TYROSINE PHOSPHATASE PEZ: A NOVEL REGULATOR OF TGFβ SIGNALLING, EPITHELIAL-MESENCHYMAL TRANSITION AND PROTEIN SECRETION IN DEVELOPMENT AND CANCER

By

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ABSTRACT

The body of work documented in this thesis defines the protein tyrosine phosphatase Pez as a novel positive regulator of TGFβ signalling, epithelial-mesenchymal transition and protein secretion. In a zebrafish model of embryonic development, Pez was found to be expressed transiently in discrete, tissue-specific regions of the developing brain, heart, pharyngeal arches and somites, and knock-down of Pez expression was found to result in architectural defects in these organs, indicating a crucial role for Pez in organogenesis during embryonic development. Over-expression of Pez in epithelial MDCK cells induced an epithelial-mesenchymal transition (EMT), as confirmed by increased expression of mesenchymal genes and EMT-associated transcription factors, concomitant with a reduction in epithelial gene expression and transition from epithelial to mesenchymal-like morphology. Pez-dependent induction of EMT was found to be a consequence of increased TGFβ signalling in Pez-expressing cells in this *in vitro* model. Furthermore, $TGF\beta_3$ mRNA was found to be co-expressed with Pez in a number of tissues during zebrafish development, and $TGF\beta_3$ expression was lost from these tissues following Pez knock-down, suggesting that Pez is required for $TGF\beta_3$ expression in these tissues.

Pez-dependent regulation of TGFβ was also found to occur in the setting of human breast cancers, with manipulation of Pez expression altering TGFβ secretion in human breast cancer-derived cell lines. Additionally, altered Pez expression led to widespread changes in the secretome of these breast cancer cell lines. In breast

and other epithelial cell types, Pez was found to localise predominantly to a peri-nuclear region that partially co-localised with markers of the golgi apparatus. Preliminary investigations suggest that Pez-dependent activation of golgi-localised Src family kinases may contribute to its effects in regulating protein secretion.

Together, these results implicate Pez as a novel regulator of embryonic development, TGFβ production and EMT, and a modulator of the tumour cell secretome. Further investigation into the molecular mechanism by which Pez mediates these effects has the potential to enhance our understanding of essential biological processes contributing to embryonic development, tissue homeostasis, and human disease states, including cancer.

AUTHOR DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Leila Belle (nee Wyatt) and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Wyatt L, Wadham C, Crocker LA, Lardelli M and Khew-Goodall Y. The protein tyrosine phosphatase Pez regulates TGFβ, epithelial-mesenchymal transition, and organ development. © Rockefeller University Press. 2007. *J.Cell Biol.* 178:1223-1235.

Wyatt, L and Khew-Goodall, Y. PTP-Pez: A novel regulator of TGFβ signaling. © Landes Bioscience. 2008. *Cell Cycle* 7:2290-2295.

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JOINT-AUTHOR CONTRIBUTIONS

Contributions to publications included in this thesis by individual co-authors are outlined below. Contributions by the candidate are divided into data obtained whilst working as a research assistant prior to commencing candidature, and data obtained and published during candidature:

The protein tyrosine phosphatase Pez regulates TGFβ, epithelial-mesenchymal transition, and organ development.

The Journal of Cell Biology 2007 **178**(7):1223-1235.

Leila Belle (nee Wyatt) (pre-candidature)

Generated data presented in Figure 1, Figure 3B-C, and Figure 4A. Generated preliminary data for Pez knock-down phenotype presented in Figure 2B-G, including cloning of regions of zebrafish Pez used to design morpholino oligos, injection of embryos with initial morpholino oligo, and crude visual analysis of Pez knock-down phenotype.

Leila Belle (nee Wyatt) (during candidature)

Contributed to conceptualisation of experimental approaches; generated, interpreted and analysed data presented in Figures 2, 3D-E, 4B-D, 5, 7 and 8, co-drafted and co-edited the manuscript.

I, …… **hereby certify that the above statement of contribution is correct**

Signed: …… **Date:** ……

Carol Wadham

Generated data presented in Figure 3A, and MDCK clones used for analysis in Figure 3B.

I, Carol Wadham hereby certify that the above statement of contribution is correct and give permission for the paper to be included in this thesis

Signed: [redacted] Date: 3/2/2011

Lesley Crocker

Generated data presented in Figure 6.

I, ... hereby certify that the above statement of contribution is correct and give permission for the paper to be included in this thesis

Signed: ..... Date: .....

Michael Lardelli

Provided access to zebrafish embryo stocks and technical assistance with embryo manipulations; assisted with conceptualisation of experimental approaches for zebrafish experiments.

I, MICHAEL LARDELLI hereby certify that the above statement of contribution is correct and give permission for the paper to be included in this thesis

Signed: [redacted] Date: 1 Feb 2011

Yeesim Khew-Goodall

Conceptualised experimental approaches; performed majority of publication-quality image acquisition; analysed and interpreted data presented in Figure 6; drafted and co-edited manuscript.

