The Role of TWIST1 in Multiple Myeloma

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&

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ABSTRACT

Multiple myeloma (MM) is an incurable haematological malignancy characterised by the uncontrolled proliferation of clonal plasma cells (PCs) in the bone marrow (BM). MM disease progression relies on the continuous trafficking of MM PCs to distant BM sites leading to multiple focal tumours throughout the skeleton at diagnosis. While metastasis is not a concept generally applied to haematological malignancies, increasing evidence suggests that like solid tumours, an epithelial-to-mesenchymal (EMT)-like process is activated in MM PCs in order to disseminate. However, an association between high-risk MM subtypes, patient prognosis and the expression of an EMT-like gene expression signature in MM PCs had not been reported.

The chromosomal translocation t(4;14), characterised by overexpression of *MMSET* and *FGFR3*, is associated with poor prognosis and aggressive tumour dissemination. This project sought to determine whether the highly aggressive phenotype observed in t(4;14) is mediated by activation of EMT-like process. Using previously published microarray data from large cohorts of newly diagnosed MM patients and RNA-sequencing data from human MM cell lines, an EMT-like expression signature in t(4;14) MM was comprehensively evaluated. Among the mesenchymal genes identified, *TWIST1* was consistently upregulated in approximately 50% of the t(4;14) MM cases and was positively correlated with expression of *MMSET*.

Using RNA-sequencing technology, the transcriptome-wide effects of TWIST1 overexpression in MM PCs were determined. Furthermore, ectopic expression of TWIST1 was found to enhance MM PC migration *in vitro*, consistent with the enrichment of genes involved in cell motility from Gene Ontology (GO) analysis. To evaluate the role of TWIST1 overexpression *in vivo*, the 5TGM1/KaLwRij murine model of myeloma was utilised. TWIST1 overexpression in 5TGM1 cells increased total tumour burden and extramedullary growth of tumour in the spleens of recipient mice. Transcriptome analysis demonstrated that TWIST1 overexpression in 5TGM1 cells led to overexpression of genes involved in cytokine production, regulation of cell death and cell motility.

These studies highlight that TWIST1, downstream of MMSET promotes MM PC motility, which, in part contributes to the aggressive phenotype of t(4;14) MM patients. Taken together, this research reveals the role of TWIST1 in MM pathogenesis and adds to the current knowledge of mechanism underlying aggressive disease progression in t(4;14) patients.

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 reference 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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Chee Man CHEONG Date:

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Thank you!

LIST OF ABBREVIATIONS

5TGM1-EV	5TGM1-pLEGO-IRES-tdTomato2
5TGM1-TWIST1	5TGM1-pLEGO-IRES-tdTomato2-TWIST1
aCGH	array comparative genomic hybridization
bHLH	basic helix-loop-helix
BM	bone marrow
BMEC	bone marrow endothelial cell
BMSC	bone marrow stromal cell
bp	base pair
BSA	bovine serum albumin
cDNA	complimentary DNA
CM	conditioned media
CSR	class switch recombination
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
DEG	differentially expressed genes
DMEM	Dulbecco's modified eadle medium
ECF	enhanced chemifluorescence
ECM	extracellular matrix
EDTA	ethylenediaminetetra-acetic acid
EMD	extramedullary myeloma disease
EMT	epithelial-to-mesenchymal transition
FACS	fluorescence-activated cell sorting
FCS	fetal calves serum
FGFR3	fibroblast growth factor receptor 3
g/ mL/ mm/ mM	mmilligram/ millilitre/ millimetre/ millimolar
GAPDH	glutaraldehyde 3-phosphate dehydrogenase
GC	germinal centre
GEP	gene expression profile
GFP	green fluorescence protein

GSEA	gene set enrichment analysis
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMCL	human myeloma cell line
IGH	immunoglobulin heavy locus
IMDM	Iscove's Modified Dulbecco's Medium
IMiD	immunomodulatory drugs
IRES	internal ribosome entry site
ISS	International Staging System
kDa	kiloDalton
LDH	lactose dehydrogenase
M	molar
MET	mesenchymal-to-epithelial transition
MFI	mean fluroscence intensity
MGUS	monoclonal gammopathy of undetermined significance
MM	multiple myeloma
MMP	matrix metalloproteinase
MMSET	MM SET domain
mRNA	messenger RNA
mRNA	messenger ribonucleic acid
nm	nanometres
PBS	phosphate bufferred saline
PBS	phosphate buffered saline
PC	plasma cell
PCL	plasma cell leukemia
R-ISS	revised-International Staging System
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute
RPMI8226-EV	RPMI8226-pRetroX-DsRed
RPMI8226-MMSET	RPMI8226-pRetroX-DsRed-MMSET
RPMI8226-MMSET-Y1118A	RPMI8226-pRetroX-DsRed-MMSET-Y1118A

