MMP
MVPS	Mitral valve prolapse syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate
NGF	Nerve growth factor

NFκB	Nuclear factor kappa B
NiTi	Nitinol
NJP	Non-junctional perforators
NMDA	N-methyl-D-aspartate
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase enzyme
NSAID	Non-steroidal anti-inflammatory drug
NV	Neovascularisation
OCP	Oestrogen/progesterone contraceptive pill
OPN	Osteopontin
OPG	Osteoprotegerin
OR	Odds ratio
OxLDL	Oxidised low density lipoprotein
PAD	Peripheral arterial disease
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease activated receptor
PAR-1	Protease activated receptor-1
PAR-4	Protease activated receptor-4
PAU	Penetrating aortic ulcer
PC	Protein C
PCA	Poly (carbonate-urea) urethane
PCI	Percutaneous coronary intervention (angioplasty)
PCWP	Pulmonary capillary wedge pressure
PDGF	Platelet-derived growth factor
PDGFβ	Platelet-derived growth factor-β
PDS	Polydioxanone
PECAM-1	Platelet-endothelial cell adhesion molecule-1
PEDF	Pigment epithelium-derived factor
PES	Paclitaxel-eluting stent

PET	Positron emission tomography
PF4	Platelet factor 4
PGI_2	Prostacyclin
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
$PGEI_2/PGI_2$	Prostaglandin I_2
PGN	Peptidoglycan
PHN	Postherpetic neuropathy
PHZ	Para-anastomotic hyper-compliant zone
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PLOD	Procollagen lysyl hydroxylase
PMCA	Plasma membrane Ca^{2+} APTases
PMN	Polymorphonuclear leukocyte
POSS	Polyhedral oligomeric silsesquioxanes
PPAR	Peroxisomal proliferation activating receptor
PPI	Proton pump inhibitor
PRV	Polycythaemia rubra vera
PS	Protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothombin time
PTCA	Percutaneous coronary angioplasty
PTFE	Polytetrafluoroethylene
PTS	Post-thrombotic syndrome
PUFA	Polyunsaturated fatty acid
PVI	Primary valvular incompetence
rAAA	Ruptured AAA
Rac	Ras activated cell adhesion molecule
RANTES	Regulated upon activation, normal T cell expressed and secreted
RAS	Renin angiotensin system
RCT	Randomised controlled trial

RF	Rheumatoid factor
RFA	Radiofrequency ablation
rhAPC	Recombinant human activated protein C
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Relative risk
RSD	Reflex sympathetic dystrophy
S1P	Sphingosine-1-phosphate
SAPK	Stress-activated protein kinase
SCF	Stem cell factor
SCS	Spinal cord stimulation
ScvO2	Superior vena cava venous oxygen saturation
SDF-1	Stromal-cell-derived factor-1
SERCA	Sarco/endoplasmic reticulum CaATPases
SEP	Serum elastin peptides
SES	Sirolimus-eluting stent
SEPS	Subfascial endoscopic perforator surgery
SFA	Superficial femoral artery
SFJ	Sapheno-femoral junction
SIRS	Systemic inflammatory response syndrome
SKCa	Small conductance calcium-activated potassium channels
SLE	Systemic lupus erythematosus
SMA	Smooth muscle alpha actin
SMC	Smooth muscle cell
SMP	Sympathetically maintained pain
SNARE	Soluble N-ethylmaleimide-sensitive factor activating protein receptors
SNP	Single nucleotide polymorphisms
SNRI	Serotonin/Noradrenaline reuptake inhibitors
SPJ	Sapheno-popliteal junction
SPP	Skin perfusion pressure
SR	Sarcoplasmic reticulum
SSRIs	Selective serotonin re-uptake inhibitors
SSV	Small saphenous vein

