A Novel Transgenic Mouse Model of the Human Multiple Myeloma Chromosomal Translocation t(14;16)(q32;q23)

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Abstract

Multiple myeloma (MM) is a currently incurable neoplasm of terminally differentiated B cells. The translocation and/or overexpression of c-MAF have been observed in human MM. Although c-MAF might function as an oncogene in human MM, there has been no report thus far describing the direct induction of MM by c-MAF overexpression in vivo. In this study, we have generated transgenic (TG) mice that express c-MAF specifically in the B-cell compartment. Aged c-Maf’ TG mice developed B-cell lymphomas with some clinical features that resembled those of MM, namely, plasma cell expansion and hypergammulinemia. Quantitative RT-PCR analysis demonstrated that Ccnd2 and Igβ7, which are known target genes of c-Maf, were highly expressed in the lymphoma cells. This novel TG mouse model of the human MM t(14;16)(q32;q23) chromosomal translocation should serve to provide new insight into the role of c-MAF in tumorigenesis. Cancer Res; 71(2): 339-48. ©2011 AACR.

Introduction

Multiple myeloma (MM) is an incurable neoplasm of terminally differentiated B cells that is characterized by monoclonal expansion of malignant plasma cells. The common premalignant stage of MM is a monoclonal gammopathy of undetermined significance (MGUS), which progresses to MM at the rate of 1% of patients per year (1). Extensive genomic analysis has revealed that approximately 50% of patients with MGUS and MM have primary translocations in the clonal plasma cells that involve the immunoglobulin heavy chain (IGH) locus on chromosome 14q32 (2). Chromosomal translocations that involve the juxtaposition of the IGH locus with the loci for CCND1 (3), FGFR3 (4), or MMSET (5) result in the overexpression of these genes. It was first reported a decade ago that c-MAF is expressed at high levels in MM cells that carry the translocation t(14;16)(q32;q23), in which the IGH locus is fused with the c-MAF gene locus (6). In addition, c-MAF is overexpressed in 50% of MM cell lines and patients (7). The maf proto-oncogene was identified originally within the genome of the avian musculoaponeurotic fibrosarcoma virus AS42 (8). The product of the Maf gene and other members of the Maf family share a conserved basic-region leucine zipper (bZIP) motif that mediates dimer formation and DNA binding to the Maf recognition element (MARE; ref. 9). Large Maf proteins, such as c-Maf, Mafb, Mafa/L-Maf/Smaf, and Nrl, also contain an acidic domain that mediates transcriptional activation and plays a key role in cellular differentiation (8–12). c-Maf encodes a T-helper cell type 2 (Th2)-specific transcription factor that activates the expression of interleukin (IL)-4 in T cells (13). It is also involved in the regulation of lens fiber cell differentiation (14–16). Recently, we have found that transgenic (TG) mice that express c-Maf specifically in the T-cell compartment develop T-cell lymphoma (17). Furthermore, 60% of human T-cell lymphomas, classified as angioimmunoblastic T-cell lymphomas, were found to overexpress c-MAF (17, 18).

On the basis of these facts, we hypothesized that overexpression of c-Maf in the B-cell lineage would enhance the development of an MM-like disease. In this study, we have generated c-Maf’ TG mice that overexpress c-Maf in the B-cell compartment, using the Igβ promoter and Eμ enhancer. We found that these mice developed B-cell lymphoma, which provided the first direct evidence that c-Maf can function as an oncogene in a murine model of the t(14;16)(q32;q23) translocation.

Materials and Methods

Mice

We made 2 constructs for the Eμ c-Maf transgene (Fig. 1A). For construct-1, we used the Eμ enhancer and the Vh promoter, as described previously (19). A 1.5-kb full-length cDNA that encoded the murine c-Maf protein was inserted into a vector that contained the Vh promoter and Eμ enhancer. Construct-1 was injected into fertilized eggs from BDF1 mice to generate TG mice and then backcrossed 4 times into...
C57BL/6J. For construct-2, the VH promoter, Eμ enhancer, and 3′Ex enhancer were used. Construct-2 was injected into fertilized eggs from C57BL/6J mice to generate TG mice. Mice were maintained with a C57BL/6J genetic background in specific pathogen-free conditions in a Laboratory Animal Resource Center. All experiments were carried out according to the Guide for the Care and Use of Laboratory Animals at the University of Tsukuba.

