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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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 agaist 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.

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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

Hnrpll: 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

RORγt: 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 it 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 thermolible 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*$^{thu/thu}$ 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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