



THE UNIVERSITY
of **ADELAIDE**

**FUNCTION OF LATENT TRANSFORMING GROWTH
FACTOR- β BINDING PROTEIN-2 (LTBP-2) IN
ELASTINOGENESIS AND MODULATION OF GROWTH
FACTOR STORAGE, EXPRESSION AND ACTIVITY IN
NORMAL AND FIBROTIC TISSUES**

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Summary

LTBP-2 is tightly associated with fibrillin microfibrils and elastic fibres in a range of tissues mainly in the lung, heart, skeletal muscle, placenta, liver and the aorta. LTBP-2 belongs to the fibrillin-LTBP superfamily of extracellular matrix proteins. Unlike other LTBPs, LTBP-2 does not covalently bind TGF-beta and its molecular function remains unclear. LTBP-2 complexes with fibulin-5, an elastin-chaperone protein critical for normal elastic fibre assembly, and it has been suggested that LTBP-2 may preferentially direct fibulin-5-elastin globules onto fibrillin-1 (rather than fibrillin-2) microfibrils during elastinogenesis. However, we have now shown that LTBP-2 inhibits rather than enhances the interaction of tropoelastin with fibulin-5 in vitro. In addition LTBP-2 inhibited elastic fibre assembly in ear cartilage chondrocyte cultures largely at the stage of elastin deposition onto the fibrillin microfibril scaffold. In parallel experiments, LTBP-2 was shown to significantly inhibit the binding of heparin to tropoelastin suggesting LTBP-2 may compete with tropoelastin for binding to certain cell surface HSPGs and contribute to controlling the release of elastin microassemblies from the cell surface. Confocal microscopy showed strong co-distribution of LTBP-2 with fibulin-5 and fibrillin-1 and partial co-distribution with HSPGs, perlecan and syndecan-4 in fibroblast matrix. Thus it is evident that LTBP-2 is a negative modulator of elastinogenesis and that LTBP-2 levels may regulate the rate and extent of elastinogenesis in some tissues.

A recent study has linked LTBP-2 gene mutations to recessive form of Weill-Marchesani syndrome which is characterised by short stature, thick fibrotic skin and ectopia lentis. Since fibrillin-1 mutations can also cause this syndrome it is now clear that LTBP-2 is linked to fibrillin biology, growth factor regulation and fibrosis. To investigate growth factor binding to LTBP-2, our laboratory screened a number of cytokines involved in the pathogenesis of fibrotic disorders and identified a very strong specific interaction of FGF-2. The activity was confined to a central region of the LTBP-2 consisting of 6 EGF-like repeats, suggesting a single binding sequence. The finding presented in this thesis found that 5-fold molar excess LTBP-2 can completely block FGF-2 stimulation of fibroblast proliferation via its receptor. In addition increased levels and extensive co-localisation of LTBP-2 and FGF-2 were observed and quantitated in human hypertrophic scars and keloids. Furthermore, qPCR confirmed consistent elevation of LTBP-2 and FGF-2 expression in samples of these fibrotic tissues. The results

support the concept that increased LTBP-2 expression in fibrotic disorders may increase FGF-2 binding and reduce FGF-2 activity, inhibiting normal repair processes.

Previously we have shown that LTBP-2 competes with LTBP-1 for binding to fibrillin *in vitro*, suggesting that LTBP-2 may modulate TGF- β storage and activation. In experiments designed to measure displacement of TGF- β complexes from fibrillin microfibrils, our laboratory discovered addition of LTBP-2, or a small bioactive fragment LTBP-2C F3 to MSU 1.1 skin fibroblasts resulted in a large increase in TGF- β levels in culture medium. However the increase in TGF- β the medium was cycloheximide sensitive indicating elevated cellular expression and secretion of TGF- β rather than release of matrix-stored TGF- β . Exogenous LTBP-2 or fragment F3 significantly increased levels of latent TGF- β in the medium after 9h peaking at 15h. The signalling mechanism appears to involve the PI3K/Akt and p38 MAPK pathways, as incubation of cells with LTBP-2 (10 μ g/ml) elevated Akt 1/2/3 Ser473 and P38 D-8 phosphorylation and inhibition of each pathway completely blocked the synthesis of TGF- β . Investigation of the cell surface receptor for the bioactive fragment of LTBP-2 was less informative. Inhibitory antibody to β 1 integrins did not affect the TGF- β upregulation but it was partially inhibited by an antibody to the integrin α V β 3 receptor, suggesting it may be involved in LTBP-2-cell interaction(s) resulting in elevated TGF- β expression.

In conclusion, these findings are consistent with LTBP-2 having novel regulatory functions in elastinogenesis, growth factor modulation and fibrosis which may lead to novel therapy development for fibrotic diseases and tissue repair.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due references has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Publication Arising During PhD Candidature

CHAPTER 2

LTBP-2 competes with tropoelastin for binding to fibulin-5 and heparin, and is a negative modulator of elastinogenesis

Mohamed A. Sideek, Clementine Menz, Mahroo K. Parsi, Mark A. Gibson

Matrix Biology 34 (2014) 114-123 (Impact factor: 5.074)

LTBP-2 inhibits elastin and fibrillin assembly in matrix of fetal bovine ear cartilage chondrocytes

Mohamed A. Sideek and Mark A. Gibson

(manuscript in preparation)

CHAPTER 3

LTBP-2 has a single high-affinity binding site for FGF-2 and blocks FGF-2-induced cell proliferation

Clementine Menz, Mahroo K. Parsi, Julian R.J. Adams, **Mohamed A. Sideek**, Zlatko Kopecki, Allison J. Cowin, Mark A. Gibson