SVT	Superficial thrombophlebitis
STIM1	Stromal interacting molecule 1
TαCE	TNFα converting enzyme
TAAD	Thoracic aortic aneurysm disease
TAD	Thoracic aortic dissection
TAFI	Thrombin-activatable fibrinolysis inhibitor
Tc-99 MDP	Technetium-99 methylene diphosphonate
TCA	Tricyclic antidepressant
TCC	Total contact cast
TCR	T-cell receptor
TENS	Transcutaneous electrical nerve stimulation
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF	Transforming growth factor
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor-beta
TGL	Triglycerides
Th	T helper
TIA	Transient ischemic attack
TIMP	Tissue inhibitors of metalloproteinase
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor-alpha
tPA	Tissue-type plasminogen activator
TRP	Transient receptor potential
TRPC	Transmembrane receptor potential canonical
TRPV1	Transmembrane receptor potential Vanilloid-type
TXA2	Thromboxane A2
uPA	Urokinase
UT	University of Texas
VCAM	Vascular cell adhesion molecule
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

VEGF-R	Vascular endothelial growth factor receptor
VIP	Vasoactive intestinal peptide
VLA-1	Very late activating antigen-1
VOCC	Voltage operated calcium channels
VPT	Vibratory perception threshold
VSMC	Vascular smooth muscle cells
VTE	Venous thromboembolism
VV	Varicose veins
vWF	von Willebrand factor
XO	Xanthine oxidase

6 • Molecular Approaches to Revascularization in Peripheral Vascular Disease

GREG S. MCMAHON, MARK J. MCCARTHY

Department of Surgery and Cardiovascular Sciences, University of Leicester, Leicester, UK

INTRODUCTION

Currently, treatment options for peripheral vascular disease include angioplasty and reconstructive surgery. An attractive, less invasive alternative could involve the revascularization of ischaemic tissue by the induction of vascular growth. It would be particularly welcome for patients in whom current approaches are difficult or prone to failure, including those with conditions that make surgical intervention unsafe, patients with diffuse occlusive disease and those in whom there is significant downstream microvascular disease. Recent years have witnessed major advances in the understanding of the molecular mechanisms underlying vascular formation and remodelling, as well as the identification of key molecules controlling these processes. Most research has focused on the induction of new vessel formation by stimulating angiogenesis and this has been the goal of the clinical trials directed at peripheral vascular disease. But, whilst the stimulation of angiogenesis may relieve microvascular disease, the bypass of occluded conduit vessels requires the formation of more substantial collateral vessels by the process of arteriogenesis. This chapter will review current understanding of the mechanisms controlling angiogenesis and arteriogenesis; approaches that are, and could be pursued to induce vessel growth in peripheral vascular disease, as well as summarizing the current status of clinical trials.

MECHANISMS OF VASCULAR GROWTH

Strategies currently being developed for the therapeutic induction of vessel growth have evolved, largely, from knowledge of the physiological mechanisms of developmental vascularisation. In development, blood vessels arise initially by the process of vasculogenesis during which precursor cells, known as angioblasts, differentiate into endothelial cells and organize into primitive vessels.[1] These vessels expand by angiogenesis, which includes both sprouting growth and non-sprouting remodelling.[1]

Vasculogenesis

The angioblasts that give rise to endothelial cells in the first stages of developmental vascularisation originate in the mesoderm under the influence of fibroblast growth factor-2 (FGF-2).[2] These differentiate into endothelial cells, which proliferate and aggregate into cords, and become lumenized

to form primitive vascular plexuses.[1] Signalling through vascular endothelial growth factor receptor-2 (VEGF-R2) is crucial for angioblast survival and establishment of these first vessels.[3]

Endothelial cell formation from precursor cells, and *in situ* differentiation into vessels, was thought to be confined to developmental vascularisation. However, it has now been shown that circulating endothelial progenitor cells (EPCs) exist in the adult and that they can contribute to vessel formation.[4] These cells originate in the bone marrow and express CD34 and VEGF-R2 as well as the orphan receptor AC133.[5] EPCs can incorporate into neovessels formed in healing wounds, ischaemic tissue and tumours.[6] The mechanisms controlling the incorporation of EPCs into neovessels are at present poorly defined although a number of growth factors, including VEGF, FGF2, granulocyte-macrophage colony stimulating factor (GMCSF), angiopoietins and cytokines have all been shown to increase the mobilization of EPCs from bone marrow.[7] Crude cell fractions that include EPCs can be expanded *ex vivo* and transplanted into ischaemic tissue in animal models where they have been reported to incorporate into neovessels.[4] Importantly, in some situations neovessels comprising only EPC-derived cells were observed. Several studies have highlighted that the increase in the number of circulating progenitors, induced by cell transfusion or enhanced mobilization, can also enhance restoration and integrity of the endothelial lining, suppress neointimal formation, and increase blood flow to ischaemic sites.[8] Whilst it is possible that EPCs, or EPC-derived cells, can form vessels *in situ*, by a process similar to vasculogenesis, current thinking suggests that the principal role of vascular stem and progenitor cells is paracrine, that is, these cells promote proliferation and migration of existing endothelial cells, as well as produce additional cytokines and chemokines to continue stem and progenitor mobilization, trafficking and adhesion.[9]

