Appendix I. Factors that regulate normal angiogenesis

Factor	Biological Actions
VEGF	Increases endothelial cell permeability
	Stimulates endothelial cell uPA/PAI-1 production
	Stimulates endothelial cell proliferation
	Inhibits endothelial cell apoptosis
	Enhances endothelial cell migration
	Stimulates in vivo angiogenesis
Ang1	Stimulates in vitro endothelial cell sprout formation
	Increases girth and stability of endothelium
Ang2	Antagonizes Ang1 signalling/destabilizes endothelium
aFGF bFGF	Stimulates endothelial cell proliferation
	Enhances endothelial cell migration
	Stimulates endothelial cell PA/collagenase production
	Stimulates endothelial cell tube formation
	Stimulates in vivo angiogenesis
PDGF	Stimulates DNA synthesis in endothelial cells
	Stimulates endothelial cells to form chords in vitro
	Stimulates proliferation of smooth muscle cells and pericytes
	Induces vWF, VEGF, and VEGFR-2 expression in cardiac endothelial cells
	Increases capillary wall stability
TGF-β	Supports anchorage-independent growth of fibroblasts
	Inhibits proliferation and migration of endothelial cells
	Stimulates/inhibits formation of endothelial cell tubes in vitro
	Produces net antiproteolytic activity via modulation of uPA/PAI-1 expression levels
	Inhibits production of other proteases/stimulates production of protease inhibitors
	Stimulates VSMA production by pericytes
	Chemotactic for monocytes and fibroblasts
	Stimulates in vivo angiogenesis in presence of inflammatory response
	Increases vessel wall stability
TNF-α	Stimulates angiogenesis in vivo
	Stimulates formation of endothelial cell tubes in vitro
	Inhibits endothelial cell proliferation
EGF, TGF-α	Stimulate endothelial cell proliferation
	Stimulate angiogenesis in vivo
G-CSF, GM-CSF	Stimulate endothelial cell proliferation and migration

Angiogenin	Stimulates angiogenesis in vivo
	Supports endothelial cell binding and spreading
Angiotropin	Stimulates random capillary endothelial cell migration
	Stimulate endothelial cell tube formation
	Stimulates in vivo angiogenesis
Tissue factor	Contributes to development of yolk sac vasculature
Factor V	Contributes to development of yolk sac vasculature
Prostaglandin	Stimulates in vivo angiogenesis
Nicotinamide	Stimulates in vivo angiogenesis
Monobutyrin	Stimulates in vivo angiogenesis
	Stimulates endothelial cell migration in vitro
Membrane-bound proteins	
$\alpha_v\beta_3$-Integrin	Highly expressed on activated endothelial cells
	Mediates endothelial cell attachment, spreading, and migration
	Present on angiogenic capillary sprouts
	Required for bFGF-stimulated angiogenesis in vivo
	Localizes MMP-2 to capillary sprouts
	Suppresses endothelial cell apoptosis
$\alpha_v\beta_5$-Integrin	Required for VEGF-stimulated angiogenesis in vivo
α_5 $_1$-Integrin	Required for non-VEGF growth factor-stimulated angiogenesis in vivo
VE-cadherin	May mediate permeability of endothelium
	Required for in vivo angiogenesis
	Prevents endothelial cell apoptosis
Eph-4B/Ephrin-B2	Colocalize at venous/arterial interfaces of developing embryo
	Required for angiogenesis of head and yolk sac and for myocardial trabeculation.
Ephrin-A1	Required for in vivo angiogenesis induced by TNF-
	Chemotactic for endothelial cells in vitro
Eph-2A	Required for endothelial cell tube formation in vitro
Biomechanical forces	
Blood flow/shear stress	Increases endothelial stress fiber formation (if laminar)
	Promotes endothelial cell division (if turbulent)
	Stimulates transcription of bFGF and TGF- genes

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Appendix II. Factors that regulate tumour angiogenesis