Figure 1. Generation of mice that overexpress c-Maf. A, constructs of the Eμ c-Maf transgene. The c-Maf cDNA was inserted into a vector that contained the VH gene promoter (Vp), and an Eμ enhancer (Eμ), or one that contained Vp, Eμ, and a 3′Ex enhancer (Ex). The probe for Southern blot analysis, the restriction enzyme sites (EcoRI; E, and HindIII; H), and the predicted sizes of the endogenous gene and the transgene are indicated. Transgene construct-1 was used for TG line 524, whereas TG lines 99 and 68 were generated with construct-2. B, a, Southern blot analysis of the endogenous and c-Maf TG genes in Eμ c-Maf TG mice. The 4.0-kb endogenous and 1.3-kb TG gene fragments are shown for TG mice. B, b, analysis of c-Maf mRNA in B cells. The amount of c-Maf mRNA in the samples from TG mice was higher than that in a sample from WT mice. B, c, Western blot analysis of splenic B220⁺ B cells from Eμ c-Maf TG mice (524, 99, and 68) and WT mice. C, immunofluorescence analysis of splenocyte subpopulations from Eμ c-Maf TG mice at 20 weeks of age. The total numbers of T cells (CD3⁺) and B cells (B220⁺) did not differ between the spleens of WT and Eμ c-Maf TG mice. There were no apparent differences in the subpopulations of B cells, namely, follicular cells (B220⁺ CD23ʰ̇CD21⁻) and marginal zone (MZ) B cells (B220⁺ CD23⁻ CD21ʰ̇). D, proliferative potential of B cells after treatment with LPS. Enriched splenic B220⁺ cell populations obtained from WT and Eμ c-Maf TG mice at 20 weeks of age were cultured in the presence of LPS, and proliferation was assayed by using a CellTiter 96 AQueous One Solution Cell Proliferation Assay. The mean absorbance at 490 nm is presented.
Southern hybridization analysis
High-molecular-weight DNA was prepared from the tail of each mouse, and 10 μg of this DNA was digested with EcoRI and HindIII and then Southern hybridization was performed, as described previously (17). To investigate Igh gene rearrangement, DNA from the tail and other tissues was examined by Southern hybridization. DNA that had been digested with EcoRI was hybridized with the JH4 fragment, as described previously (20).

Isolation of mouse B220<sup>+</sup> cells and reverse transcriptase PCR to analyze transgene expression
B220<sup>+</sup> splenic B cells were isolated using magnetic microbeads from Miltenyi Biotec. Total RNA was prepared from the B220<sup>+</sup> splenic B cells of 10 weeks old TG mice or their wild-type (WT) littermates using TRIzol reagent according to the manufacturer’s instructions (Invitrogen). Total RNA (1 μg) was reverse transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen), and 1 μL of this 20 μL reaction mixture was used for PCR. The c-Maf sequence was amplified using the primers 5′-CTGCCGGCTTCAAGAGGGTGTCAGC-3′ and 5′-TCCTGGTCTACACTCACATG-3′, which yielded a 225-bp product. The sequence of Hprt (hypoxanthine-guanine phosphoribosyl transferase), which was used as a control, was amplified using the primers 5′-CAAACTTTGCTTTCCCTGGT-3′ and 5′-CAAGTGACCCATATCCAAACA-3′, which yielded a 250-bp product.

