c-Myc controls the development of CD8αα TCRαβ intestinal intraepithelial lymphocytes from thymic precursors by regulating IL-15–dependent survival

Wei Jiang, Isabel Ferrero, Elisa Laurenti, Andreas Trumpp, and H. Robson MacDonald

The murine gut epithelium contains a large population of thymus-derived intraepithelial lymphocytes (IELs), including both conventional CD4+ and CD8αβ+ T cells (expressing T-cell receptor αβ [TCRαβ]) and unconventional CD8αα+ T cells (expressing either TCRαβ or TCRγδ). Whereas conventional IELs are widely accepted to arise from recirculation of activated CD4+ and CD8αβ+ T cells from the secondary lymphoid organs to the gut, the origin and developmental pathway of unconventional CD8αα IELs remain controversial. We show here that CD4-Cre-mediated inactivation of c-Myc, a broadly expressed transcription factor with a wide range of biologic activities, selectively impairs the development of CD8αα TCRαβ IELs. In the absence of c-Myc, CD4+ CD8αα+ TCRγδ+ thymic precursors of CD8αα TCRαβ IELs are present but fail to develop on adoptive transfer in immunocompetent hosts. Residual c-Myc-deficient CD8αα TCRαβ IEL display reduced proliferation and increased apoptosis, which correlate with significantly decreased expression of interleukin-15 receptor subunits and lower levels of the antiapoptotic protein Bcl-2. Transgenic overexpression of human BCL-2 resulted in a pronounced rescue of CD8αα TCRαβ IEL in c-Myc-deficient mice. Taken together, our data support a model in which c-Myc controls the development of CD8αα TCRαβ IELs from thymic precursors by regulating interleukin-15 receptor expression and consequently Bcl-2–dependent survival. (Blood. 2010;115(22):4431-4438)

Introduction

T lymphocytes can be broadly divided into 2 categories. Conventional T cells, expressing T-cell receptor αβ (TCRαβ) and either CD4 or CD8 coreceptors, arise in the thymus via a complicated differentiation process involving positive and negative selection and are subsequently exported to the periphery where they primarily reside in secondary lymphoid organs, such as lymph nodes and spleen. On the other hand, unconventional T cells (which can express either TCRαβ or TCRγδ with or without coreceptors) can be frequently found in nonlymphoid organs, such as the skin, liver, or intestine. Well-characterized examples of unconventional T cells in the mouse include dendritic epidermal T cells in the skin, Vα14 invariant (Vα14i) natural killer T (NKT) cells in the liver, and a subset of intraepithelial lymphocytes (IELs) expressing CD8αα homodimers in the gut. Unconventional T cells are generally accepted to be thymus-dependent and to undergo agonist selection during development, but details of their differentiation pathway and selection requirements are less well understood than for conventional T cells.1,4

Unconventional CD8αα IELs in the intestinal epithelium are composed of subsets expressing TCRαβ or TCRγδ. Despite early reports suggesting an extrathymic origin for these cells,5 more recent studies indicate that the majority of CD8αα TCRγδ T cells and (especially) CD8αα TCRαβ IELs are derived from thymic precursors that subsequently migrate to the gut.6,7 However, the differentiation pathway followed by CD8αα IELs remains controversial. According to one model, both CD8αα TCRγδ and CD8αα TCRαβ IELs originate from immature CD4−CD8− (DN) thymic precursors, which exit the thymus at the DN2-DN3 stage8 and complete their development in gut cryptopatches (CPs), which provide a unique microenvironment for the terminal differentiation of these cells.9 In contrast, another hypothesis proposes that CD8αα TCRαβ IELs ultimately arise from a unique CD8αα-expressing subset of CD4+ CD8αβ+ thymocytes (referred to as triple positive [TP]). According to this scenario, TP thymocytes undergo agonist selection to become DN TCRαβ+ cells, which subsequently leave the thymus and migrate to the gut to become CD8αα TCRαβ IELs under the local influence of interleukin-15 (IL-15).10 Although these conflicting studies appear difficult to reconcile (particularly with respect to the intrathymic differentiation pathway of CD8αα TCRαβ IELs), it should be noted that they are based on rather complex experimental protocols involving intrathymic injections and transplantsations of thymocytes or whole thymi into immunodeficient recipients. Thus, there is a need for alternative approaches to investigate the origin and developmental pathway of CD8αα IELs.

