



**UNIVERSITY OF
ADELAIDE**

School of Medicine

**HANSON
INSTITUTE**

Vascular Biology

Laboratory

Human Immunology

IMVS

Characterisation of a novel gene p73RhoGAP in angiogenesis.

BY

Zhi Jian SU
MB BS MD

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TABLE OF CONTENTS

TABLE OF CONTENTS	1
ABSTRACT	9
DECLARATION.....	11
ACKNOWLEDGEMENT	12
ABBREVIATIONS	14
CHAPTER 1: INTRODUCTION.....	19
1.1 REVIEW OF ANGIOGENESIS.....	22
1.1.1 The “angiogenic switch”.....	22
1.1.1.1 Angiogenesis stimulators	24
1.1.1.1.1 Vascular endothelial growth factor	24
1.1.1.1.2 Basic fibroblast growth factor	29
1.1.1.2 Angiogenesis inhibitors.....	31
1.1.2 <i>In vitro</i> 3-dimensional collagen model for capillary tube formation	34
1.1.3 Mechanisms of angiogenesis	35
1.1.3.1 Initiation of the angiogenic response	36
1.1.3.2 Cell invasion and migration	36
1.1.3.2.1 Role of matrix metalloproteinases	36
1.1.3.2.2 Role of integrins	40
1.1.3.3 Vacuole formation.....	42
1.1.3.4 Maturation of the capillary sprout.....	43
1.1.3.4.1 Tie receptors and angiopoietins	43
1.1.3.4.2 Ephrins and Eph receptors	46
1.1.4 Isolation of genes involved in angiogenesis	48
1.1.5 Clinical trials of angiogenic stimulators	49

1.1.6	Clinical trials of angiogenic inhibitors.....	50
1.1.6.1	Inhibition of VEGF and their receptors.....	51
1.1.6.1.1	Anti-VEGF monoclonal antibodies	51
1.1.6.1.2	Small molecule inhibitors	52
1.1.6.1.3	Soluble VEGF receptor.....	54
1.1.6.2	Intervention of adhesion and migration	54
1.1.6.2.1	Inhibition of matrix metalloproteinases	54
1.1.6.2.2	Inhibition of integrin signalling	56
1.1.6.3	Endogenous angiogenic inhibitors	57
1.1.7	Summary.....	58
1.2	REVIEW OF THE RHOGTPASE FAMILY	60
1.2.1	Rho upstream effectors	61
1.2.2	Rho downstream effectors	63
1.2.3	Functional changes influenced by RhoGTPases.....	64
1.2.3.1	Actin cytoskeleton.....	64
1.2.3.2	Cell migration	65
1.2.3.3	Focal adhesions.....	65
1.2.3.4	Proliferation and apoptosis.....	66
1.2.3.5	Membrane trafficking	66
1.2.3.6	Angiogenesis.....	67
1.3	HYPOTHESIS.....	69
1.4	SPECIFIC AIMS.....	69
CHAPTER 2: MATERIALS AND METHODS.....		70
2.1	MATERIALS.....	71
2.2	METHODS	75
2.2.1	Isolation of genes involved in 3D tube formation.....	75
2.2.1.1	Isolation and purification of total RNA.....	75
2.2.1.2	Suppression subtractive hybridisation.....	75

2.2.1.3	Colony array and cDNA array	76
2.2.1.4	Northern blot analysis	76
2.2.1.5	Virtual Northern blot analysis	76
2.2.2	General cloning procedures	77
2.2.2.1	PCR amplification.....	77
2.2.2.2	PCR amplification A-Tailing	78
2.2.2.3	Agarose gel electrophoresis	78
2.2.2.4	Sequencing	79
2.2.2.5	Restriction endonuclease digestion.....	79
2.2.2.6	Extraction of digested DNA from the agarose gel	80
2.2.2.7	Ligation of vector with inserts	80
2.2.2.8	Transformation of <i>E.coli</i> DH5 α with plasmid DNA.....	80
2.2.2.9	Small scale purification of plasmid DNA	81
2.2.2.10	Large scale purification of plasmid DNA	81
2.2.3	Tissue culture.....	82
2.2.3.1	Endothelial cells.....	82
2.2.3.2	HEK293 cells.....	82
2.2.3.3	Lipofectamine™ 2000 transfection of HEK293 cells.....	83
2.2.3.4	Lipofectamine™ 2000 transfection of HUVEC.....	83
2.2.4	Generation of recombinant retroviral constructs and retrovirus	83
2.2.4.1	Generation of retroviral constructs.....	83
2.2.4.2	Generation of recombinant retrovirus	84
2.2.4.2.1	Calcium phosphate precipitation transfection of BING cells.....	84
2.2.4.2.2	Infection of mammalian cells.....	84
2.2.4.2.3	Selection for cells expressing the neomycin resistance (NeoR) gene	85
2.2.5	Generation of recombinant adenoviral constructs and adenovirus	85
2.2.5.1	Generation of adenoviral constructs.....	85
2.2.5.2	Generation of recombinant adenovirus	86
2.2.5.2.1	Transfection of HEK293 cells	86
2.2.5.2.2	Harvesting HEK293 cells and generation of adenovirus containing lysate.....	87
2.2.5.2.3	Upscaling the production of viral particles: infection of HEK293 cells	87