Western blot analysis
Nuclear extracts were prepared from the B220<sup>+</sup> splenic B cells of 10 weeks old TG or WT mice. The extracts were fractionated by size on a 10% SDS-polyacrylamide gel, transferred to a polyvinylidene difluoride membrane (Fluoro Trans; Pall BioSupport Division), and incubated with primary and secondary antibodies. To detect the c-Maf protein, a rabbit antibody against mouse c-Maf was used as the primary antibody and peroxidase-conjugated goat anti-rabbit IgG (Zymed Laboratories) was used as the secondary antibody. The anti-c-Maf antibody was kindly provided by Dr Masaharu Sakai (University of Hokkaido, Sapporo, Japan). To normalize the results with respect to the amount of protein in each sample, a goat antibody against mouse Lamin B (Santa Cruz Biochemicals) was used.

Flow cytometric analysis
Single-cell suspensions were prepared from the spleen and the bone marrow of each mouse. Multicolor flow cytometric analysis was performed using an LSR Flow Cytometer and CellQuest software (Becton Dickinson). The phycoerythrin (PE)-labeled rat antibodies against mouse CD3, CD138, CD23, and IgD, the FITC-labeled rat antibodies against mouse CD21, CD220, and IgM, and APC-labeled rat antibody against mouse B220 were obtained from BD Pharmingen. After 16 hours of stimulation, the viable cell number was determined using a CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega). Aliquots of 40 μL of CellTiter 96 AQueous One Solution were added to each well and incubated for an additional 3 hours before the final absorbance at 490 nm was determined.

Measurement of serum immunoglobulins, paraprotein determination, and X-ray analysis
The amounts of IgG, IgM, and IgA were determined by ELISA, as described previously (21). Paraproteins (M spikes, extra gradients) were determined using cellulose acetate electrophoresis methods by SRL, Inc. X-ray analysis of bones was carried out using a Faxitron X-ray Specimen Radiographic System and Kodak X-OMAT-TL film.

Histopathologic analysis
Each mouse was bled under ether anesthesia. At autopsy, organs were fixed with 10% formalin in 0.01 mol/L phosphate buffer (pH 7.2) and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) stain for histopathologic examination by light microscopy. For dual immunofluorescent staining, the rabbit antibody against mouse c-Maf, FITC-labeled goat anti-rabbit IgG (green), and PE-labeled rat anti-mouse CD138 antibody (red) were used. The anti-CD138 and anti-B220 antibodies were purchased from BD Pharmingen. The immunohistochemical analysis of CD138 and anti-B220 were performed by the avidin-biotin-peroxidase complex staining technique. For immunofluorescent analysis, frozen sections were stained with FITC-labeled goat antibodies against mouse immunoglobulins IgG, IgM, IgA, κ light chain, and λ light chain (ICN Pharmaceuticals).

Quantitative reverse transcriptase PCR
Total RNA (1 μg) was reverse transcribed into cDNA. Each reaction was done in duplicate. The quantity of cDNA in each sample was normalized to the amount of hprt cDNA. For the PCR, we used SYBR Premix Ex Taq II (TAKARA Bio) according to the manufacturer’s instructions. The amplification was carried out in a Thermal Cycler Dice Real Time System (TAKARA Bio). The following primer pairs were used: c-Maf forward: 5′-CTGCCGGCTTCAAGAGGGTGACG-3′ and c-Maf reverse: 5′-GATCTCTGCTGAGTGTC-3′; Ccdn2 forward: 5′-CTCTTTCTTCAAACACTTTTGTGTC-3′ and Ccdn2 reverse: 5′-CTACCTCCGACGTTCCTTCT-3′; Itgb7 forward: 5′-CAACCTGGAGCTACCAAAGAAG-3′ and Itgb7 reverse: 5′-CTACCTCCCAGTGTTTCCTT-3′; Prdm1 forward: 5′-AAGCCGAGGCATCTTACC-3′ and Prdm1 reverse: 5′-GGCCATGCAGGTGATTCC-3′; Xbp1 forward: 5′-GATTTCCTTTGGGAGTC-3′ and Xbp1 reverse: 5′-CTGCCGCTTCAAGAGGGTGCAGC-3′; and Xbp1 forward: 5′-CAGCGTGTCC-3′ and Xbp1 reverse: 5′-GGAGCAGL light chain, and λ light chain (ICN Pharmaceuticals).