Angiogenesis

Establishment of the vascular network in development, as well as new vessel formation in the adult, requires angiogenesis. The two processes of sprouting and non-sprouting angiogenesis are responsible for remodelling of the primitive vascular plexus into a complex functional network. In sprouting angiogenesis, endothelial cells are activated by growth factors to undergo migration, proliferation and morphogenesis into new vessels, and VEGF is the major physiological activator.

Ischaemia is the primary initiator of sprouting angiogenic growth. Low oxygen tension activates expression of a wide range of angiogenic factors including VEGF, VEGF receptors, angiopoietin-2 and platelet-derived growth factor (PDGF). Genes for these factors contain hypoxia responsive elements in their promoters and some, like VEGF, have been shown to be direct targets of the transcriptional regulator hypoxia inducible factor (HIF). HIF-1 is a heterodimer composed of HIFα and HIFβ subunits.[10] Under normoxic conditions HIF-1α is held at low intracellular concentrations by proteosomal degradation. With decreased oxygen tension HIF-1α becomes hydroxylated preventing its association with the von Hippel-Lindau ubiquitin ligase complex that is responsible for directing HIF-1α for degradation.[11] Thus, HIF-1α accumulates in the cell allowing it to activate transcription of hypoxia-inducible genes via the HIFα:β dimer.

VEGF expressed in response to HIF is secreted by ischaemic cells and acts on endothelial cells in adjacent microvessels. In these previously quiescent microvessels, endothelial activation, proliferation and migration are

normally suppressed by signals from abluminal perivascular support cells; pericytes. This suppression needs to be relieved before angiogenesis can proceed.[12] The close interaction between endothelial cells and pericytes is promoted by the ligand angiopoietin-1, which is produced by the pericyte and acts on the endothelial receptor Tie2.[13] Disruption of this signalling interaction is likely to involve angiopoietin-2, another hypoxia-responsive molecule.[14] Angiopoietin-2 can act to inhibit angiopoietin-1-induced activation of Tie2.[15] Once released from the pro-stabilizing effects of pericytes, the endothelial cells are free to invade the perivascular space, aided by proteases that degrade extracellular matrix. Many metalloproteinases have been implicated in angiogenic sprouting, including matrix metalloproteinases 2, 3, 7 and 9 as well as other proteolytic enzymes such as urokinase-type plasminogen activator.[16] Migration of the activated endothelial cells is aided by plasma proteins that extravasate from the activated microvessels in response to the vasodilatory and pro-permeability effects of VEGF. This growth factor is a potent chemoattractant and mitogen for endothelial cells, and directs their migration and proliferation. Interestingly, *in vivo* endothelial cells in the developing vascular sprouts respond differentially to VEGF, with the cells at the tip migrating and those behind the tip proliferating.[17] Migration and proliferation give rise to endothelial cords that become lumenized, a process which is poorly understood, though is known to be enhanced by angiopoietin-1.[18]