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CHAPTER 4

Co-localization of LTBP-2 with FGF-2 in fibrotic human keloid and hypertrophic scar

Mohamed A. Sideek, Abdulrahman Teia, Zlatko Kopecki, Allison J. Cowin, Mark A. Gibson

Journal of Molecular Histology (Impact factor: 1.815)

CHAPTER 5

LTBP-2 stimulates the expression of TGF- β via Akt & p38 MAPK signalling pathway in human fibroblast

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Scientific Communications

INTERNATIONAL:

2011

The Elastin and Elastic Fibers Gordon Research Conference, University of New England, Biddeford, ME, United States (oral and poster)

NATIONAL AND LOCAL:

2015

Australian Society of Medical Research (ASMR) South Australia Annual Scientific Meeting, National Wine Centre, Adelaide, Australia (poster)

2014

Matrix Biology Society of Australia and New Zealand (MBSANZ) 38th Annual Scientific Meeting, Queenscliff, Victoria, Australia (oral and poster)

Florey International Postgraduate Research Conference, Faculty of Health Sciences (FHS) Postgraduate Research Conference 2014, The University of Adelaide, Adelaide, Australia (poster)

Australian Society of Medical Research (ASMR) South Australia Annual Scientific Meeting, Adelaide Convention Center, Adelaide, Australia (poster)

2013

Matrix Biology Society of Australia and New Zealand (MBSANZ) 37th Annual Scientific Meeting, McCracken Country Club, South Australia, Australia (poster)

Faculty of Health Sciences (FHS) Postgraduate Research Conference 2013, Adelaide, Australia (poster)

Australian Society of Medical Research (ASMR) South Australia Annual Scientific Meeting, Adelaide Convention Center, Adelaide, Australia (poster)

1st Malaysian Postgraduate Student Symposium of South Australia 2013, Adelaide, Australia (oral and poster)

2012

Matrix Biology Society of Australia and New Zealand (MBSANZ) 36th Annual Scientific Meeting, Mantra Legends, Gold Coast, Queensland, Australia (poster)

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2013

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2012

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Abbreviations

BCIP-	5-bromo-4-chloro-3-indolylphosphate toluidine salt
BMP-	bone morphogenetic protein
BSA-	bovine serum albumin
C-	carboxy-terminus
C-6-S-	chondroitin-6-sulphate
Ca ²⁺ -	calcium ions
CaCl ₂ -	calcium chloride
cbEGF-	calcium binding epidermal growth factor
cDNA-	complementary deoxyribonucleic acid
CHX-	cycloheximide
CS-	chondroitin sulphate
DAPI-	4',6-Diamidino-2-phenylindole dihydrochloride
ddH ₂ O-	double distilled water
DMEM-	Dulbecco's Modification of Eagles Medium
DMSO-	dimethyl sulphoxide
DNA-	deoxyribonucleic acid
DTT-	dithiothreitol
ECM-	extracellular matrix
EDTA-	ethylene diamine tetraacetic acid
EGF-	epidermal growth factor
ELISA-	enzyme-linked immuno-sorbent assay
EMILIN-	elastinmicrofibril interface located protein
FCS-	foetal calf serum
FGF-2	fibroblast growth factor -2
FGFRs	FGF receptors
FITC-	fluorescein isothiocyanate
GAG-	glycosaminoglycan
GuHCL-	guanidine hydrochloride
HAC-	heparin-albumin conjugates
HEK-	human embryo kidney
HFF-	human foreskin fibroblasts
his ₆ -	6-histidine
HS-	heparan sulphate
HSPGs-	heparan sulphate proteoglycans
HTS-	hypertrophic scar
IgG-	Immunoglobulin G
IL-	interleukin
K _d -	dissociation constant

kDa-	kiloDalton
LAP-	latency-associated protein
LLC-	large latent complex
LTBP-	latent transforming growth factor- β binding protein
LTBP-2C (H)-	LTBP-2 C-terminal
LTBP-2NT (H)-	LTBP-2 N-terminal
M-	molar
MAGP-	microfibrillar-associated glycoprotein
MAPK-	mitogen-activated protein kinases
MFS-	Marfan syndrome
min-	minutes
mM-	millimolar
MMP-	matrix metalloprotease
mRNA-	messenger RNA
N-	amino-terminus
NaCl-	sodium chloride
NBCS-	new born calf serum
NBT-	nitro-blue tetrazolium chloride
NEAA-	non-essential amino acids
ng-	nanogram
Ni-	nickel
NRS-	normal rabbit serum
PBS-	phosphate-buffered saline
PCR-	polymerase chain reactions
PG-	proteoglycan
PVDF-	polyvinylidene difluoride
r-	recombinant
RGD-	arginine-glycine-aspartic acid motif
RNA-	ribonucleic acid
RT-	room temperature
SD-	standard deviation
SDS-PAGE-	sodium sulphate polyacrylamide gel electrophoresis
SLC-	small latent complex
SMA-	smooth muscle actin
SMC-	smooth muscle cell
TBS-	tris buffered saline
TGF- β -	transforming growth factor- β
TIMP-	tissue inhibitor of metalloproteinases

TMB-	tetramethylbenzidine
v or vol-	volume
w-	weight
WMS-	Weill-Marchesani syndrome
α -	alpha
$\alpha V\beta 3$ -	alpha V beta 3
$\alpha V\beta 5$ -	alpha V beta 5
β -	beta
μ l-	microliter
Δ	delta
8-cys-	8-cysteine containing motif