Cell culture of B cells
B220<sup>+</sup> cells (2 × 10<sup>5</sup>) were plated in triplicate on 96-well plates before stimulation with lipopolysaccharide (LPS; 10 μg/mL; Sigma) and anti-mouse CD40 (1 μg/mL; BD Pharmingen). After 16 hours of stimulation, the viable cell number was determined using a CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega). Aliquots of 40 μL of CellTiter 96 AQueous One Solution were added to each well and incubated for an additional 3 hours before the final absorbance at 490 nm was determined.

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forward: 5’-TTGTGTGGATATGCCTTGACTA-3’ and Hprt reverse: 5’-AGGCCATGGGCCCACAGGACTA-3’.

Statistical analysis
Results were expressed as the mean ± SE. Data were compared by 1-way ANOVA and the Bonferroni correction was applied routinely. Significant differences between the groups of mice were analyzed using the Wilcoxon test for paired samples, and values of P < 0.05 were considered statistically significant. Survival rates were compared using the Kaplan–Meier method.

Results
Generation of Eμ c-Maf TG mice that express c-Maf specifically in B cells
To generate TG mice that express high levels of c-Maf specifically in B cells, the murine c-Maf coding sequence was inserted into a vector that contained the Vμ gene promoter, an Eμ enhancer sequence, and a 3’Ex enhancer sequence (Fig. 1A). We generated 2 constructs (the first did not contain the 3’Ex enhancer) and obtained 17 F0 TG mice with a BDF1 or C57BL/6J genetic background. Subsequently, 3 TG mouse lines with a C57BL/6J genetic background were generated and screened by PCR, using tail DNA as the template. Genomic DNA was analyzed by Southern blotting to confirm the integrity and copy number of the transgene in each TG line. The EcoR1/HindIII fragment that contained the c-Maf transgene was 1.3 kb in length, whereas the corresponding fragment for the endogenous c-Maf gene was 4.0 kb. The results of densitometric analyses revealed that TG line 524, which was generated using construct-1, contained approximately 4 copies of the transgene, whereas TG lines 99 and 68, which were generated with construct-2, both contained approximately 2 copies (Fig. 1B, a). These transgenes were stably transmitted to the progeny.

Overexpression of c-Maf in TG mice
To confirm that the transgene was expressed, the levels of c-Maf mRNA and protein in splenic B cells from the TG lines were monitored by reverse transcriptase PCR (RT-PCR) and Western blotting (Fig. 1B, b and c). The c-Maf mRNA was overexpressed in all the TG mice that were tested (Fig. 1B, b). Correspondingly, Western blot analysis of B220⁺ splenic B cells confirmed that the levels of c-Maf protein were elevated (Fig. 1B, c). c-Maf protein was not detected in WT B cells in this analysis.

B-cell development and proliferative potential in Eμ c-Maf TG mice
To investigate the effect of c-Maf overexpression on B-cell differentiation, we carried out flow cytometric analysis of splenocytes isolated from TG mice at 20 weeks of age. Flow cytometry showed that the total numbers of T cells (CD3⁺) and B cells (B220⁺) in the spleens of Eμ c-Maf TG mice were unaltered relative to WT mice (Fig. 1C). In addition, we could not find any apparent differences in the subpopulations of B cells, namely, follicular cells (B220⁺CD23loCD21⁻) and marginal zone (MZ) B cells (B220⁺CD23⁺CD21hi; Fig. 1C). Next, we analyzed the proliferative potential of WT and Eμ c-Maf TG B cells in vitro. The proliferative potential of Eμ c-Maf TG B cells was relatively high, but there was no substantial difference in the response to LPS (Fig. 1D) or CD40 (data not shown) between the two groups.

