Investigation of PI3Kγ signaling downstream of IGF-1R-CXCR4 transactivation in metastatic MDA-MB-231 breast cancer cells

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A thesis submitted to the University of Adelaide in fulfillment of the requirements for the degree of Doctor of Philosophy

February 2012

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Abstract

Breast cancer metastasis is a multi-step process regulated by a number of homeostatic factors. The insulin-like growth factor 1 tyrosine kinase receptor (IGF-1R) and the chemokine G-protein coupled receptor, CXCR4 have been shown to play an important role in breast cancer metastasis. More recently, accumulating evidence suggest that these two distinct receptors may regulate breast cancer cell migration through receptor transactivation. However, the underlying molecular mechanisms by which IGF-1R-CXCR4 transactivation regulates breast cancer cell metastasis remain unclear. Since phosphoinositide 3 kinases (PI3Ks) are known to be key signaling molecules governing cell migration, PI3K signaling downstream of IGF-1R-CXCR4 transactivation was investigated. In the present study the expression of class I PI3K isoforms was investigated in metastatic MDA-MB-231 breast cancer cells compared to that in non-metastatic MCF-7 cells. The data show that high levels of class IB PI3K catalytic subunit, p110γ are restricted to the highly metastatic cell types, correlating with the metastatic potential of the cell lines. Moreover, PI3Kγ is the major PI3K isoform regulating cell migration and activation of Akt downstream of IGF-1R-CXCR4 transactivation in metastatic MDA-MB-231 cells. Finally, several downstream targets that are dependent on PI3Kγ were identified using 2-D Fluorescence Difference Gel Electrophoresis (DIGE) and mass spectrometry analysis, including eukaryotic elongation factor 2 (eEF2), pyruvate kinase isozymes M1/M2 (PKM1/M2) and phosphoglycerate kinase 1 (PGK1) with PI3Kγ being shown to regulate phosphorylation of eEF2. In summary, the data in this study demonstrate a novel role for PI3Kγ in regulating cell migration downstream of IGF-1R-CXCR4 transactivation, potentially by attenuating cell proliferation via inhibition of eEF2 activation. The understanding of molecular mechanisms underlying receptor transactivation, including PI3K signaling transduction pathways in the progression of breast cancer metastasis and invasion may lead to development of more effective diagnostic and therapeutic strategies.

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 Meizhi Niu 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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February 2012

Acknowledgements

I would like to thank my supervisor Professor. Shaun McColl for giving me the opportunity to partake in such an interesting research project. Your support, guidance and encouragement during the completion of my Ph.D. studies will be forever appreciated. Working in your lab has been a rewarding and enjoyable experience. Thank you also for the hours of editing you have dedicated to this thesis. I would also like to thank my co-supervisor Dr. Manuela Klinger-Hoffmann. I am very grateful for all the time and efforts you have taken to guide me through, especially during my late Ph.D. Your scientific ideas and kind friendship will always be cherished at the bottom of my heart. I also extend a thankyou to my co-supervisor Professor John Wallace for his considerable help, especially for introducing me to Professor. Shaun McColl.

I would like to thank all of the past and present members of McColl Laboratory. You have all made the experience that much more enjoyable. I thank Dr. Marina Kochetkova and Dr. Iain Comerford for continuous advice and help. And Iain, thank you so much for your critical editing of my chapter. I also thank my fellow Ph.D. students: Chareeporn Akekawatchai, Sarah Haylock-Jacobs, Julie A. Brazzatti, Wendel Litchfield, Yuka Harata-Lee, Matt Higgins, Mark Bunting, and all those McColl group, Adriana, Michelle, Kevin, Valentina, Sarah, Carly and Ervin.

I would like to acknowledge Dr. Marina Kochetkova for the production of CXCR4 knockdown MDA-MB-231 cells, Julie A. Brazzatti for the production of p110γ knockdown MDA-MB-231 cells, Dr. Briony E. Forbes for providing important reagents and assistance with KIRA assay, Dr. Peter Hoffmann, Dr. Florian Weiland and Mr. James Eddes for conducting 2-D DIGE and MS analysis.

For all my friends in Adelaide who have made growing up in Adelaide such a great experience. Especially, thank you Koi for holding my hands when I first started my Ph.D. and keeping encouraging me when I was writing my thesis. Thank you Yuan for your accompany especially when I was extremely stressed and making the lunch time fun time. Thank you Shih-Hsun for sharing the late Ph.D feelings. Thanks also go to Gemma,

Anastasia, Jennifer, Rachelle, Rethish, and Caroline for the wonderful time we have spent together.