Originally defined as an oncogene, c-Myc is a transcription factor that can play a role in proliferation, survival, apoptosis, or malignant transformation of diverse cell types depending on the tissue or context.11,12 In the thymus, early c-Myc inactivation in immature DN thymocytes leads to a severe block in T-cell development because of inhibition of pre–TCR–induced proliferation.13,14 In contrast, later inactivation of c-Myc in CD4+ CD8− (DP) thymocytes has little effect on the subsequent development of conventional T cells. Interestingly, however, the development of several unconventional T-cell subsets, including Vα14i NKT cells and peripheral CD8 T cells with a memory phenotype (CD44[high] CD122+), is selectively impaired when c-Myc is inactivated at the DP thymocyte stage.15,16
Because CD8αα IELs share several properties with other unconventional T-cell subsets, such as Vα14i NKT cells and memory phenotype CD8 T cells (including an activated phenotype and dependence on IL-15 for development and/or survival), we investigated the role of c-Myc in CD8αα IEL development. Our data demonstrate that CD8αα TCRαβ (but not CD8αα TCRγδ) IELs are selectively decreased when c-Myc is inactivated at the DP thymocyte stage, probably because of a requirement for c-Myc in IL-15–dependent survival of these cells. The implications of these findings for current models of CD8αα IELs development will be discussed.

Methods

Mice

CD4-Cre c-myc<sup>flx/flx</sup> (c-myc<sup>CD4</sup>) mice have been previously described. 16 CD4-Cre<sup>+</sup> c-myc<sup>flx/flx</sup> littermates were used as wild-type (WT) control mice. Transgenic mice expressing human BCL-2 under the control of the H2K promoter have been previously described. 17 Rag2<sup>−/−</sup>γc<sup>−/−</sup> mice were purchased from Taconic Farms. c-myc<sup>N/N</sup> mice 18 were kindly provided by Dr. Frederick W. Alt (Boston, MA). IL15<sup>−/−</sup> mice 19 were originally provided by Immunex. All mice are C57BL/6 background. Control C57BL/6 mice for the IL15<sup>−/−</sup> analysis were obtained from Harlan. Six- to 8-week-old mice were used in all experiments. All animal experiments were conducted under the authorization and with approval of the review board of the Veterinary Service from Canton de Vaud, Lausanne, Switzerland.

Cell preparations

Thymocyte and spleen cell suspensions were prepared by grinding the organs through mesh filters. CD8-depleted thymocytes were prepared as previously described. 16 IELs were isolated as previously described. 20 Briefly, Peyer patches were excised, and the small intestine was opened longitudinally and cut into 1-cm-long pieces. Then the specimens were washed twice in phosphate-buffered saline (PBS) containing 100 U/mL penicillin and 100 µg/mL streptomycin. The pieces were then stirred at 37°C in prewarmed Dulbecco modified Eagle medium containing 100 U/mL penicillin, 100 µg/mL streptomycin, and 5% fetal calf serum for 30 minutes. The supernatants were separated on a 40% to 70% Percoll density gradient (GE Healthcare). The cells that layered between the 40% and 70% fractions were collected as IELs.

Antibodies and flow cytometry

The following monoclonal antibody (mAb) conjugates were used: TCRγδ (GL-3)-fluorescein isothiocyanate (FITC) and -phycoerythrin (PE); TCRβ (H57)-PE-Cy5, -Alexa647 and -allophycocyanin (APC)-Alexa750; CD4 (GK1.5)-FITC, and -PE-Cy7; CD8α (53.6.7)-PE, -PE-Cy7, -Alexa647, and -APC-Alexa750; CD8β (H55)-PE and -Alexa647; CD44 (IM781)-PE-Cy7 and -APC-Alexa750; CD62L (Mel-14)-FITC; CD122 (IL-2Rβ, TM-β1)-PE and -biotin; CD132 (γc, 4G3(biotin); CD161 (PK136)-peridinin chlorophyll protein-Cy5.5; B220 (RA3.6B2)-PE-Cy7; IL-15Rα-biotin. All FITC conjugates were prepared in our laboratory using standard protocols. Alexa 647 and CD8α PE conjugates were also prepared in our laboratory using Alexa 647 protein labeling kit (Invitrogen) and the Prozyme PE conjugation kit (Europa Bioproducts), respectively. CD161 was obtained from BD Biosciences. IL-15Rα (BAF551) mAb was purchased from R&D Systems. CD122 and CD132 were purchased from BD Biosciences. The remaining mAbs were purchased from eBioscience. For revealing biotin-conjugates, PE-Streptavidin (eBioscience) was used. CD8α-expressing thymocytes were detected with PE-labeled TL-tetramer 21 (kindly provided by Dr. H. Chmourat, La Jolla Institute for Allergy and Immunology, San Diego, CA), which was added before other mAbs used in the staining. TL-tetramer was detected with PE-Streptavidin from Invitrogen.

