

The role of 14-3-3 ζ in cytokine receptor signalling

by

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Table of Contents

	<i>Page #</i>
List of Figures and Tables	IV
Summary	VI
Declaration	IX
Publications	X
Abbreviations	XI
Acknowledgements	XIV
Chapter 1: General introduction	
1.1. Signal transduction through phosphotyrosine and phosphoserine/threonine-dependent pathways	1
1.1.a. Cell signalling	1
1.1.b. Phosphotyrosine-mediated signalling mechanisms	2
1.1.c. Phosphoserine/threonine-mediated signalling mechanisms	4
1.1.d. Integration of phosphotyrosine and phosphoserine/threonine-mediated signalling mechanisms	6
1.2. GM-CSF, IL-3 and IL-5	11
1.2.a. Biological functions and pathophysiology of GM-CSF, IL-3 and IL-5	11
1.2.b. Mechanisms of GM-CSF, IL-3 and IL-5-dependent signal transduction	12
1.3. The 14-3-3 family of proteins	20
1.3.a. General characteristics of 14-3-3 proteins	20
1.3.b. Mechanisms of 14-3-3 binding	20
1.3.c. Mechanisms of action of 14-3-3 proteins	24
1.3.d. Role of 14-3-3 proteins in disease	26
1.3.e. Role of 14-3-3 proteins in GM-CSF, IL-3 and IL-5-dependent signalling	27
1.4. Aims	29
Chapter 2: Materials and Methods	
2.1. Cell lines, culture conditions and transfections/electroporations	30
2.1.a. Cell culture conditions	30
2.1.b. Generation of CTL-EN cells expressing human GM-CSF receptor	30
2.1.c. Calcium phosphate transfection	31
2.1.d. Electroporation of CTL-EN cells	31
2.1.e. Stimulation with GM-CSF	32
2.2. Cell survival assay	32
2.3. Cell proliferation assay	32
2.4. Bioinformatics	33

	<i>Page #</i>
2.5. DNA constructs and generation of 14-3-3ζ mutants	34
2.6. Antibodies and other reagents	37
2.7. Expression and purification of the SH2 domain of Shc	38
2.8. Expression and purification of recombinant 14-3-3ζ	38
2.9. Immunoprecipitations, pull-downs and immunoblot analysis	39
2.10. PI3K assay	42
2.11. Src kinase assay	43
Chapter 3: The adaptor Shc and Src kinases are important for the assembly of 14-3-3/PI3K signalling complexes	
3.1. Introduction	45
3.2. Aims	50
3.3. Results	51
3.3.a. 14-3-3 ζ interacts with PI3K in a cytokine-dependent manner	51
3.3.b. Shc interacts with 14-3-3 ζ in a cytokine-dependent manner and is important for the assembly of the 14-3-3/PI3K signalling complex	55
3.3.c. c-Src interacts with 14-3-3 ζ and mediates the assembly of a 14-3-3/PI3K signalling complex	60
3.4. Discussion	63
Chapter 4: 14-3-3ζ is tyrosine-phosphorylated in a cytokine-dependent manner	
4.1. Introduction	67
4.2. Aims	69
4.3. Results	69
4.3.a. 14-3-3 ζ is tyrosine-phosphorylated in HEK 293T cells	69
4.3.b. 14-3-3 ζ is tyrosine-phosphorylated in a GM-CSF-dependent manner	71
4.3.c. Src tyrosine kinases phosphorylate 14-3-3 ζ	74
4.3.d. Bioinformatic analysis of potential tyrosine phosphorylation sites on 14-3-3 ζ	76
4.3.e. Individual tyrosine to phenylalanine mutations on 14-3-3 ζ do not reduce overall phosphotyrosine signal	80
4.4. Discussion	82
Chapter 5: Simultaneous recruitment of phosphoserine and phosphotyrosine molecular pathways by 14-3-3ζ is necessary for PI3K activation	
5.1. Introduction	86
5.2. Aims	87
5.3. Results	88
5.3.a. Tyr179 of 14-3-3 ζ is important for its interaction with Shc	88

