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

by

Fernando Augusto Felquer

Thesis submitted in fulfilment of the requirement
for the Degree of Doctor of Philosophy

Cytokine Receptor Laboratory
Department of Human Immunology
The Hanson Institute, Institute of Medical and Veterinary Science
Adelaide, South Australia, Australia

Faculty of Health Sciences
Department of Medicine
University of Adelaide, South Australia, Australia

June 2006

Table of Contents

Page #

List of Figures and Tables

Page #

Chapter 5: Simultaneous recruitment of phosphoserine and phosphotyrosine molecular pathways by 14-3-3ζ is necessary for PI3K activation.

Chapter 6: Tyr179 of 14-3-3ζ is important for Akt/PKB activation and cell survival in response to GM-CSF stimulation.

Chapter 7: General discussion.

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 14-3-3ζ, previously reported to bind to Ser585 of the GM-CSF receptor, undergoes tyrosine phosphorylation. Using a panel of 14-3-3ζ 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 14-3-3ζ 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 14-3-3ζ 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 14-3-3ζ 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-hydroxylethyl)-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
μF	Microfarad
μg	Microgram
mg	Milligram
μl	Microlitre
ml	Millilitre
mm	Millimetre
μM	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	*Pyrococcus furiosus* DNA polimeRase
pmol	Picomol(s)
PMSF	Phenylmethylsulphonylfluoride
PTB	Phosphotyrosine-binding
RIPA	Radioimmunoprecipitation (buffer)
SDS	Sodium dodecylsulphate

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

Acknowledgments

I would like to thank the Department of Human Immunology, The Hanson Institute, Institute of Medical and Veterinary Sciences, Adelaide for allowing me to pursue my studies.

I am extremely grateful to Professor Angel Lopez and Dr Mark Guthridge for their daily and overall supervision, encouragement and support throughout my candidature. Angel has established a great laboratory and I thank him for his excellent guidance and tuition.

I would like to thank all the members of the Cytokine Receptor Laboratory, both past and present who have always been willing to help. I specially would like to thank Dr Joanna Woodcock for her advice and support during my candidature.

I would also like to thank the Department of Medicine, University of Adelaide.

I would also like to thank my family for their love and support.

Finally, I would like to dedicate this thesis to my wife Lidia for her understanding, love, support and patience throughout this candidature.