Statistical analysis was performed using the Student t test.

Results

Selectively reduced numbers of CD8αα TCRαβ IELs in c-myc<sup>CD4</sup> mice

We have previously reported that inactivation of c-Myc at the DP stage of thymocyte development in c-myc<sup>CD4</sup> mice does...
not affect further development of conventional CD4+ and CD8+ mature thymocytes or peripheral T cells. However, unconventional T-cell subsets, such as Vα14i NKT cells and memory phenotype (CD45RBhi CD122+) CD8+ T cells, were selectively decreased in c-mycACD4 mice. Because the CD8α TCRβ subset of IEL shares several properties with these unconventional T cells (including an activated phenotype and dependence on IL-15 for development), we decided to investigate IEL development in c-mycACD4 mice. As shown in Figure 1A, TCRβ IEL development was impaired in c-mycACD4 mice, whereas TCRγδ IELs were present in normal (or even slightly increased) numbers. Further analysis of TCRβ IELs in c-mycACD4 mice revealed that the unconventional CD8α subset was dramatically (~20-fold) reduced, whereas the CD8β subset was only slightly (~3-fold) decreased and the CD4 subset was normal (Figure 1A). These data thus indicate a critical and selective role for c-Myc in CD8α TCRβ IEL development. Interestingly, quantitative PCR analysis of c-myc transcripts in residual CD8α TCRβ IELs in c-mycACD4 mice revealed only a 2-fold decrease compared with WT controls (Figure 1B), suggesting that these may represent rare cells that have survived by escaping c-myc deletion on one allele.

**Normal numbers of putative thymic precursors of CD8α TCRβ IELs in c-mycACD4 mice**

Recent evidence suggests that CD8α TCRβ IELs are derived from thymic precursors by a process involving antigen selection. According to this model, TP thymocytes expressing CD8α homodimers (which can be detected by staining with TL tetramers) are the preselection precursors of CD8α TCRβ IELs. After antigen selection, TP thymocytes differentiate to become DN NK1.1− TCRβ+ (DN TCRβ+) thymocytes, which can give rise to CD8α TCRβ IELs when transferred into RAG2−/− mice.10 We therefore investigated whether either of these putative IEL precursor populations was affected in c-mycACD4 mice. As shown in Figure 2, both TP thymocytes (Figure 2A) and DN TCRβ+ thymocytes (Figure 2B) were present in normal frequencies and absolute numbers in c-mycACD4 mice.

**Impaired IL-15–dependent differentiation of DN TCRβ+ thymic precursors of CD8α TCRβ IELs in c-mycACD4 mice**

The final maturation of CD8α TCRβ IELs, including the induction of CD8α expression, occurs only after thymus export in the IL-15–rich environment of the gut. Because intrathymic precursor numbers appeared normal in c-mycACD4 mice, we therefore asked whether c-Myc affects postthymic CD8α TCRβ IEL differentiation. To this end, we transferred sorted DN TCRβ+ thymocytes from WT or c-mycACD4 mice to Rag2−/−γc−/− mice and analyzed the IEL subsets of recipients 1 month later. In contrast to WT, transferred cells from c-mycACD4 mice could not give rise to CD8α TCRβ IELs (Figure 3A). Because IL-15 is important for the final maturation of CD8α TCRβ IELs in the gut,25,26 we examined the expression of IL-15Rα, β, and γ chains on DN TCRβ+ thymocytes in c-mycACD4 mice. Compared with control mice, the expression of IL-15Rα, β, and γ chains was decreased significantly in DN TCRβ+ thymocytes from c-mycACD4 mice (Figure 3B), which might result in reduced sensitivity of these cells to IL-15 signaling. To test this hypothesis directly, we cultured sorted DN TCRβ+ thymocytes from WT or c-mycACD4 mice with recombinant IL-15 for 6 days. The results showed that DN TCRβ+ thymocytes from WT mice can give rise to CD8α progeny in the presence of IL-15, whereas DN TCRβ+ thymocytes from c-mycACD4 mice cannot (Figure 3C). Taken together, these results suggest that the lower expression of IL-15R components on DN TCRβ+ thymocytes from c-mycACD4 mice might be responsible for the impaired postthymic maturation of CD8α TCRβ IELs.