2.2.5.2.4	Purification and dialysis of adenovirus	88
2.2.5.2.5	Tissue Culture Infectious Dose 50 (TCID ₅₀)	88
2.2.5.2.6	Titration of recombinant adenovirus for infection of HUVEC	89
2.2.6	<i>In vitro</i> angiogenesis assays.....	89
2.2.6.1	Proliferation assay.....	89
2.2.6.2	Migration assay	90
2.2.6.3	Attachment assay	90
2.2.6.4	Matrigel capillary tube formation assay.....	91
2.2.6.5	Endothelial permeability assay.....	91
2.2.7	<i>In vivo</i> angiogenesis assay	92
2.2.7.1	Gelfoam assay for <i>in vivo</i> angiogenesis.....	92
2.2.8	Fluorescence staining.....	92
2.2.8.1	F-actin staining.....	92
2.2.8.2	DAPI staining.....	93
2.2.9	Immunoblotting	93
2.2.9.1	Western blot analysis	93
2.2.9.2	Rho, Rac and Cdc42 activity assays.....	94
2.2.9.3	Caspase 3 activity assay	95

CHAPTER 3: ISOLATION OF REGULATED GENES DURING CAPILLARY

TUBE FORMATION 96

3.1 RESULTS..... 97

3.1.1	Generation of subtracted angiogenic cDNA libraries	97
3.1.2.	Isolation of regulated genes by colony array	98
3.1.3	Isolation of regulated genes by cDNA array.....	99
3.1.4	Identification and classification of regulated genes	100
3.1.5	Virtual Northern blot analysis to confirm regulated gene expression patterns.....	101
3.1.6	Cell specific expression and response to growth factors of selected genes	102
3.1.7	Regulated GTPase-related genes identified by microarray.....	102

3.2 DISCUSSION 103

3.2.1	Matrix metalloproteinases.....	103
3.2.2	Early growth response gene 1	104
3.2.3	Phospholipase A2 and Cyclooxygenase-2	105
3.2.4	Jagged 1	106
3.2.5	Cysteine rich angiogenic protein 61 and connective tissue growth factor	106
3.2.6	Polymerase I and transcript-release factor	107
3.2.7	The RhoGTPase Family.....	108
3.2.8	Novel Genes.....	109
3.3	SUMMARY	110

CHAPTER 4: GENERATION OF P73RHOGAP RECOMBINANT VIRAL AND FUSION CONSTRUCTS 111

4.1	RESULTS.....	112
4.1.1	Generation of a p73 coding cDNA	112
4.1.2	Generation of a non-functional RhoGAP domain mutant of p73	113
4.1.3	Generation of recombinant retroviral vectors	114
4.1.4	Generation of recombinant adenoviral vectors	114
4.1.4.1	Generation of p73 sense and antisense adenoviral constructs.....	114
4.1.4.2	Generation of p73 mutant adenoviral constructs.....	115
4.1.4.3	Generation of adenoviral particles	115
4.1.5	Generation p73 fusion constructs.....	116
4.1.5.1	Generation of 5'FLAG-tagged p73	116
4.1.5.2	Generation of pcDNA3-GFP-p73 and pcDNA3-GFP-p73(Δ CC)	117
4.2	DISCUSSION	117
4.3	SUMMARY	119

CHAPTER 5: IDENTIFICATION AND CHARACTERISATION OF P73RHOGAP 120

5.1	RESULTS.....	121
5.1.1	Isolation and identification of a novel gene	121
5.1.2	Characterisation of p73 using bioinformatics	122
5.1.3	Distribution of p73 in HUVEC.....	124
5.1.4	Overexpression of p73 in HEK293 cells.....	125
5.1.5	Knockdown of p73 protein by antisense of p73	125
5.1.6	Determination of the substrate specificity of p73	126
5.2	DISCUSSION	126
5.2.1	p73 is a RhoGAP protein.....	126
5.2.2	Characteristics of p73	128
5.3	SUMMARY	130
 CHAPTER 6: FUNCTIONAL ANALYSIS OF P73RHOGAP		131
6.1	RESULTS.....	132
6.1.1	The role of p73 in cell morphological change	132
6.1.1.1	Antisense of p73 increases cell size	132
6.1.2	The role of p73 in the cytoskeleton.....	133
6.1.2.1	Antisense of p73 increases stress fibres.....	133
6.1.2.2	Antisense of p73 increases basal permeability.....	134
6.1.2.3	Antisense of p73 decreases cell attachment through regulation of FAK.....	135
6.1.3	The role of p73 on <i>in vitro</i> hallmarks of angiogenesis.....	136
6.1.3.1	Effect of p73 on capillary tube formation	136
6.1.3.2	Effect of p73 on migration.....	137
6.1.3.3	Effect of p73 on proliferation.....	138
6.1.4	The role of p73 on <i>in vivo</i> angiogenesis	138
6.1.5	The role of p73 in apoptosis	139
6.1.5.1	Antisense of p73 induces apoptosis in HUVEC.....	139
6.1.5.2	Antisense of p73 increases caspase 3 activity	140
6.1.5.3	Antisense of p73 inhibits Akt phosphorylation	140
6.1.5.4	Regulation of Bcl-2 family members by antisense to p73	140