	<i>Page #</i>
5.3.b. Tyr179 of 14-3-3 ζ is critical for its interaction with Shc in response to GM-CSF stimulation	94
5.3.c. Tyr179 of 14-3-3 ζ is important for its interaction with PI3K	96
5.3.d. Tyr179 of 14-3-3 ζ is critical for its interaction with PI3K in response to GM-CSF stimulation	97
5.3.e. grb2 and Gab2 are not required for the recruitment of Shc and PI3K to Tyr179 of 14-3-3 ζ	100
5.3.f. 14-3-3 ζ binds to Ser585 of β c and simultaneously recruits Shc and PI3K through Tyr179	102
5.4. Discussion	105
Chapter 6: Tyr179 of 14-3-3ζ is important for Akt/PKB activation and cell survival in response to GM-CSF stimulation	
6.1. Introduction	109
6.2. Aims	113
6.3. Results	113
6.3.a. Akt/PKB activation in response to GM-CSF requires Tyr179 of 14-3-3 ζ	113
6.3.b. GM-CSF-stimulated cell survival requires Tyr179 of 14-3-3 ζ	115
6.3.c. ERK activation in response to GM-CSF does not require Tyr179 of 14-3-3 ζ	118
6.3.d. Tyr179 of 14-3-3 ζ is not required for GM-CSF-induced cell proliferation	118
6.3.e. Tyr179 of 14-3-3 ζ is required for Ψ 2 cells growth	121
6.4. Discussion	124
Chapter 7: General discussion	129
References	151
Appendix	
The Greek alphabet	194
3 letter and single-letter amino acid code	195

List of Figures and Tables

	<i>Page #</i>
Chapter 1: General Introduction	
Figure 1.1. Phosphotyrosine-dependent signalling pathways.	3
Table 1.1. Distribution of phosphotyrosine and phosphoserine/threonine modules in human proteins.	8
Figure 1.2. Activation of signalling pathways by the GM-CSF/IL-5/IL-3 receptor family.	14
Figure 1.3. Domain organization of the catalytic and regulatory subunits of PI3K.	16
Figure 1.4. Model for the regulation of survival by GM-CSF, IL-3 or IL-5.	19
Figure 1.5. Multiple sequence alignment of various isoforms of 14-3-3 proteins from different species.	21
Figure 1.6. Crystal structure of human 14-3-3 ζ .	23
Figure 1.7. Mechanisms of action of 14-3-3 proteins.	25
Chapter 2: Materials and Methods	
Figure 2.1. Vector map of pRc/CMV-14-3-3-myc.	35
Table 2.1. Mutagenesis primers used in the generation of 14-3-3 ζ -myc mutants.	36
Figure 2.2. Summary of the process of generation of r14-3-3 ζ .	40
Chapter 3: The adaptor Shc and Src kinases are important for the assembly of 14-3-3/PI3K signalling complexes	
Figure 3.1. Domain organization of the Shc proteins.	47
Figure 3.2. Domain organization of c-Src.	49
Figure 3.3. 14-3-3 ζ associates with p85 in mammalian cells.	52
Figure 3.4. 14-3-3 ζ associates with PI3K in response to GM-CSF stimulation.	53
Figure 3.5. 14-3-3 ζ associates with Shc in mammalian cells.	56
Figure 3.6. 14-3-3 ζ associates with Shc in response to GM-CSF stimulation.	57
Figure 3.7. Shc mediates the association of 14-3-3 ζ with p85.	59
Figure 3.8. 14-3-3 ζ associates with Src in mammalian cells.	61
Figure 3.9. 14-3-3 ζ associates with Src in mammalian cells (2).	62
Figure 3.10. Src tyrosine kinases mediate the association of 14-3-3 ζ with p85.	64
Chapter 4: 14-3-3ζ is tyrosine-phosphorylated in a cytokine-dependent manner.	
Figure 4.1. 14-3-3 ζ is tyrosine-phosphorylated in HEK 293T cells.	70
Figure 4.2. 14-3-3 ζ is tyrosine-phosphorylated in response to GM-CSF stimulation.	72
Figure 4.3. Src tyrosine kinases phosphorylate 14-3-3 ζ <i>in vitro</i> .	75
Table 4.1. Bioinformatic analysis of tyrosine residues of 14-3-3 ζ .	77

