INSULIN-LIKE GROWTH FACTOR RECEPTORS IN COLORECTAL CANCER

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&

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Abstract

The IGF system is a crucial regulator of normal growth and development, however dysregulation of the system on multiple levels is associated with the incidence of a wide variety of malignancies including the breast, thyroid, lung, and colon, making the IGF system an important anti-cancer therapeutic target. Due to its role in mediating cellular proliferation, protection from apoptosis, and metastasis, traditional focus has been set on examining the role of the type 1 IGF receptor [IGF1R] in cancer. However there is mounting evidence to suggest the insulin receptor [IR] may also be involved in the potentiation and pathogenesis of cancers.

The observation that IGF-II is overexpressed, compared to normal tissues, by cancers suggests signaling via target receptors by this ligand has important implications on cancer pathogenesis. Indeed, both the IGF1R and IR have been demonstrated to be up-regulated in a variety of malignancies. In regards to IR isoform, the IGF-II binding IR-A is preferentially expressed by a number of cancer cell types. Together with the observation that an autocrine proliferative loop exists between IGF-II and the IR-A in malignant thyrocytes and cultured breast cancer cells, suggests signaling via the IR-A may play a role in cancer cell growth and survival. However, very few studies on the IR-A have been conducted in cells co-expressing the IGF1R. This is mainly due to the difficulties associated with discrimination between signaling arising from IGF1R homodimers, IR-A homodimers, and IGF1R/IR-A hybrid receptors.

It is not known how the IR-A interacts, and functions in conjunction with the other receptors of the IGF system to signal biologically relevant outcomes, especially in terms of anti-cancer therapeutics that aim to block and down-regulate the IGF1R. Current anti-cancer therapies targeting the IGF system have concentrated on blocking IGF signaling via the IGF1R, due mostly to the functional properties of the receptor, but also in part due to the metabolic consequences associated with blockade and inhibition of the IR. This individual targeting of the IGF1R potentially leaves a pathway by which IGF-II secreted by the tumour can circumvent current IGF1R based therapies. Consequently, this thesis investigated whether the IR-A could compensate for the targeted loss of the IGF1R and how the IR-A interacts with the IGF1R in cells co-expressing these two receptors. In addition, the individual ability of the IR isoforms to signal biological outcomes in response to IGF stimulation was assessed.

The main experimental techniques used throughout this body of work included; assessment of protein expression and activation by Western blot, siRNA mediated gene silencing, and measures of cell proliferation, survival, and migration.

The key areas of investigation included:

1. Investigation of the individual ability of the IR isoforms to signal biological outcomes in response to IGF stimulation
2. Identification of an appropriate cell line model in which to investigate the interactions between the IR-A and IGF1R
3. Optimisation of siRNA mediated knock-down of the IR-A and IGF1R in SW480 colorectal adenocarcinoma cells
4. Determination of the biological role of the IR-A in SW480 cells co-expressing the IGF1R

The key findings from this work included:

1. The IR-A could not compensate for IGF1R depletion in SW480 cells
2. Dual silencing of the IR-A and IGF1R indicated signaling via the IGF1R was dominant to signaling via the IR-A in SW480 cells
3. Signaling via IR-A/IGF1R hybrid receptors may not be as potent as signaling via IGF1R homodimers
4. IGF-I at physiological concentrations can stimulate biological responses via both isoforms of the IR.

Declaration

This thesis contains no material that has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, it contains no material that has previously been published by any other person except where due reference is made.

I give consent for my thesis, when deposited in the library of the University of Adelaide to be available for loan and photocopying

Gemma Victoria Brierley

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Here we are, at the end of it all. A year ago I thought I would be struggling to write a very different thesis. What now forms this thesis makes for a pleasant and welcome surprise. I'd like to start by acknowledging and thanking Prof. John Wallace [Department of Biochemistry, University of Adelaide], Dr. Leah Cosgrove [CSIRO, Molecular and Health Technologies, Adelaide], and Dr. Lance Macaulay [CSIRO, Molecular and Health Technologies, Melbourne] for their supervision. Particular thanks to Lance for his thoughtful and detailed feedback throughout the preparation of this document. I also acknowledge the input of Dr. Briony Forbes at the beginning of this project.