Factor	Role in Tumor Neovascularization
VEGF	Secreted by many tumor cells in vitro
	Highly upregulated in most human cancers
	Expression correlates with intratumoural microvessel density and poor prognosis in cancer patients
	Inhibition decreases tumor vessel density and tumor growth
FGF	Inhibition suppresses generation of tumor vessels in vitro and in vivo and tumor growth in vivo
	Important for maintenance, vs. induction, of tumor angiogenesis
	Synergizes with VEGF to promote angiogenesis in vitro and in vivo
	Induces VEGF expression in tumor cells and VEGF receptor expression in endothelial cells
Heparanase	Stimulates invasion and vascular sprouting of endothelial cells
	Releases bFGF from extracellular matrix
	mRNA and protein are enriched in metastatic tumor cell lines and human tumors vs. normal tissues
	Overexpression renders nonmetastatic cell lines metastatic in vivo and increases tumor neovascularization
Ang 2	Induced in endothelial cells of preexisting vessels co-opted by a tumor, leading to vessel regression
	Induced in endothelial cells of newly formed vessels of tumor, leading to vessel plasticity and VEGF-mediated growth
IL-8	Mitogenic and chemotactic for HUVECs in vitro
	Stimulates angiogenesis in vivo
	mRNA is upregulated in neoplastic tissues vs. normal ones in vivo; expression correlates with extent of neovascularization
	Overexpression increases invasiveness, tumourigenicity, neovascularization, and metastatic potential of tumor cells
	Mediates stimulation of MMP-2 gene transcription
MMP-2	Directly modulates melanoma cell adhesion and spreading on extracellular matrix
	Mediates tumor growth and neovascularization in CAM

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ADDENDUM I. List of congresses where parts of the work were presented.

1. **Mabeta P**. Shelver G and Dippenaar N. Development of an HPLC method for the determination of bleomycin profiles in urine. Paper at the International Immunopharmacology congress, Pilanesburg, South Africa. **2001**.

2. **Mabeta P,** Shelver G and Dippenaar N. An assay method for the determination of bleomycin profiles in hemangioma patients. Paper at the congress of the Physiology Society of Southern Africa. Stellenbosch, South Africa. **2002**.

3. **Mabeta P,** Shelver G, Dippenaar N, Davis PF. and Tan ST. Levels of bleomycin in haemangioma patients undergoing intralesional bleomycin injection (IBI). Poster at the congress of the International Society for the study of vascular anomalies. Wellington, New Zealand. **2004**.

4. **Mabeta P,** Soley JT and Davis PF. Assessment of the effects of bleomycin and interferon α-2a on angiogenesis in a human vascular tumour model. Paper presented at the congress of the Microscopy Society of Southern Africa. Port Elizabeth. **2006**.

5. **Mabeta P,** Davis PF. and Pepper MS. Interferon alpha and bleomycin exert antiangiogenic activity through different mechanisms. Poster at the Nature Biotechnology Winter Symposium. Miami, USA. **2006**.

6. **Mabeta P**, Pepper MS. The effect of Paclitaxel on neovessel formation *in vitro.* Poster at the congress of the Physiology Society of Southern Africa. Muldersdrift, South Africa. **2007**.

7. **Mabeta P,** Davis PF. and Pepper MS. Assessment of the mechanisms of antiangiogenic action of bleomycin and interferon alpha. Poster at the congress of the Physiology Society of Southern Africa. Muldersdrift, South Africa. **2007**.

ADDENDUM II. List of abstracts and articles published from this work.

1. **Mabeta P,** Soley JT and Davis PF. Assessment of the effects of bleomycin and interferon α-2a on angiogenesis in a human vascular tumour model. Abstract. Microscopy Society of Southern Africa – Proceedings 2006; 36:6.

2. **P. Mabeta**, P.F. Davis. The mechanism of bleomycin in inducing haemangioma regression. SAMJ 2008; 98:5389-539**.**

3**.** Ionescu G, **Mabeta P**, Dippenaar N, Muir T, Fourie P, Shelver G. Bleomycin plasma spill-over levels in paediatric patients undergoing intralesional injection for the treatment of haemangiomas. SAMJ 2008; 98:539-540.

ADDENDUM III. List of awards.

1. South African Women in Science Award, 2005 – Gender responsive research – Finalist (second place).

2. Microscopy Society of Southern Africa, 2006 – Innovative Research – 1st position.

3. Junior researcher of the year, 2007, Faculty of Health Sciences, University of Pretoria – 3rd Place.