	<i>Page #</i>
Figure 4.4. Tyrosine residues on various isoforms of 14-3-3 proteins from different species.	78
Figure 4.5. Crystal structure of human 14-3-3 ζ (with highlighted tyrosine residues)	79
Figure 4.6. Individual tyrosine mutations of 14-3-3 ζ -myc do not reduce overall tyrosine phosphorylation signal.	81
Chapter 5: Simultaneous recruitment of phosphoserine and phosphotyrosine molecular pathways by 14-3-3ζ is necessary for PI3K activation.	
Figure 5.1. Tyr179 of 14-3-3 ζ is required for binding to the SH2 domain of Shc.	89
Figure 5.2. Tyr179 of 14-3-3 ζ is important for its binding to Shc.	91
Figure 5.3. Tyrosine mutations do not affect phosphoserine-binding properties of 14-3-3 ζ .	93
Figure 5.4. Tyrosine 179 of 14-3-3 ζ is critical for its interaction with Shc in response to GM-CSF stimulation.	95
Figure 5.5. Tyrosine 179 of 14-3-3 ζ is important for binding to PI3K.	98
Figure 5.6. Tyrosine 179 of 14-3-3 ζ is required for the GM-CSF-stimulated recruitment of PI3K.	99
Figure 5.7. grb2 and Gab2 are not required for the recruitment of Shc and PI3K to Tyrosine 179 of 14-3-3 ζ .	101
Figure 5.8. 14-3-3 ζ can simultaneously bind to Ser585 of β c and recruit Shc and PI3K.	103
Chapter 6: Tyr179 of 14-3-3ζ is important for Akt/PKB activation and cell survival in response to GM-CSF stimulation.	
Figure 6.1. Tyrosine 179 of 14-3-3 ζ is required for GM-CSF-stimulated activation of Akt/PKB.	114
Figure 6.2 Tyrosine 179 of 14-3-3 ζ is necessary to promote cell survival in response to GM-CSF.	116
Figure 6.3. Tyrosine 179 of 14-3-3 ζ is not required for GM-CSF-stimulated activation of ERK.	119
Figure 6.4. Tyrosine 179 of 14-3-3 ζ is not necessary to promote cell proliferation in response to GM-CSF.	120
Figure 6.5. Tyrosine 179 of 14-3-3 ζ is required for Ψ 2 cells growth.	123
Chapter 7: General discussion.	
Figure 7.1. The role of Tyr179 of 14-3-3 ζ in GM-CSF receptor signalling.	137

Summary

The ability of a cell to respond to extrinsic stimuli critically depends on its ability to regulate specific intracellular protein-protein interactions in a reversible manner and allow the temporal-spatial characteristics of the signal to be accurately transduced to downstream targets. Growth factor and cytokine receptors provide a link by which extracellular stimuli are propagated within the cell to accomplish specific cellular functions. Ligand-stimulation of these receptors activates cascades of intracellular events that transduce the signals that lead to a variety of cellular responses. The specificity of these signals and the fidelity with which they are communicated within the cell are critical for the fate of an organism as deregulation or misbalance of signalling networks is commonly associated with a wide range of pathologies and diseases.

Cytokines are important regulatory proteins that regulate diverse cellular functions through their ability to bind to specific cell surface receptors. Most cytokines are pleiotropic effectors that regulate multiple cellular functions. For example, many cytokines can regulate diverse biological activities such as cell survival, proliferation and differentiation and in many cases these different biological activities can be independently regulated. The regulation of pleiotropic biological responses is mediated through the modulation of multiple intracellular signalling pathways. These pathways often present a high level of redundancy in terms of the biological functions that they control. However, pleiotropic cytokines have the ability to independently activate signalling pathways that lead to the regulation of specific biological functions such as survival, proliferation, differentiation or activation. The molecular mechanisms by

which cytokines can regulate pleiotropic biological responses from the activation of a limited number, often redundant, of intracellular signalling pathways have not been fully resolved and remains one of the most important unanswered questions in cell biology. In particular, proteins and molecular mechanisms responsible for specifying different biological responses remain largely unidentified.

In many cases, activation of multiple signalling pathways and integration of the signals they transduce is needed in order to modulate a biological function. One important mechanism by which signalling pathways are assembled within the cell is through the action of protein scaffolds that contain phosphotyrosine (e.g. SH2, PTB) or phosphoserine/threonine (e.g. 14-3-3, WW, FHA, PBD, BCRT) binding modules. Interestingly, although phosphotyrosine and phosphoserine/threonine-dependent signalling pathways are highly integrated within the cell, scaffold proteins containing both phosphotyrosine and phosphoserine or phosphothreonine-binding domains (i.e. SH2/PTB and WW/FHA/PBD/BCRT) have not been identified.

The broad aim of this thesis is to study the fundamental molecular mechanisms by which cytokines, through the binding of cell surface receptors, are able to activate and integrate signalling pathways that regulate and specify cellular responses. In particular, these studies examine the role of the 14-3-3 family of adaptor proteins in the assembly of signalling networks that couple the activated receptors of the haematopoietic cytokines IL-3, IL-5, and GM-CSF to downstream signalling targets and specific cellular functions such as survival and proliferation. The specific aims of this thesis are to examine the composition, molecular mechanisms of assembly and functional roles of signalling complexes that use the adaptor or scaffold protein 14-3-3 and are important for signal transduction in response to GM-CSF.

