Dissecting Signalling Contributions of the Alpha and Beta Subunits of the GM-CSF Receptor

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Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I consent to the thesis being made available for photocopying and loan if accepted for the award of the degree.

Michelle Perugini

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Abbreviations

aa	Amino acid
Ab	Antibody
Abs	Absorbance
ALL	Acute lymphoid leukaemia
AML	Acute myeloid leukaemia
bp	Base pairs
BM	Bone marrow
BSA	Bovine serum albumin
cDNA	Complementary DNA
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleic acid triphosphates
E. coli	*Escherichia coli*
EDTA	Ethylenediamine tetra-acetate
EGTA	Ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid
ERK	Extracellular regulated kinase
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal bovine serum
FITC	fluorescein isothiocyanate
g	Gram
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMR	Granulocyte-macrophage colony-stimulating factor receptor
GMRα	GMR alpha subunit
GST	Glutathione-S-transferase
$h\beta_c$	Human beta common
hr	Hour
HRP	Horse radish peroxidase
IκB	I-kappa-B
IKKβ	I-kappa-B kinase beta
IMDM	Iscove's modified Dulbecco's medium
IPTG	Isopropylthio-beta-D-galactosidase
JAK2	Janus kinase 2
kDa	Kilo Dalton

LB	Luria broth
M	Molar
MAPK	Mitogen-activated protein kinase
MEK	MAPK/ERK kinase
MFI	Mean fluorescence intensity
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimolar
NFκB	Nuclear factor-kappa-B
OD	Optical density
PB	Peripheral blood
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI3K	Phosphoinositol 3 kinase
RIPA	Radioimmunoprecipitation buffer
rpm	revolutions per minute
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SDM	Site-directed mutagenesis
STAT5	Signal transducer and activator of transcription 5
TEMED	N,N,N',N'-Tetramethylethylenediamine
μ	Micro (10^{-6})
μg	Micro gram
μL	Micro liter
μM	Micro molar
WCL	Whole cell lysate
WT	Wild-type
w/v	weight to volume

Abstract

Normal tissue homeostasis and appropriate responses to injury and infection are dependent on cellular communication mediated by cell surface receptors that respond to extrinsic stimuli. The GM-CSF receptor was the major focus of this project. This receptor shares a common signalling subunit, β_c, with the IL-3 and IL-5 receptors. The unique GM-CSF receptor α-subunit (GMRα) confers ligand binding specificity to the complex and is essential for GM-CSF receptor signalling, although the full complement of signalling events mediated by GMRα remains elusive. Through cloning of candidate interacting proteins, expression and co-immunoprecipitation studies, we have confirmed interactions for two proteins previously reported to interact with the GMRα, p85 and IKKβ. Additionally, we identified the Src family kinase, Lyn, as a novel direct interacting partner of GMRα and provide insights into possible roles of this kinase in initiating signalling from the GM-CSF receptor. In addition to GMRα associated events we aimed to further characterise the role of the common β_c subunit in GM-CSF mediated signalling. We utilised two classes of consitutively active β_c mutants (extracellular or transmembrane) which transform the bi-potential myeloid FDB1 cell line to either factor-independent growth and survival, or granulocyte-macrophage differentiation, respectively. Here we report a comprehensive biochemical analysis of signalling by these two classes of mutants in this cell line. The two activated GMR mutants displayed distinct and non-overlapping signalling capacity. In particular, expression of a mutant with a substitution in the transmembrane domain (V449E) selectively activated JAK/STAT5 and MAPK pathways resulting in a high level of sensitivity to JAK and MEK inhibitors. In contrast, expression of a mutant with a 37

amino acid duplication in its extracellular domain (FIΔ) selectively activates the PI3K/AKT and IKKβ/NFκB pathways. Cells responding to this mutant display a relative high level of sensitivity to two independent PI3K inhibitors and relative resistance to inhibition of MEK and JAK2. The non-overlapping nature of signalling by these two activated mutants suggests that there are alternative modes of receptor activation that differentially dependent on JAK2 and that act synergistically in the mature liganded cytokine receptor complex. Further detailed analysis of these mutants will facilitate the dissection of the signalling pathways involved in the GM-CSF response that mediate proliferation, survival and differentiation.

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