Aged Eμ c-Maf TG mice developed lymphoma
The Eμ c-Maf TG mice appeared healthy up to 50 weeks of age. However, they subsequently developed tumors. The mean age at diagnosis as lymphoma by histologic analysis was 80.1 weeks. We confirmed that 28% (18/64) of the Eμ c-Maf TG mice developed lymphoma (Fig. 2A). We could not detect any apparent difference in survival between the mice that were generated with the two different constructs, construct-1 for TG524 and construct-2 for TG 99 and 68.

Lymphomas observed in Eμ c-Maf TG mice were of B-cell origin
Eμ c-Maf TG mice appeared healthy up to 50 weeks of age, but they developed tumors subsequently. We performed histologic analysis to identify the origin of the lymphoma cells. The spleens of the Eμ c-Maf TG mice were enlarged approximately 2- to 5-fold relative to those of the WT mice. The spleens of the mice with lymphoma were infiltrated by numerous cells, which partially destroyed the normal tissue architecture (Fig. 2B). Cells that had infiltrated showed plasma cell-like morphology (Fig. 2B). Moreover, immunohistochemical analysis revealed that the infiltrating cells were B220⁺ (Fig. 2C) and also expressed CD138 (Fig. 2C). We confirmed that the infiltrating cells were positive for both CD138 and c-Maf by immunofluorescence staining (Fig. 2C). To investigate the characteristics of the lymphoma cells, we performed flow cytometry (Fig. 2C and D). We found the number of B220⁺CD138⁺CD21⁺IgM⁺IgD⁻ cells was increased in the TG mice relative to the WT mice. We also found that the number of mature plasma cells (B220⁺CD23⁻/CD138⁻) was increased in the TG mice (Fig. 2C).

Hypergammaglobulinemia in aged Eμ c-Maf TG mice
Approximately 50% (7/14) of the Eμ c-Maf TG mice of line 524 showed a clonal M spike in the serum between 20 and 50 weeks of age (Fig. 3A). At 120 weeks old, 35% (5/14) of these TG mice had developed lymphoma. The remaining mice that showed a clonal M spike (2/14) showed no evidence of lymphoma at 120 weeks. The plasma levels of both IgG and IgM, as measured by ELISA, were increased significantly in aged Eμ c-Maf TG mice (Fig. 3B). The mean amount of total IgG in the TG mice was 1,760 ± 302 (mg/dL), whereas in the control mice this value was 934 ± 142 (mg/dL). In addition, the mean amount of total IgM in the TG mice was 564 ± 92 (mg/dL), whereas in the control mice this value was 302 ± 223 (mg/dL). However, there was no obvious elevation of total IgA in the TG mice. It must be noted that only 1 of the 14 mice showed high levels of both IgG and IgM (Fig. 3B). Notably, these changes in the serum were associated
with increased numbers of mature plasma cells (B220lowCD138+) in the bone marrow; the proportion of plasma cells as a percentage of the total number of bone marrow cells was about 5% to 20% in the TG mice compared with less than 5% in the non-TG controls (Fig. 3C). We also found the number of B220+CD138+ cells in the bone marrow was increased (Fig. 3C). However, we could not identify any obvious osteolytic lesions by X-ray analysis (Fig. 3C).

**Eμ-c-Maf** TG mice showed clinical features that resembled those of patients with plasma cell disorders

In **Eμ-c-Maf** TG mice with hyperglobulinemia that were older than 60 weeks, renal tubular casts and tubular obstruction were observed (Fig. 4A), which resembled human myeloma kidney (so-called cast nephropathy; ref. 22). Moreover, glomerular changes that were characterized by mesangial widening due to the deposition of PAS-positive material were observed in aged **Eμ-c-Maf** TG mice (Fig. 4A); this resembled monoclonal immunoglobulin deposition disease (22). These renal lesions were similar to the pathologic manifestations that are present in human MM and other plasma cell disorders in which the systemic chronic overproduction of immunoglobulins and the accumulation of light chains, paraproteins, and other immunoglobulin fragments are observed (22). The depositions contained immunoglobulin light and heavy chains, which consisted of either polyclonal heavy and light chains or clonal IgG or IgM heavy chains and κ light chains (Fig. 4B).
Autonomous proliferation in nude mice of lymphoma cells from \( E\text{\textmu} \) c-Maf TG mice