Neovessel maturation

Whether by vasculogenesis or angiogenesis, newly formed blood vessels are highly unstable, and are prone to haemorrhage, thrombosis and spontaneous regression in the absence of elevated growth factors.[19] Such vessels are characteristically found in pathological vascularisation and their phenotype can directly contribute to the disease process. Newly formed primitive vascular channels are maintained by local high concentrations of VEGF, withdrawal of which leads to endothelial apoptosis and neovessel regression.[20] Transformation to a functional vessel requires interaction of endothelial cells in the nascent vessel with pericytes, which originate as mesenchymal cells that are recruited to the developing vessel and differentiate into pericytes on contact with the endothelium.[21] Proliferation and migration of partially or fully differentiated pericytes along established microvessels also contributes to mural cell acquisition by sprouting neovessels, and sprouts can themselves recruit mesenchymal cells.[22] Migration and proliferation of pericytes is regulated mainly by platelet-derived growth factor-β (PDGFβ) secreted by endothelial cells. Interaction between mesenchymal cells and endothelial cells in a developing vessel produces phenotypic changes in both cell types. The mesenchymal cell is directed toward a pericyte or smooth muscle phenotype and the endothelial cell adopts the phenotype required for formation of a stabilized microvessel.[1] Pericytes supply anti-apoptotic ligands, including angiopoietin-1,[23] to underlying endothelial cells allowing neovessels to survive the decrease in VEGF concentrations that occur as the ischaemia is relieved by increased perfusion. In addition, pericytes suppress endothelial proliferation and migration, and increase deposition of the perivascular basement membrane, all of which contribute to switching the fragile nascent vessel into a quiescent functional microvessel.[24] Transforming growth factor-β, produced by proteolytic cleavage from a precursor form as a result of endothelial:pericyte interaction,[25] has a central role in these effects.

Microvascular network maturation

Maturation of vascular channels into functional vessels is accompanied by maturation of the neovessel network. This involves optimization of the new vessel configuration, density, branching pattern and vessel hierarchy. Spatial distributions of angiogenic initiators, like VEGF, have a major influence on the direction of the initial branches. There are six members of the VEGF family and VEGF-A is expressed as a number of alternatively spliced variants; in humans these are mainly forms with 121, 165, 189 and 206 amino acids.[26] VEGF165, 189 and 206 possess heparin-binding domains that allow these forms to interact with the extracellular matrix. The ability of VEGF isoforms to be retained by the matrix is important in regulating the spatial organization of vessel branching.[27]

Patterning also occurs through selective loss of certain vessels by regression. Another major determinant of network maturation is the branching and 'splitting' of vessels by non-sprouting angiogenesis. This occurs by the process of intussusception in which vessel lumens are internally divided by insertion, and subsequent growth and stabilization, of transcapillary tissue pillars.[28] The mechanism of intussusception and factors that regulate it are poorly understood, though the Tie receptors are known to have a role.[29]

Arteriogenesis

Further expansion and muscularization of vessels occurs by the process of arteriogenesis; the development of large calibre collateral arteries from a pre-existing network, in response to occlusive disease. This involves recruitment of additional mural cells and their proliferation, as well as expansion of the abluminal extracellular matrix. Whilst angiogenesis is driven by ischaemia, the initiator of arteriogenesis is increased fluid shear stress, which acts directly upon gene expression involved in the endothelial cell cycle.[30] PDGFβ with its effects on smooth muscle cell recruitment and proliferation, and TGFβ, a known regulator of vascular extracellular matrix synthesis, are likely to be key regulators.

Arteriogenesis of collateral vessels has been demonstrated in a number of animal models following the occlusion of major vessels.[31] In humans, well-developed collateral vessels that bypass occluded arteries have been found frequently and those patients with the best developed collaterals often have minimal symptoms.[32] Arteriogenesis of collaterals in response to the occlusion of primary supply vessels occurs in two phases. An initial increase in the lumen size occurs within a few days and this is followed by a slower remodelling of smooth muscle cell and extracellular matrix cover.[31] The early phase of this adaptive arteriogenesis is associated with inflammation. There is monocyte attachment to endothelium secondary to release of monocyte chemoattractant protein-1 (MCP-1), GMCSF and stromal-cell-derived factor-1 (SDF-1), which recruit CD14+ monocytes to the activated endothelial cell surface as well as extravasation and accumulation in the adventitia and perivascular space, with mast cells and T lymphocytes.[33] Monocytes have a critical role in adaptive arteriogenesis as experimental suppression of monocyte numbers decreases arteriogenesis.[34] These cells provide growth factors to stimulate vessel enlargement and proteases that can act on the extracellular matrix to accommodate increased vessel size. Changes to the wall of the vessel involve remodelling of the media, with increased turnover of medial smooth muscle cells and a shift towards a synthetic phenotype, assuming a contractile phenotype after enlargement of arterial diameter.[33]

A new internal elastic lamina is established and vessel wall thickening results from increased extracellular matrix deposition.

