

*T cell deficiencies resulting from aberrant
pre-mRNA alternative splicing caused by
a novel splicing silencer hnRNP LL in an
ENU mutant mouse strain thunder*

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July 2008

A thesis submitted for the degree of Doctor of Philosophy
of the Australian National University



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Statement

The research contained within this study was performed in the Immunogenomics Laboratory under the supervision of Dr. Gerard Hoyne and Professor Chris Goodnow. The data presented is my own work, with all contributions from others clearly stated in the acknowledgements and methods.

This thesis is dedicated to my father-in-law, Professor Dezheng Liang, who encouraged me to face challenges, but sadly passed away in my first year of PhD.

Acknowledgements

I had my medical degree fifteen years ago and had most of my postgraduate training in the focus of clinical haematology. After a long period of clinic practice, I found what really interest me is to discover the truth lying behind the diseases so that we can find better strategies to fight against them. However, after 2 decades extensive studies and rapid progress of genomics and molecular immunology, I found that everything is new to me when I finally decided turn to basic research and started my PhD project. This is the reason I would like to start my acknowledgements with my supervisors. I would like to express my deepest appreciation to Gerard and Chris, who are the greatest supervisors and nicest gentlemen in every way. Thank you very much, Gerard, for your generous contribution of your time in the early days, teaching me Immunological theories and basic laboratory skills from experiment design to data analysis. Thank you for your huge supports and always willing to share me with your creative and rational thoughts all through last four years. From Chris I learned how a genius leader contributes with his perspective insights. I can always rely on you when I have some difficulties to proceed, especially in the exon array analysis. Thank you very much, Chris, for always leading this interesting but complicate project to the right track.

There are a lot of colleagues from the Immunogenomics lab I need to thank. Thank Lina, Anselm, Steph for many stimulating discussions and helpful suggestions; special thanks to Anselm and Steph for reading some of the chapters of my thesis and frankly giving critical opinions; Di helped me a lot with recombination and retrovirus experiments; Lisa, Keisuke and Di also helped with western blot; Debbie did really good jobs in Bone Marrow Chimeras; Adam completed genome scan and found primary chromosome linkage; Belinda gave a lot helpful suggestions in mapping, sequencing and helped with *thunder* mutation genotyping; David taught me microsatellite PCR; Suzanne, David, Adam, Rong, and Sayema genotyped most of the mice; Nathan, Katrina, Holly, and Sharon looked after thunder mice at different stages; Kelly, Judi, and Holly bled the mice. It is well appreciated for Mitchell Townsend organising sample shipment to overseas and lab managements to make lab work easily and friendly. Also thanks to Rosemary for ordering and lab maintenance.

Thanks also go to previous PhD in the group, Rui, Lisa, and Adrain, I am sure their successful experience inspire most of our PhD fellows with the enthusiasm in researches. My student fellows Di, Nick, Mick, Daniel, Kim, Eleanor, Yovina, Mitchell, Katrina, Sally, John, Charis, and Jack, thank you all for making study in the lab full of fun.

John Curtin School provide some fantastic facilities and services. Harpreet and Sarah from FACS lab helped me with the usage of Flow cytometry and contribute a lot of work in sorting cells for RNA isolation. Carmeron from BRF helped with sequencing; Melbourn gave some helpful suggests on Realtime-PCR; Kaiman helped with RNA quality check; Steve provided some help in using Partek Genomics Suite software; Christine helped me with the usage of Partek too.

Our collaborators made some important contributions to this study as well. Professor Alan Aderem from the Institute of System Biology generously provided Affymetrix chips for mouse all exon sense target (ST) arrays; Bruz Marzolf and Kathrine Kennedy are really good collaborators in quick responses, paying very well attention to run long term experiments, and taking seriously responsibility to look after the samples in array experiments. Dan from ISB too provided some important suggestions in array data analysis.

Dr. Xinying Jia and Professor Gottfried Otting from the Research School of Chemistry in ANU helped with the NMR structure work of RRM domain and RNA binding affinity experiments.

Most importantly, I want to thank my lovely wife Rong Liang and my daughter Aileen Y Wu. Thanks Rong for your constant support and heart-warming encouragement in my hard time; in most circumstance Aileen is my first and best audience to tell the story of T cells. Thank my family that I left behind in China, Dad and Mum, sisters and brothers, and my parents-in-law who encouraged me to face the new challenges when moving to Australia.

Publications, presentations, and awards arising from the study

Publications:

1. **Zuopeng Wu***, Xingying Jia, Bruz Marzolf, Daniel Zak, Alan Aderem, Di Yu, Belinda Whittle, Gottfried Otting, Christopher C Goodnow & Gerard Hoyne. Memory T cell mRNA splicing is reprogrammed by heterogeneous nuclear ribonucleoprotein L-like hnRNPLL. (Submitted)
2. **Zuopeng Wu***, Adele Yates, Christopher C Goodnow & Gerard F Hoyne. CD45 expression and alternative splicing jointly regulate Lck activity in T cells. (Manuscript in preparation)

Conference presentations:

04/2005, Genome-Phenome workshop, John Curtin School of Medical Research, Canberra.

Poster: Characterisation of immune regulatory gene in an ENU mutagenised mouse strain.

09/2006, European Congress of Immunology, Paris, France.

Poster: T cell deficiency resulting from aberrant pre-mRNA alternative splicing.

12/2006, Australian Society of Immunology Annual Meeting, Auckland, New Zealand.

Oral presentation: T cell deficiency resulting from pre-mRNA alternative splicing regulated by a novel hnRNP protein.)