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He who learns must suffer,
Even in our sleep, pain which cannot forget,
Falls drop by drop upon the heart,
Until, in our own despair,
Against our will,
Comes wisdom
- Aeschylus

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Publications arising from this thesis

List of Publications

Peer reviewed publications [see appendix]
* Denotes joint first authorship

Denley A*, Carroll JM*, **Brierley GV**, Cosgrove LJ, Wallace JC, Forbes BE, Roberts CT Jr. Differential activation of insulin receptor substrates 1 and 2 by insulin-like growth factor-activated insulin receptors. *Molecular Cell Biology* 2007 May; 27[10]: 3569 - 77

Denley A, **Brierley GV**, Carroll JM, Lindenberg A, Booker GW, Cosgrove LJ, Wallace JC, Forbes BE, Roberts CT Jr. Differential activation of insulin receptor isoforms by insulin-like growth factors is determined by the C domain. *Endocrinology* 2006 Feb; 147[2]: 1029 – 1036

Conference Proceedings

Brierley GV, Forbes BE, Macaulay L, Siddle K, Wallace JC, Cosgrove LJ. The biological role of insulin-like growth factors signalling via IR-A and IR-A/IGF1R hybrid receptors in cell survival, migration, and invasion. *The role of the IGF system in Cancer – Taormina, Sicily, Italy. November 2005*

Brierley GV, Forbes BE, Wallace JC, Cosgrove LJ. The biological role of insulin-like growth factors signalling via IR-A and IR-A/IGF1R hybrid receptors in cell survival, migration, and invasion. *Gordon Research Conference: Insulin-like growth factors in physiology and disease – Ventura, California, U.S.A. April 2005*

Abbreviations

^oC	degrees celcius
μg	microgram
μl	microlitre
μm	micrometre
3'	three prime
5'	five prime
95% CI	ninety five percent confidence interval
bp	base pair
Ab	antibody
ACF	aberrant crypt foci
APC	Adenomatosis Polyposis coli
ASO	anti-sense oligonucleotide
ATP	adenosine triphosphate
BMI	body mass index
BuA	butyrate
BRET	bioluminescence resonance energy transfer
BSA	bovine serum albumin
CO_2	carbon dioxide
cDNA	complementary DNA
c.f	compared with
COX-2	cyclooxygenase-2
CR	cystine-rich
CRC	colorectal cancer
CT	carboxy-terminal tail
C-terminus	Carboxyl-terminus
dATP	2`-Deoxyadenosine 5`-triphosphate
DEPC	diethyl pyrocarbonate
DEPC-MQ H_2O	dethyl pyrocarbonate milliQ water
DMEM	Dulbecco's modified eagles medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dL	decilitre
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
dTTP	2'-deoxythymidine 5'-triphosphate
dNTPs	deoxynucleotide triphosphates
DTT	dithiothreitol
EC_{50}	50% effective concentration
EDTA	ethylene diamine tetra-acetic acid
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
EPIC	European Prospective Investigation into Cancer and Nutrition
Erk-1/2	extracellular signalling-related kinase – 1/2
Eu	Europium
FACS	fluorescent-activated cell sorting
FCS	foetal calf serum
FITC	fluorescein isothiocyanate
F_n	fibronectin domain
GH	growth hormone
Grb2	Growth factor receptor-bound protein 2
H_2O	water
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HER-2	Human Epidermal Growth Factor-2
Hybrid-A	IGF1R/IR-A hybrid receptor
Hybrid-B	IGF1R/IR-B hybrid receptor
IB	immunoblot
IC_{50}	concentration of inhibitor which reduces binding by 50%
IGF1R	Type 1 IGF receptor
$IGF1R_{\alpha}$	Type 1 IGF receptor alpha-subunit
$IGF1R_{\beta}$	Type 1 IGF receptor beta-subunit
IGF2R	Type 2 IGF receptor
IGFBPs	Insulin-like growth factor binding proteins
IGF-I	Insulin-like growth factor I
IGF-I CII	IGF-I containing the IGF-II C domain
IGF-I DII	IGF-I containing the IGF-II D domain
IGF-I CIIDII	IGF-I containing the IGF-II C and D domains
IGF-II	Insulin-like growth factor II
IGF-II CI	IGF-II containing the IGF-I C domain
IGF-II DI	IGF-II containing the IGF-I D domain
IGF-II CIDI	IGF-II containing the IGF-I C and D domains
Ins	insert domain
IP	immunoprecipitate
IR	Insulin receptor