This work shows that the phosphoserine/threonine-binding scaffold protein 14-3-3 ζ , previously reported to bind to Ser585 of the GM-CSF receptor, undergoes tyrosine phosphorylation. Using a panel of 14-3-3 ζ mutants a particular tyrosine residue, Tyr179, was found to be critical for the binding of the SH2 domain of Shc, the assembly of a PI3K signalling complex, the activation of the Akt/PKB signalling pathway and the control of cell survival in response to GM-CSF stimulation. Tyr179 of 14-3-3 ζ was also found to be important for specifying GM-CSF-mediated biological responses as it was found to play an important role in the control of cell survival versus cell proliferation. Furthermore, it was found that 14-3-3 ζ is able to simultaneously bind to Ser585 of the GM-CSF receptor and recruit Shc and PI3K through Tyr179, thus integrating phosphoserine/threonine and phosphotyrosine-dependent signalling pathways.

The findings described in this thesis helped to identify a novel mechanism by which cytokine receptors achieve both integration in signalling and specificity in biological outcomes. The discovery that phosphoserine/threonine-binding proteins (i.e. 14-3-3) are themselves tyrosine phosphorylated and able to recruit phosphotyrosine-binding molecules provides a new insight into how intracellular signal integration is achieved.

Understanding how signal transduction is carried out within the cell is paramount to successful drug development in many therapeutic areas. The new insights in GM-CSF signalling provided by this work may help to successfully develop treatments to target diseases such as asthma, rheumatoid arthritis and leukaemia, where GM-CSF appears to play a pathogenic role.

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 and, to the best of my knowledge and belief contains no material previously published or written by another person.

I give my consent to this thesis, when deposited in the University library, being available for loan and photocopying.

Signed:

Date:

Publications

Related publications arising from the work presented in this thesis:

1. Guthridge, M. A., Barry, E. F., **Felquer, F. A.**, McClure, B. J., Stomski, F. C., Ramshaw, H., Lopez, A. F. (2004) The phosphoserine-585-dependent pathway of the GM-CSF/IL-3/IL-5 receptors mediates hematopoietic cell survival through activation of NF-kappaB and induction of bcl-2. *Blood* 103, 820-827
2. Guthridge, M. A., Powell, J. A., Barry, E. F., Stomski, F. C., McClure, B. J., Ramshaw, H., **Felquer, F. A.**, Dottore, M., Thomas, D. T., To, B., Begley, C. G., Lopez, A. F. (2006) Growth factor pleiotropy is controlled by a receptor Tyr/Ser motif that acts as a binary switch. *EMBO J* 25, 479-485
3. **Felquer F.A.**, Hercus T.R., Lopez F.A., Guthridge M.A. Simultaneous recruitment of of phosphoserine and phosphotyrosine molecular pathways by 14-3-3 ζ is necessary for PI 3-kinase activation and cell survival. (Manuscript in preparation).

Abbreviations

ATP	Adenosine triphosphate
BrdU	5-Bromo-2_-deoxyuridine
BSA	Bovine Serum Albumin
cm	Centimeter
C-terminal	Carboxy-terminal
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethyleneglycolbis(β -aminoethyl ether)tetraacetic acid
FACS	Fluorescence activated cell sorting
FCS	Fetal calf serum
g	gram
GM-CSF	Granulocyte/macrophage colony-stimulating factor
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP	Horseradish peroxidase
IL-2	Interleukin-2
IL-3	Interleukin-3
IL-5	Interleukin-5
IPTG	Isopropyl-beta-D-thiogalactopyranoside
IRES	Internal ribosomal entry site
kD	Kilodaltons

Kd	Dissociation constant
kV	Kilovolt
M	Molar
mA	Milliampere
μ F	Microfarad
μ g	Microgram
mg	Milligram
μ l	Microlitre
ml	Millilitre
mm	Millimetre
μ M	Micromolar
mM	Millimolar
mmol	Millimol
ng	Nanogram
NP40	Nonident P-40
N-terminal	Amino-terminal
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	PolymeRase Chain Reaction
PDB	Protein Data Base
Pfu	<i>Pyrococcus furiosus</i> DNA polimeRase
pmol	Picomol(s)
PMSF	Phenylmethysulphonylfluoride
PTB	Phosphotyrosine-binding
RIPA	Radioimmunoprecipitation (buffer)
SDS	Sodium dodecylsulphate

SH2	Src homology-2
SH3	Src homology-3
TLC	Thin layer chromatography

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