RT-qPCR	reverse transcription-quantitative polymerase chain reaction
SD	standrad deviation
SDS-PAGE	sodium dodecyl sulfate polycrylamide gel electrophoresis
SEM	standard error of the mean
SHM	somatic hypermutation
siRNA	small interfering ribonucleic acid
SMM	smouldering multiple myeloma
SNP	single nucleotide polymorphism
Tween 20	polyethylene glycol sorbitan monolaurate
TWIST1	Twist family basic helix-loop-helix transcription factor 1
w/v	weight per volume
WES	whole exome sequencing
WHSC1	Wolf-Hirschhorn syndrome candidate 1
WL2-EV	WL2-pRUF-IRES-GFP
WL2-TWIST1	WL2-pRUF-IRES-GFP-TWIST1
WST-1	4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulphonate
x g	times gravity

LIST OF PUBLICATIONS

Scientific manuscripts

1. **Cheong CM**, Vandyke K, Mrozik KM, Kok CH, To LB, Licht JD, Zannettinno ACW. MMSET promotes the acquisition of an epithelial-to-mesenchymal-like gene expression signature in t(4;14) multiple myeloma (2017). *Manuscript in preparation.*
2. **Cheong CM**, Mrozik KM, Kok CH, Vandyke K, Hewett DR, Noll JE, Fitter S, Zannettino ACW. TWIST1 promotes tumour migration and dissemination in multiple myeloma (2017). *Manuscript in preparation.*
3. Vandyke K, Zeissig MN, Hewett DR, Martin SK, Mrozik KM, **Cheong CM**, Diamond P, To LB, Gronthos S, Peet DJ, Croucher PI, Zannettino ACW Hypoxia Inducible Factor 2 alpha (HIF-2α) drives plasma 1 cell dissemination in multiple myeloma by regulating CXCL12/CXCR4 and CCR1 (2016). *Submitted.*
4. Mrozik KM, **Cheong CM**, Hewett D, Chow AW, Blaschuk OW, Zannettino ACW, Vandyke K. Therapeutic targeting of N-cadherin is an effective treatment for multiple myeloma. *Br J Haematol* 2015 Nov; **171**(3)**:** 387-399
5. **Cheong CM**, Chow AW, Fitter S, Hewett DR, Martin SK, Williams SA, To LB, Zannettino ACW, Vandyke K. Tetraspanin 7 (TSPAN7) expression is upregulated in multiple myeloma patients and inhibits myeloma tumour development in vivo. *Exp Cell Res* 2015 Mar 1; **332**(1)**:** 24-38.

Conference proceedings

1. 10th Florey International Postgraduate Research Conference, Adelaide, Australia, September 2016. *TWIST1 promotes tumour progression and metastasis in multiple myeloma via cytoskeleton remodeling*. Poster. Awarded the John Barker Prize for Cancer Research
2. 2016 Australian Society for Medical Research (ASMR) SA Annual Scientific Meeting, Adelaide, Australia, June 2016. *RNA-sequencing reveals pathways regulated by TWIST1 to promote cell migration in multiple myeloma plasma cells.* Oral presentation.

3. TEMTIA-VII The EMT International Association Conference, Melbourne, Australia, October 2015. Meta-analysis of microarray datasets identifies EMT-like gene expression signature in t(4;14) multiple myeloma patients. Poster.
4. 2015 SAHMRI Research Showcase, Adelaide, Australia, September 2015. Meta-analysis of microarray datasets identifies EMT-like gene expression signature in t(4;14) multiple myeloma patients. Poster.
5. 2015 Australian Society for Medical Research (ASMR) SA Annual Scientific Meeting, Adelaide, Australia, June 2015. *Meta-analysis of microarray datasets identifies EMT-like gene expression signature in t(4;14) multiple myeloma patients.* Poster.
6. 2014 Annual Scientific Meetings of the HAA (Haematology Society of Australia and New Zealand, Australian & New Zealand Society of Blood Transfusion and the Australasian Society of Thrombosis and Haemostasis), Perth, Australia 2014. *Meta-analysis of microarray datasets identifies EMT-like gene expression signature in t(4;14) multiple myeloma patients.* Poster.
7. 2014 Australian Society for Medical Research (ASMR) SA Annual Scientific Meeting, Adelaide, Australia, June 2014. Tetraspanin 7 (TSPAN7) inhibits tumour development in vivo and regulates multiple myeloma cell transendothelial migration and adhesion in vitro. Poster.