

**TYROSINE PHOSPHATASE PEZ: A NOVEL REGULATOR OF TGF $\beta$   
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 TGF $\beta$  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 TGF $\beta$  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 TGF $\beta$  was also found to occur in the setting of human breast cancers, with manipulation of Pez expression altering TGF $\beta$  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 perinuclear 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, TGF $\beta$  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 TGF $\beta$ , 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 TGF $\beta$  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 TGF $\beta$ , 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.

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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

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

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Date: 1 Feb 2011

Yeessim Khew-Goodall

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Date: .....

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

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

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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

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

<b>EGF</b>	epidermal growth factor
<b>EGFR</b>	epidermal growth factor receptor
<b>EGTA</b>	ethylene glycol tetra-acetic acid
<b>ELISA</b>	enzyme-linked immunosorbent assay
<b>ENA</b>	Epithelial Neutrophil Activating Peptide 78
<b>EMT</b>	epithelial-mesenchymal transition
<b>ER</b>	endoplasmic reticulum <b>or</b> estrogen receptor
<b>EST</b>	expressed sequence tag
<b>EV</b>	empty vector
<b>Fab</b>	antigen binding fragment
<b>FAK</b>	focal adhesion kinase
<b>FBS</b>	foetal bovine serum
<b>FERM</b>	band <u>4</u> .1, <u>e</u> zrin, <u>r</u> adixin, <u>m</u> oesin homology protein domain
<b>FGF</b>	fibroblast growth factor
<b>GAPDH</b>	glyceraldehyde 3-phosphate dehydrogenase
<b>GFP</b>	green fluorescent protein
<b>GLUT4</b>	glucose transporter 4
<b>GPI</b>	glycosylphosphatidylinositol
<b>GRO</b>	growth related oncogene
<b>GSK3</b>	glycogen synthase kinase 3
<b>H&amp;E</b>	hematoxylin and eosin stain
<b>HEK</b>	human embryonic kidney
<b>HRP</b>	horseradish peroxidase
<b>hpf</b>	hours post fertilisation
<b>HUVEC</b>	human umbilical vein endothelial cells

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

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

<b>TAM</b>	tumour-associated macrophage
<b>TBS</b>	tris-buffered saline
<b>TEMED</b>	tetramethylethylenediamine
<b>TGF<math>\beta</math></b>	transforming growth factor $\beta$
<b>TGN</b>	trans-golgi network
<b>TIMP</b>	tissue inhibitor of metalloproteinases
<b>TNF<math>\alpha</math></b>	tumour necrosis factor $\alpha$
<b>UTR</b>	untranslated region
<b>VEGF</b>	vascular endothelial growth factor
<b>VEGFR</b>	vascular endothelial growth factor receptor
<b>VSMC</b>	vascular smooth muscle cell
<b>VSVG</b>	vesicular stomatitis viral glycoprotein
<b>zf</b>	zebrafish
<b>ZFIN</b>	zebrafish information network
<b>Zv5/ 7/ 8</b>	progressive versions of the zebrafish whole-genome assembly

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