06/2007, AMRC Yong Investigator Forum, Canberra.

Poster: T cell deficiency resulting from pre-mRNA alternative splicing regulated by a novel hnRNP protein.

10/2007, “Cytokine in Health and Disease” 15th Annual Meeting of the International Cytokine Society” San Francisco, USA

Oral presentation: Roles of IL-7R expression in naïve T cell homeostasis in a pre-mRNA alternative splicing deficient mouse strain.

Awards:

Postgraduate International Travel Award 2007,
Australian Society of Immunology:

Vice-Chancellor Postgraduate Student Travel Award, 2007
The Australian National University:

Outstanding Scholar Award, 3rd place. 10/2007.
The International Cytokine Society

Abbreviations

ACAD: activated cell autonomous death
AICD: activation induced cell death
ARS: activation responsive sequence
APCs: antigen presenting cells
BH: Bcl-2 homology
Bim: Bcl-2 interacting molecule
CD45R: CD45 restricted epitope
CFSE: Carboxyfluorescein succinamide ester
Csk: C-terminal Src kinase
DISC: death inducing signalling complex
DN: CD4-CD8- double negative cells
DP: double-positive cells
Egr3: Early growth response gene 3
ELISA Enzyme-linked immunosorbance assays
EMS: ethylmethane sulfonate
ENU: N-ethyl-N-nitrosourea
ES: embryonic stem cells
ESE/ISE: exonic/intronic splice enhancers
ESS/ISS: exonic/intronic splice silencers
FACS: Flow cytometry analysis
FADD: FAS-associated death domain protein
hnRNPs: heterogeneous nuclear ribonucleoproteins
Hnrp11: heterogeneous nuclear ribonucleoprotein L-like (protein semble: hnRNP LL)
IL-7/IL-7R: Interleukin-7/Interleukin-7 Receptor
ISP: immature single-positive
ITAMs: immunoreceptor tyrosine-based activation motifs
ITIM: inhibitory tyrosine immunoreceptor motifs
KH: hnRNP K homolog
MAPK: Mitogen activated protein kinase
PCD: programmed cell death
PKC: Protein kinase C

PTB: Polypyrimidine Tract Binding protein (hnRNP I)

PTP: protein tyrosine phosphatases

ROR γ t: orphan nuclear hormone receptor

RRM: RNA recognition motif

SCID: severe-combined immunodeficiency

SF: splicing factor

SHP1: SH2-domain-containing PTP1

SLE: systemic lupus erythematosus

SNPs: single nucleotide polymorphisms

snRNPs: small nuclear ribonucleoprotein particles

snRNAs: small nuclear RNAs

SR proteins: Serine/Arginine rich proteins

SSLP: simple sequence length polymorphism

TCR: T-cell antigen receptor

T_{FH}: T follicular helpers

Tregs: regulatory T cells

Tap2: Transporter of Antigen Presentation 2

ZAP70: ζ chain associated protein kinase of 70 kDa

Abstract

ENU mutagenesis screening is a phenotype-driven approach to identify genes in a nonbiased manner. Through this approach we identified a previously uncharacterised gene, *Hnrpll*, which is involved in nascent mRNA alternative splicing. *Thunder* strain carries a point mutation in the *Hnrpll* gene (407T->A) in its first RNA recognition motif (RRM) which changes its function in regulating nascent mRNA alternative splicing.

One of *Hnrpll* target genes is CD45 that undergoes alternative splicing in T cells depending on their development stages and activation status. We demonstrated that *Hnrpll*^{thu/thu} T cells fail to silence 3 variable exons of CD45 and result in constitutive expression of CD45RA, B, and C epitopes on the cell surface. Retroviral based expression of wild-type *Hnrpll* cDNA in the *Hnrpll*^{thu/thu} T cells compensates the effects of loss of function in the mutation. The mutated RRM1 domain remains the ability to bind the regulatory element of activation responsive sequence (ARS) within CD45 pre-mRNA but cripples the protein function by destabilising the proteins to unfold in a thermolabile sensitive manner.

We also found that *Hnrpll*^{thu/thu} mutation disrupts peripheral T cell subsets. *Thunder* mice have normal T cell development in the thymus but specifically lost naïve T cells in the peripheral lymphoid tissues, whereas memory T cells are not affected. *Hnrpll*^{thu/thu} naïve T cells can homeostatically proliferate but fail to persist for a long term *in vivo*, suggesting that *thunder* mutation influences naïve T cell longevity.

We observed that *Hnrpll*^{th^u/th^u} naïve T cells express lower level of IL-7Ra and lower Bcl-2, together with the stronger pro-apoptotic BimS isoform due to alternative splicing. This highlights the nonredundant role of the *Hnrpll* gene in regulating peripheral T cell homeostasis.

CD45 isoforms are widely used markers to distinguish naïve and memory T cell subsets. CD45 splicing does not account for the loss of naïve T cells in the *thunder* mice, however, CD45RABC shows stronger phosphatase activity than CD45RO when the amount of CD45 is dramatically reduced to 5% remaining on T cell surface, suggesting jointly regulation of CD45 catalytic activity by its expression and alternative splicing.

Immunological memory is the hallmark of the adaptive immune system. Through Affymetrix mouse all exon arrays, we found that hnRNP LL protein plays a critical role in controlling an extensive program of alternative splicing as naïve T cells differentiate to the memory cell fate. It acts as an either trans- or cis- acting factor in regulating multiple nascent mRNA alternative splicing. This study provides an unprecedented insight into the extent of alternative splicing in the generation of immune memory.

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