IR_{α}	Insulin receptor alpha-subunit
IR_{β}	Insulin receptor beta-subunit
IR-A	A isoform of the human insulin receptor [exon 11-]
IR-B	B isoform of the human insulin receptor [exon 11+]
IRS-1	Insulin Receptor Substrate -1
IRS-2	Insulin Receptor Substrate -2
JM	juxtamembrane domain
kDa	kilodalton
L1	large domain 1
L2	large domain 2
L-chain	light chain
LHS	left-hand side
LOI	loss of imprinting
M	molar
mAb	monoclonal antibody
MALDI-TOF	matrix-assisted laser desorption/ionisation – time of flight
MAPK	Mitogen-activated protein kinase
Max.	maximum
mg	milligram
$MgCl_2$	magnesium chloride
ml	millilitre
mM	millimolar
MMP	matrix metalloprotienase
MOPS	3-(N-morpholino)propanesulfonic acid
mRNA	messenger ribonucleic acid
N/A	not applicable
NaCl	sodium chloride
NaF	sodium fluoride
$Na_4O_7P_2$	sodium orthovanidate
Na_3PO_4	sodium pyrophosphate
ng	nanogram
NH_4	ammonia
nm	nanometer
nM	nanomolar
n/s	not significant
NSAIDs	non-steroidal anti-inflammatory drugs
nt	nucleotide
N-terminus	amino-terminus
OD_{260}	optical density at 260 nm
OD_{280}	optical density at 280 nm
p53	protein 53
pAb	polyclonal antibody
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFA	paraformaldehyde
PI3K	phosphoinositide kinase-3
pIRS-1	phosphorylated IRS-1
pIRS-2	phosphorylated IRS-2
PKB	protein kinase B
PMSF	phenylmethanesulphonyl fluoride
PTEN	phosphatase and tensin homologue
R^-	IGF1R negative NIH 3T3-like mouse fibroblasts
R^-IGF1R	IGF1R negative NIH 3T3-like mouse fibroblasts recombinantly expressing human IGF1R
R^-IRA	IGF1R negative NIH 3T3-like mouse fibroblasts recombinantly expressing human IR-A
R^-IRB	IGF1R negative NIH 3T3-like mouse fibroblasts recombinantly expressing human IR-B
RHS	right-hand side
RISC	RNA-inducing silencing complex
RNA	ribonucleic acid
RNAi	RNA interference
rpm	revolutions per minute
RR	relative risk
RT-PCR	reverse-transcriptase PCR
SCFA	short-chain fatty acid
SDS	sodium dodecyl sulphate
SH2	Src-homology domain
siRNA	short interfering RNA
SEM	standard error of the mean
TAE	Tris-acetate EDTA
TK	tyrosine-kinase domain
Tris-Cl	Tris (hydroxymethyl) aminomethane
Tyr	tyrosine
VEGF	vascular epidermal growth factor
vs.	versus
WB	Western blot
WCL	Whole cell lysate