THERAPEUTIC INDUCTION OF VASCULAR GROWTH

Most studies on the therapeutic induction of vessel growth at a pre-clinical level, and all clinical trials, have focused on angiogenesis. Clinical trials aimed at relieving peripheral vascular disease by therapeutic angiogenesis have had limited success. This is perhaps not surprising given the very limited ability of angiogenic growth to compensate for the loss of conductance vessels. It is now being generally recognised that revascularisation in peripheral vascular disease, as in coronary heart disease, would be best achieved by the therapeutic activation of collateral arteriogenesis. Nevertheless, the ability to activate angiogenesis therapeutically would be valuable where significant microvascular disease exists. In addition, the early work on therapeutic angiogenesis has provided data and approaches that may be valuable in future studies aimed at the activation of arteriogenesis.

Delivery of molecular activators of vascular growth

Induction of angiogenesis in the appropriate ischaemic areas and in future local arteriogenesis at suitable sites for collateral development, requires activating agents to be delivered in a manner that ensures controllable local activity and minimizes systemic side effects. Growth factors are readily degraded and if administered systemically would have to be used at high concentrations in order to ensure enough active growth factor reached the appropriate site. There are risks associated with non-local delivery, for example, systemically delivered VEGF produces severe hypotension in animal models.[35] In addition to localization, activators must be present for sufficient time to induce optimal vascular growth.

The two principal methods used to deliver angiogenic activators in pre-clinical studies as well as clinical trials have been as recombinant proteins or as the genes that encode these proteins. Activators can be delivered locally via intramuscular catheters, direct injection into the muscle or use of coated stents. An important consideration in the use of peptide growth factors is ensuring sufficient longevity of the molecules at the desired site. Where recombinant proteins are injected this would necessitate multiple injections during the course of treatment. An alternative strategy is the use of local reservoirs of recombinant protein, such as biodegradable microspheres.[36] A major limitation to the use of recombinant protein, however, is the expense and difficulty in obtaining large enough quantities of appropriate purity, especially when cocktails of growth factors are required.

Delivery of angiogenic factors by gene transfer has significant advantages over the administration of recombinant protein. It is relatively easy to produce high purity DNA in large quantities and the transfected genes remain active over a period of several days to several weeks. In contrast to gene therapy aimed at correcting genetic diseases, gene transfer as a means of providing short term local expression of therapeutic proteins has been successful. Surprisingly, small amounts of DNA plasmid vectors can be taken up by muscle cells *in vivo* and are reported to result in significant gene expression in humans.[37] Improvements in transfer efficiency have been sought by the use of liposomal carriers and viral vectors. Adenovirus is the most common viral vector used for the delivery of angiogenic genes, and since genes transferred in this way do not integrate into

chromosomes of transduced cells, transient expression is provided.

There have been reports of an inflammatory reaction to adenoviral vectors in human trials, but no long term safety problems at doses appropriate for angiogenic therapy. Second generation adenoviral vectors with deletions of E1 and E4 regions have better transfection efficiency and elicit a decreased inflammatory response[38] and further improved adenoviral vectors can be expected.[39] The adeno-associated viruses (AAV) offer an alternative viral means for gene delivery. AAV efficiently transduce skeletal muscle and vasculature.[40] However, along with retroviruses and lentiviruses, AAV integrate into the recipient genome requiring the development of regulatory systems if they are to provide controllable expression of vascular growth genes. As with recombinant protein, local delivery of genes can be accomplished by direct intramuscular injection, implantation of coated stents or catheters. It may also be possible to utilize tissue-specific endothelial surface molecules for targeting vectors to particular vascular beds. Implantation of cells transfected *ex vivo* offers an additional route of local delivery.