**c-Myc acts upstream of IL-15 signaling in DN TCRβ+ thymocytes**

The reduced expression of IL-15R components and impaired IL-15 responsiveness in vitro of c-Myc–deficient DN TCRβ+ thymocytes suggest that c-Myc may be acting upstream of IL-15 signaling to inhibit postthymic maturation of these cells into CD8α TCRβ IELs. Alternatively, it remains theoretically possible that c-Myc acts downstream of IL-15 signaling during CD8α TCRβ IEL development, as suggested previously for
memory CD8 T-cell development. To accommodate this latter scenario, one must hypothesize that interference with downstream IL-15 signaling in the absence of c-Myc would initiate a homeostatic feedback loop that ultimately results in downregulation of expression of IL-15R components on DN TCRαβ+ thymocytes. To distinguish between these possibilities, we examined IL-15R expression in DN TCRαβ+ thymocytes from IL-15−/− mice, which have impaired CD8αα TCRαβ IEL development because of lack of IL-15 signaling but no defect in c-Myc. As shown in Figure 3D, DN TCRαβ+ thymocytes were present in normal numbers in IL-15−/− mice and expressed similar levels of IL-15Rα, β, and γ chains as control mice, despite the absence of IL-15 signaling. These data exclude an indirect role for c-Myc in regulating IL-15R expression via a putative homeostatic IL-15-dependent feedback loop and thus support the more straightforward hypothesis that c-Myc acts upstream of IL-15R components in DN TCRαβ+ thymic precursors to regulate IL-15 signaling during CD8αα TCRαβ IEL development.
with this, CD8αα TCRαβ IELs in c-myc<sup>CD<sub>4</sub></sup> mice showed poorer proliferation and higher apoptosis (Figure 4B-C). These results suggest that c-Myc affects postthymic differentiation of CD8αα TCRαβ IELs by regulating IL-15-dependent proliferation and/or survival.

**Figure 4. c-Myc deficiency affects IL-15R expression, proliferation, and survival of CD8αα TCRαβ IELs.** (A) Expression of IL-15R chains on CD8αα and CD8αβ TCRαβ IEL subsets from WT and c-myc<sup>CD<sub>4</sub></sup> mice. The number in each histogram corresponds to MFI of the IL-15R staining (empty histogram) minus MFI of the unstained control (gray filled histogram) (mean ± SD; n = 4). (B) Cell-cycle analysis of TCRαβ IELs in WT and c-myc<sup>CD<sub>4</sub></sup> mice. Dot plots show Ki67 versus Hoechst DNA content. (C) Bar graph represents the percentage of apoptotic annexin V<sup>+</sup> cells in the indicated populations from WT and c-myc<sup>CD<sub>4</sub></sup> mice (mean ± SD; n = 6). *Statistically significant difference (P < .05).