6.1.6	Mutagenesis of p73.....	141
6.1.6.1	Generation of a dominant negative p73 mutant	141
6.1.6.2	p73M increases Rho activity.....	142
6.1.6.3	p73M increases stress fibres.....	142
6.1.6.4	p73M inhibits capillary tube formation.....	143
6.1.6.5	p73M inhibits proliferation	143
6.1.6.6	p73M induces apoptosis.....	143
6.1.6.7	p73M increases caspase 3 activity	143
6.1.6.8	p73M does not significantly alter the cell size.....	144
6.2	DISCUSSION	144
6.2.1	Effect on tube formation	145
6.2.2	Effect on migration	147
6.2.3	Effect on proliferation.....	147
6.2.4	Effect on apoptosis	148
6.2.4.1	Antisense of p73 induces apoptosis due to overexpression of Rho.....	148
6.2.4.2	Antisense of p73 induces apoptosis via the FAK-PI3K-Akt pathway	149
6.2.4.3	Rho induces apoptosis via different downstream effectors.....	151
6.2.4.4	Rho induces apoptosis involving the Bcl-2 family members	152
6.2.5	Effect on the cytoskeleton.....	153
6.2.5.1	Effect on stress fibres and permeability	153
6.2.6	Effect on cell size change	154
6.3	SUMMARY	155
CHAPTER 7: GENERAL DISCUSSION		156
7.1	PROPOSED MECHANISM OF P73 MEDIATED EFFECTS AND FUTURE DIRECTIONS..	157
7.1.1	Rho downstream effectors and apoptosis.....	158
7.1.2	Role of p73 in cell size change	159
7.2	FINAL SUMMARY AND CONCLUSIONS	160

REFERENCES	162
PUBLICATIONS AND PRESENTATIONS	189
PUBLICATION:.....	189
CONFERENCE PRESENTATIONS:	189
APPENDIX: BUFFERS AND SOLUTIONS.....	190

ABSTRACT

Angiogenesis is the formation of new blood vessels from pre-existing vessels. It is highly regulated in a normal physiological system. Dysregulated angiogenesis is associated with a number of pathological states such as cancer and rheumatoid arthritis. Thus angiogenesis is proposed as a target for control of such diseases. The identification of novel genes involved in angiogenesis will improve the likelihood of the development of efficacious drugs.

To identify genes essential to angiogenesis, we used an *in vitro* model of capillary tube formation and a PCR based subtraction hybridisation approach for isolation of regulated genes. We identified 125 different genes including 96 known and 29 novel genes and of these, 84 genes were confirmed to be regulated during the formation of capillary tubes.

From the 125 isolated genes, one novel gene displayed characteristics suitable for further investigation. The gene, p73RhoGAP is a new member of the RhoGAP family. It is highly upregulated at 3 and 6 h in the tube formation process but only in an angiogenic milieu with little or no regulation seen under non-angiogenic conditions, and is restricted in its expression to vascular cells. Thus, we propose that p73RhoGAP is called VASGAP. Bioinformatics analysis of the protein sequence revealed the presence of a putative RhoGAP domain, indicating a potential role in the activation of RhoGTPases. Our study suggests that VASGAP displays RhoGAP activity for Rho but not Rac or Cdc42. RhoGTPase activity assays demonstrated that mutation of the conserved arginine at residue 82 (R82A) in the RhoGAP domain is crucial to the p73 effect on Rho. VASGAP localises to actin stress fibres.

To characterise the function of VASGAP, knockdown of the protein was achieved by adenovirus delivery of VASGAP antisense into EC. Such VASGAP depleted cells showed enhanced actin stress fibres and basal permeability consistent with alteration of Rho activity. Cell migration, proliferation and capillary tube formation were inhibited, and enhanced apoptosis was evident. In an *in vivo* model of angiogenesis, antisense of VASGAP inhibited blood vessel invasion.

Thus, the results suggest VASGAP is an important and novel modulator of angiogenesis and cell survival and displays many of the features worthy of a drug target.