Angiogenic activators

Perhaps the simplest approach to activating angiogenesis is the administration of a soluble angiogenic activator. VEGF is relatively specific for endothelial cells and physiologically relevant. Although VEGF-A, -B, -C, -D and –E, as well as the VEGF-R1 ligand placental growth factor, have all been shown to activate angiogenesis when administered in animal models, most studies have concentrated on VEGF-A. Administration of VEGF alone results in a high percentage of malformed capillaries in animal models.[41] This growth factor also induces vessel permeability resulting in local hypotension and oedema.[35] Neovessels induced by VEGF are transient, with regression occurring on growth factor withdrawal.[42] These data indicate formation of a sustained microvessel network may require relatively long term exposure to the angiogenic initiator.

The fibroblast growth factors have also been examined as potential therapeutic agents to induce angiogenesis in clinical trials. There are 23 members of the FGF family and FGF-2 and FGF-4 have been used in clinical trials. FGF-1, -2, -4 and -9 are highly mitogenic for endothelial cells, although these growth factors are also active on non-endothelial cells.[43] Another growth factor that induces angiogenesis and is in early trials is hepatocyte growth factor; again its effects are not confined to endothelial cells.[44]

With the recognition that physiological angiogenesis requires a spatially and temporally co-ordinated repertoire of signals, attempts have been made to improve capillary formation by providing cocktails of growth factors. Indeed combination of VEGF with the pro-stabilizing Tie2 agonist angiopoietin-1 in a mouse model does produce microvessels with increased lumen size, less thrombosis and increased perfusion compared with VEGF alone.[18] These vessels are also less permeable than those formed in response to VEGF alone.[45] Another approach aimed at providing a more physiological range of angiogenic factors is the targeting of HIF. Expression of a form of HIF-1α that is resistant to oxygen-induced degradation in mouse skin led to up-regulation of HIF-sensitive angiogenic genes and the stimulation of microvessel formation.[46] Again the vessels produced were not associated with oedema. The cell permeable peptide, PR39, inhibits proteosomal degradation and can stabilize HIF-1α.[47] PR39 stimulates angiogenesis in mouse heart, although further studies are required to determine its specificity.[47]

Pre-clinical and early clinical studies have shown that angiogenesis can be induced *in vivo* by a variety of approaches. The challenge is to devise a means to stimulate the conversion of these neovessels into optimally organized, persistent and functional microvascular networks.

Arteriogenic activators

Little is known about the molecular mechanisms of arteriogenesis. In contrast to the ischaemic tissue microenvironment in which angiogenesis occurs, collateral arteriogenesis in the limb takes place in normoxic conditions.[48] In adaptive arteriogenesis studied in animal models, the biochemical effects of the increased flow that the collaterals experience as a result of occlusion of conductance vessels plays a major role. These effects include increased wall shear stress as well as tangential and axial stresses. Increased shear can up-regulate vascular cell adhesion molecule-1 and intracellular adhesion molecule-1, as well as MCP-1 which contributes to monocyte recruitment.[49] Growth factors are undoubtedly involved in adaptive arteriogenesis, but again more work is required to identify the key factors and their roles. In animal models FGF-1 and FGF-2 were found to be unchanged during adaptive arteriogenesis, although there was a transient increase in expression of FGF-receptor-1.[50]

TGFβ is increased during collateral development and can enhance arteriogenesis in animal models. Several factors have been found to enhance arteriogenesis when administered to animals, including FGF-2, VEGF, placental growth factor, angiopoietin-1 and MCP-1, although the exact mechanisms of action remain undefined.[31] Many appear to have indirect actions, for example, VEGF and placental growth factor infusions are likely to enhance arteriogenesis via their monocyte chemoattractive activity. Given the great potential of therapeutic induction of collateral arteriogenesis for the treatment of peripheral vascular disease, it is important that a better understanding of the molecular mechanisms be gained. Identification of key regulators that can induce or enhance arteriogenesis of collaterals is a priority.