I wholeheartly thank my parents and parents-in-law, for whom I am eternally grateful for supporting me with whatever decisions I have made over the years and offering dramatic helps to look after my children, making my life much easier and allowing me to achieve my goals.

Finally, I thank my beloved husband Qing, who has been a loving and inspiring partner since I begun my Ph.D. studies. Without your unconditional love and support over these years, none of these would be possible.

Thank you everyone!!!

Publications arising from this work

Journals

M. Niu, M. Klingler-Hoffmann, J. A. Brazzatti, C. Akekawatchai, B. E. Forbes, P. Hoffmann and S. R. McColl. An IGF-1R-CXCR4 heterodimer mediates PI3Kγ-dependent phosphorylation of eukaryotic elongation factor 2 (eEF2) in MDA-MB-231 breast cancer cells. Biochem J. 2011 (Submitted)

J. A. Brazzatti, M. Klingler-Hoffmann, S. Haylock-Jacobs, Y. Harata-Lee, **M. Niu**, M. D. Higgins, M. Kochetkova, P. Hoffmann and S. R. McColl. Differential roles for the p101 and p84 regulatory subunits of PI3kγ in tumour growth and metastasis. Oncogene. 2011 (In press)

Conference Proceedings

Annual Scientific Meeting for Medical Research Adelaide Australia (2009)
Transactivation of PI3Kγ by IGF-I in MDA-MB-231 breast cancer cells
Poster Presentation

The 14th International Congress of Immunology Kobe Japan (2010)
Transactivation of PI3Kγ by IGF-I in MDA-MB-231 breast cancer cells
Poster Presentation

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Abbreviations

Ab	antibody
BCA	bicinchoninic acid
bp	base pair
BSA	bovine serum albmin
°C	degrees Celsius
CCL	CC chemokine ligand
CCR	CC chemokine receptor
cDNA	complementary deoxyribonucleic acid
CXCL	CXC chemokine ligand
DIGE	Difference Gel Electrophoresis
DMEM	Dulbecco' s modifeied Eagle' s medium
DMSO	dimethyl sulfoxide
DNA	Deoxynucleic triphosphate
dNTPs	deoxynucleic triphosphates
eEF2	Eukaryotic elongation factor 2
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	estrogen receptor
FCS	fetal calf serum
g	gram
GPCR	G-protein coupled receptor
G protein	GTP-binding protein
GRK	G-protein coupled receptor kinase
GTP	guansine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
HER2	human epidermal growth factor receptor 2
HRP	horseradish peroxidase
Ig	immunoglobulin
IGF-I or II	insulin-like groth factor I or II
IGF-1R	insulin-like groth factor-1 receptor

IGF-2R	insulin-like groth factor-2 receptor
IGFBP	insulin-like groth factor binding protein
IR	insulin receptor
IRS	insulin receptor substrate
JAK	Janus-family tyrosone kinase
kDa	kiloDalton
KIRA	kinase receptor activation assay
l	liter
MS	mass spectrometry
mTOR	mammalian target of rapamycin
m	metre
mA	milliampare
MAPK	mitogen-activated protein kinase
mg	milligram
ml	milliliter
mM	millimolar
mRNA	messenger RNA
µ	micron
µg	microgram
µl	microliter
n	nano
nm	nanometer
nM	nanomolar
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PI3K	phosphatidylinositol 3-kinase
PKB	protein kinase B
PKC	protein kinase C
PMSF	phenylmethylsulfonylfluoride

PTEN	phosphatase and tensin homolog deleted on chromosome ten
PTX	pertussis toxin
Raf	Ras activated factor
RNA	ribonucleic acid
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institue medium
RTK	receptor tyrosine kinase
SDS	sodium dodecyl sulphate
Shc	src homology proteins
SOS	son of sevenless
S1P	sphingosine 1-phophate
S1P1	sphingosine 1-phophate receptor
STAT	signal transducer and activator of transcription
TAE	tris acetate EDTA
TBS	tris buffered saline
TEMED	N,N,N'-tetramethyl ethylenediamine
Tween-20	polyoxyethylene sorbitan monolaurate
V	volt
v/v	volume per volume
w/v	weight per volume