I, ... hereby certify that the above statement of contribution is correct and give permission for the paper to be included in this thesis

Signed: ..... Date: .....

PTP-Pez: A novel regulator of TGFβ signaling.
Cell Cycle 2008 **7**(15):2290-2295.

(An invited "Extra-Views" article based on 2007 JCB publication)

Leila Belle (nee Wyatt) (during candidature)

Drafted, revised and submitted the manuscript.

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Yeesim Khew-Goodall

Received invitation to submit article. Evaluated and co-edited the manuscript

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GLOSSARY OF ABBREVIATIONS USED

AJ	adherens junctions
AP	alkaline phosphatase
APS	ammonium persulphate
BBA	benzylbenzoate / benzyl alcohol mix
BCIP	5-bromo-4-chloro-3-indolyl-phosphate toluidine salt
bp	base pairs
BSA	bovine serum albumin
CAF	cancer-associated fibroblast
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
Chr	chromosome
CM	conditioned medium
CTL	cytotoxic T-lymphocyte
DAPI	4',6-diamidino-2-phenylindole
DEPC	diethylpyrocarbonate
DIG	digoxigenin
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethylsulfoxide
dpf	days post fertilisation
EC	endothelial cell
ECL	enhanced chemiluminescence
ECM	extracellular matrix
EDTA	ethylenediamine tetra-acetic acid
EEA1	early endosome antigen 1

EGF epidermal growth factor

EGFR epidermal growth factor receptor

EGTA ethylene glycol tetra-acetic acid

ELISA enzyme-linked immunosorbent assay

ENA Epithelial Neutrophil Activating Peptide 78

EMT epithelial-mesenchymal transition

ER endoplasmic reticulum **or** estrogen receptor

EST expressed sequence tag

EV empty vector

Fab antigen binding fragment

FAK focal adhesion kinase

FBS foetal bovine serum

FERM band 4.1, ezrin, radixin, moesin homology protein domain

FGF fibroblast growth factor

GAPDH glyceraldehyde 3-phosphate dehydrogenase

GFP green fluorescent protein

GLUT4 glucose transporter 4

GPI glycosylphosphatidylinositol

GRO growth related oncogene

GSK3 glycogen synthase kinase 3

H&E hematoxylin and eosin stain

HEK human embryonic kidney

HRP horseradish peroxidase

hpf hours post fertilisation

HUVEC human umbilical vein endothelial cells

IFNγ	interferon-γ
IGF	insulin-like growth factor
IL	interleukin
KD	knock-down
LAMP1	lysosome associated membrane protein 1
LEC	lymphatic endothelial cells
LTBP	latent TGFβ binding protein
MCP-1	monocyte chemotactic protein 1
MDCK	Madin Darby canine kidney cells
miR	microRNA
MMP	matrix metalloproteinase
MO	morpholino oligonucleotides
MQ	Milli-Q deionised water
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetra-zolium
MW	molecular weight
NBT	4-nitroblue tetrazolium chloride
NC	non-targeting control
NFκB	nuclear factor κB
nt	nucleotides
NRPTP	non-receptor protein tyrosine phosphatase
OE	over-expressed
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor

PDGFR	platelet-derived growth factor receptor
PDZ	post synaptic density protein, disc large tumor suppressor, zonula occludens protein domain
PI3K	Phosphatidylinositol 3-kinase
PlGF	Placental growth factor
PIPES	1,4-piperazinediethanesulfonic acid
PMS	phenazine methosulfate
Pre-hyb	pre-hybridisation solution
PTP	Protein tyrosine phosphatase
pY	phospho-tyrosine
RE	restriction enzyme
RO	reverse osmosis (water)
RT	room temperature
RT-PCR	reverse-transcription polymerase chain reaction
RTPTP	receptor type protein tyrosine phosphatase
SDS	sodium dodecylsulphate
SDS-PAGE	sodium dodecylsulphate polyacrylamide gel electrophoresis
SFK	Src family kinases
SFM	serum-free medium
shRNA	short-hairpin RNA
SS	statistically significant
ST	substrate-trapping
STAT3	signal transducer and activator of transcription 3
TACE	Tumor necrosis factor Alpha-Convertase
TAE	tris-acetate, EDTA buffer

TAM	tumour-associated macrophage
TBS	tris-buffered saline
TEMED	tetramethylethylenediamine
TGFβ	transforming growth factor β
TGN	trans-golgi network
TIMP	tissue inhibitor of metalloproteinases
TNFα	tumour necrosis factor α
UTR	untranslated region
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VSMC	vascular smooth muscle cell
VSVG	vesicular stomatitis viral glycoprotein
zf	zebrafish
ZFIN	zebrafish information network
Zv5/ 7/ 8	progressive versions of the zebrafish whole-genome assembly

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