**Figure 5. CD8αα TCRαβ IEL development in c-myc<sup>CD<sub>4</sub></sup> mice is partially restored by enforced BCL-2 expression.** (A) Intracranial mouse Bcl-2 expression in the indicated populations from WT and c-myc<sup>CD<sub>4</sub></sup> mice. Dashed histogram represents the staining with IgG control. Bar graph represents MFI of mouse Bcl-2 staining (mean ± SD; n = 5). *Statistically significant difference (P < .05). (B) Intracranial mouse Bcl-2 or human BCL-2 expression in CD8αα TCRαβ IELs from WT, BCL-2 Tg, c-myc<sup>CD<sub>4</sub></sup>, and c-myc<sup>CD<sub>4</sub></sup> BCL-2 Tg mice. The number in each histogram indicates the MFI of Bcl-2 staining. (C) Bar graph represents the absolute number of cells in the indicated IEL subsets in WT, BCL-2 Tg, c-myc<sup>CD<sub>4</sub></sup>, and c-myc<sup>CD<sub>4</sub></sup> BCL-2 Tg mice (mean ± SD; n = 5). *Statistically significant difference (P < .05). (D) Thymic V<sub>α</sub>14i NKT cells and memory phenotype CD8 splenic T cells in WT, BCL-2 Tg, c-myc<sup>CD<sub>4</sub></sup>, and c-myc<sup>CD<sub>4</sub></sup> BCL-2 Tg mice. CD8αα-depleted thymocytes were stained with TCRαβ and CD1d-dimer. Percentage of V<sub>α</sub>14i NKT cells (TCRαβ<sup>+</sup> CD1d-dimer<sup>+</sup>) is indicated in the upper dot plots. Total spleen cell suspensions were stained with CD8αα, CD4<sub>2</sub>L, CD122<sup>+</sup>, and CD44. Lower dot plots represent CD122 versus CD44 profile of the CD8αα<sup>+</sup> CD4<sub>2</sub>L<sup>+</sup> splenic T cells. The percentage of memory phenotype T cells (CD122<sup>+</sup> CD44<sup>+</sup>) is indicated. Data are representative of 3 independent experiments.
CD8α TCRβ IELs in c-myc^ΔCD4 mice are partially rescued by enforced BCL-2 expression

Because CD8α TCRβ IELs in c-myc^ΔCD4 mice preferentially underwent apoptosis, we examined the expression of the antiapoptotic protein Bcl-2 in these cells. Indeed, Bcl-2 levels were significantly reduced in CD8α TCRβ IELs from c-myc^ΔCD4 mice but remained normal in CD8αβ TCRβ IELs as well as in DN TCRβ+ thymocytes (Figure 5A). To directly examine the role of Bcl-2 in IEL development, we introduced a human BCL-2 transgene driven by an H2K promoter into c-myc^ΔCD4 mice. Expression of exogenous human BCL-2 in CD8α TCRβ IELs was comparable in both BCL-2 Tg and c-myc^ΔCD4 BCL-2 Tg mice, and expression levels of endogenous mouse Bcl-2 were not affected by the presence of the transgene (Figure 5B). Importantly, the enforced expression of BCL-2 selectively restored the numbers of CD8α TCRβ IELs in c-myc^ΔCD4 mice (10-fold increase) without rescuing the CD8αβ TCRβ IEL subset (Figure 5C).

The rescue of CD8α TCRβ IELs by BCL-2 in c-myc^ΔCD4 was not the result of restoration of IL-15R expression because levels of all IL-15R chains remained very low on CD8α TCRβ IELs and DN TCRβ+ thymocytes in c-myc^ΔCD4 BCL-2 Tg mice (data not shown). Moreover, the promotion of survival by BCL-2 in c-myc^ΔCD4 mice was restricted to the CD8α TCRβ IEL subset because no rescue of Vα14i NKT cells or memory phenotype (CD44<sup>high</sup> CD122<sup>+</sup>) CD8<sup>+</sup> splenic T cells was observed (Figure 5D).

**N-myc expressed from the c-myc locus can rescue CD8α TCRβ IEL development**

The failure of other Myc family members to rescue CD8α TCRβ IEL development (as well as the development of Vα14i NKT cells and CD44<sup>high</sup> CD122<sup>+</sup> CD8<sup>+</sup> T cells) in c-myc^ΔCD4 mice could be interpreted to mean that c-Myc has a unique function during the development of unconventional T-cell subsets. However, both N-Myc and L-Myc are expressed at extremely low levels in DP thymocytes and mature T cells, which could quantitatively preclude their ability to replace c-Myc in the T-cell lineage. To address this issue directly, we took advantage of the availability of c-myc^ΔNN knock-in mice, which express N-Myc coding sequences from the c-myc locus on both alleles, whereas c-Myc expression is completely disrupted. Previous studies have shown that c-myc^ΔNN mice are viable and that development of conventional T and B cells proceeds normally in these mice. As shown in Figure 6A, c-myc^ΔNN mice are devoid of detectable c-myc transcripts in thymocytes as well as in Vα14i NKT cells and IELs. Moreover, N-myc transcripts are dramatically increased in c-myc^ΔNN mice and exhibit the same pattern of expression as c-myc transcripts in normal mice (c-myc<sup>+/+</sup>; Figure 6A). Importantly, analysis of IELs in c-myc^ΔNN mice and littermate controls demonstrates normal percentages and absolute numbers of all subsets, including CD8α TCRβ IELs (Figure 6B). In addition, Vα14i NKT cells and CD44<sup>high</sup> CD122<sup>+</sup> CD8<sup>+</sup> splenic T cells were present at the same levels in c-myc^ΔNN mice compared with littermate controls (Figure 6C). Together with previous studies, these data demonstrate that c-Myc and N-Myc are potentially functionally redundant during unconventional and conventional T-cell development, provided that they are expressed under the control of the same genetic regulatory elements.