Clinical trials for angiogenic therapy of peripheral vascular disease

There have been a number of phase 2 and 3 clinical trials aimed at relieving peripheral vascular disease by angiogenic therapy. The Therapeutic Angiogenesis with Recombinant Fibroblast Growth Factor-2 for Intermittent Claudication (TRAFFIC) study used single or repeated doses of recombinant FGF-2 delivered by arterial puncture and cross-over catheter in patients with intermittent claudication. Patients receiving a single FGF-2 dose showed a significant improvement in peak walking time at 90 days.[51] In the Regional Angiogenesis with Vascular Endothelial Growth Factor (RAVE) study, VEGF121 gene transfer by adenovirus was utilized but failed to produce a significant improvement in peak walking time at 12 weeks.[52] In contrast, in a different study adenoviral delivered VEGF165 given during angioplasty did produce an increased angiographically-assessed vascularity at 3 months.[53] The Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis (VIVA) trial failed to show a difference between the treatment and placebo groups for the primary endpoint of walking time.[54] The TALISMAN collaborators evaluated a plasmid-based angiogenic gene delivery system for the local expression of FGF-1 in patients with non-healing ulcers who were not suitable for invasive re-vascularisation. Whilst there was no difference observed in the primary endpoint of complete ulcer healing, there was a reduced risk of major amputation.[55] Initial

reports from the ongoing Bone Marrow Outcome Trial in Critical Limb Ischemia (BONMOT-CLI), show promise for the intramuscular injection of autologous bone marrow stem cells into ischaemic limbs, with significant improvements in Rutherford categories and a trend against major limb amputation.[56]

A meta-analysis of 5 randomised controlled trials investigating the role of gene therapy as an option for the treatment of peripheral vascular disease concluded that the current literature does not demonstrate a clinical benefit for patients with peripheral vascular disease.[57] Although these trials have met with limited success, together with phase 1 studies and earlier small trials, they demonstrate the feasibility and safety of molecular approaches to therapeutic modulation of vascular growth. The trials have also been valuable in aiding development of techniques for delivering therapeutic agents, as well as helping clinicians refine aspects of trial design for future clinical work on arteriogenesis and angiogenesis.

The realization that therapeutic induction of collateralization by arteriogenesis would be most appropriate for occlusive disease, whilst angiogenic therapy would benefit patients with microvascular defects, should help improve selection of the most appropriate populations for use in future trials. Clear clinical end-points are required in such work. Where angiogenesis is the aim, establishment of optimal treatment modalities will depend on further pre-clinical work focused on determining ways to establish mature, correctly patterned vascular networks. This may involve defined cocktails of stimulators, or activation of transcriptional factors triggering coordinated expression of stimulators. In both cases distinct spatial and temporal expression patterns are likely to be required.

CONCLUSIONS

The prospects for a molecular approach to stimulate vascular growth as a means of relieving tissue ischaemia in peripheral vascular disease are promising. Early clinical work, together with a better understanding of vascular growth mechanisms, has allowed identification of the key areas in which progress is required in order to bring therapeutic vascular growth to the clinic. Yet Isner predicted in therapeutic angiogenesis 'a new frontier for vascular therapy' in 1996.[58] That today the underlying cellular mechanisms and processes continue to be elucidated is testament to the complexity of this area of vascular medicine. Bypassing the occluded conductance vessels is now recognised to require collateral growth by arteriogenesis, rather than angiogenesis. In comparison to angiogenesis our understanding of arteriogenesis remains rudimentary. Significant work is needed therefore to understand the mechanisms regulating physiological arteriogenesis as well as adaptive arteriogenesis, and to identify key molecular regulators. Activation of angiogenic growth will be valuable where microvascular disease is prevalent. Indeed, situations in which activation of arteriogenesis to restore conductance level flow together with activation of angiogenesis to relieve microvascular defects can be envisaged. It is clear that optimum microvascular growth will require correctly patterned, functional and persistent mature microvessel networks. Further work on the basic biology of angiogenesis is needed to determine the best means of inducing this therapeutically.

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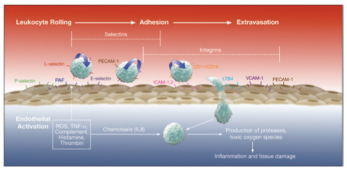

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