**Discussion**

The data presented here demonstrate that c-Myc plays a critical and selective role in the development of CD8α TCRβ IELs. Inactivation of a floxed c-myc allele in DP thymocytes via a CD4-Cre transgene had little effect on the further development of conventional CD4<sup>+</sup> and CD8<sup>+</sup> mature thymocytes and peripheral T cells. However CD8<sup>+</sup> TCRβ IELs were significantly reduced, largely resulting from a 20-fold reduction in absolute numbers of...
the CD8α TCRαβ subset. Moreover, residual CD8α TCRαβ IELs in c-mycACD4 mice displayed reduced proliferation and increased apoptosis, which correlated with decreased expression of IL-15R subunits and significantly lower levels of expression of the antiapoptotic protein Bcl-2. Importantly, transgenic overexpression of human BCL-2 led to a pronounced (~10-fold) increase in the absolute numbers of CD8α TCRαβ IELs in c-mycACD4 mice. Taken together with other studies,25,26 our results support a model in which c-Myc regulates the survival of CD8α TCRαβ IELs in the intestine by controlling IL-15R expression, which in turn regulates levels of intracellular Bcl-2.

In addition to identifying a selective role for c-Myc in CD8α TCRαβ IEL development, our data have interesting implications in light of the current controversy concerning the origin of this unusual cell population. Although CD8α TCRαβ IELs are generally considered to be ultimately of thymic origin (at least in euthymic mice), the precise identification of intrathymic precursors generally considered to be ultimately of thymic origin (at least in unusual cell population. Although CD8α thymocytes of CD8α transgene should occur relatively late in thymus development (10-fold increase in absolute numbers) by exogenous BCL-2 expression, whereas Vo14i NKT and CD44high CD122+ CD8+ T cells are unaffected. The reason for this discrepancy is not clear but could potentially reflect a preferential effect of c-Myc deficiency on survival in the CD8α TCRαβ IEL lineage (which could be rescued by BCL-2) compared with proliferation15 or other functions in Vα14i NKT cells and memory CD44high CD122+ CD8+ peripheral T cells (which would not be rescued by BCL-2).

Analysis of the role of c-Myc in the development of other IL-15-dependent cell subsets, such as CD8α NK1.1+ T cells,20 might help to clarify this issue.

Finally, our data are also of interest with regard to the potential functional redundancy of the Myc protein family. Of the 3 family members (c-Myc, N-Myc, and L-Myc), only c-Myc is expressed at significant levels in the T-cell lineage from the DP thymocyte stage onward,16 perhaps explaining why c-Myc deficiency in unconventional T cells cannot be rescued by N-Myc or L-Myc. Direct evidence in favor of Myc functional redundancy was obtained using knock-in mice that express N-myc from both alleles of the c-myc locus. Despite the total absence of c-Myc in these mice, not only CD8α TCRαβ IELs but also Vo14i NKT cells and CD44high CD122+ CD8+ peripheral T cells were present at comparable levels as in WT littermate controls. Thus, adequate levels of N-Myc are sufficient to substitute for c-Myc in the development of 3 independent lineages of unconventional T cells. In an earlier study, conventional T-cell development was found to be normal in c-mycNN mice, but some subtle defects in T-cell (and B-cell) activation were observed.18 Further studies are thus required to unambiguously determine whether Myc family members function in a “generic” fashion or exhibit unique tissue-specific activities.

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**Authorship**

Contribution: W.J. designed, performed, and analyzed the majority of the experiments of this work; I.F. designed, performed, and analyzed the experiments of real-time PCR and contributed to other experiments; E.L. carried out the breeding of c-mycΔN mice and contributed to the analysis of these mice; A.T. provided critical mice for this study; H.R.M. directed the study, planned experiments, and wrote the manuscript; and W.J. and I.F. helped with the writing and edited the manuscript.

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**References**

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