To ascertain whether the infiltrating tumor cells in \( E\text{\textmu} \) c-Maf TG mice could proliferate autonomously, we isolated mononuclear cells from the spleens of these mice and injected the isolated cells into nude mice via the tail vein (10^6 cells per mouse). Recipient mice displayed prominent hepatomegaly, splenomegaly, and/or enlarged lymph nodes throughout their bodies within 12 weeks of transplantation. A typical example is shown in Figure 5A. The tumor cells could also be transplanted into syngeneic C57BL/6J mice. To assess clonality, we examined the rearrangement of the Igh locus in the c-Maf TG lymphoma cells. Southern blot analysis was carried out using a murine \( JH4 \) probe (Fig. 5B). We extracted DNA from the tails and spleens of the mice. Monoclonal rearrangement of the Igh locus was found in DNA that had been isolated from the spleens of the \( E\text{\textmu} \) c-Maf TG mice (Fig. 5B, lanes 3–6). Histologic analysis of the spleen from these recipient mice revealed that the tumor cells showed a plasma cell-like morphology, that is, the same as the original lymphoma cells (Fig. 5C).

Expression of c-Maf target genes in \( E\text{\textmu} \) c-Maf TG mice

To gain insight into the molecular mechanism by which plasma cell dyscrasia develops, we measured the level of
mRNA expression from c-Maf and its target genes in Em c-Maf TG mice, using a quantitative RT-PCR assay (Fig. 6). In human MM, CCND2 and ITGB7 are known to be targets of c-MAF (7, 23). As we have described previously, deregulated expression of Ccnd2 and Itgb7 is also observed in the T-cell lymphomas of TG mice that overexpress c-Maf in the T-lymphoid compartment (17). Therefore, we analyzed the expression of Ccnd2 and Itgb7 in Em c-MafTG mice. The level of c-Maf expression in the lymphoma-infiltrated lymph nodes of Em c-MafTG mice was 3- to 5-fold greater than the level in WT B cells (B220+; Fig. 6). We confirmed the percentage of B220+ cells in the lymph nodes was more than 70%. We also found that the expression of both Ccnd2 and Itgb7 was elevated in Em c-Maf TG mice (Fig. 6), which was consistent with the results of previous reports in human MM (7).

Next, we examined the levels of the B-cell–associated transcription factors, Prdm1 (Blimp-1), Xbp1, and Pax5. Blimp-1 is a transcriptional repressor that plays a critical role in the terminal differentiation of B cells into antibody-secreting plasma cells (24). Overexpression of Blimp-1 has been shown to drive plasma cell differentiation (24–26). Xbp1 is a bZIP transcription factor and a major regulator of plasma cell differentiation (27). It is known that Em Xbp1s mice develop MM/MGUS (28). In addition, Pax5 is known to inhibit plasmacytic development and also to repress the expression of Xbp1, which is needed for plasma cell formation and immunoglobulin secretion (29). By RT-PCR analysis (Fig. 6), we found that Prdm1 and Xbp1 expression were increased but Pax5 expression was decreased in the TG mice as compared with the control mice, which might correlate with the plasma cell differentiation.

It is known that the dysregulation of MYC (c-MYC) expression can induce the progression of MGUS to MM (1). In addition, it has been reported that myeloma cells are completely dependent on the transcription factor IRF4 (interferon regulatory factor 4), despite that fact that most myeloma cells
do not harbor mutations, translocations, or amplifications of the IRF4 locus (30). Moreover, IRF4 itself is a direct target of MYC that generates an autoregulatory circuit in myeloma cells (30). However, neither Myc nor Irf4 expression was elevated in the Eμ c-Maf TG mice. We also analyzed Spp1 (osteopontin) expression, because we could not find any obvious osteolytic lesions in the Eμ c-Maf TG mice. SPP1, which is highly expressed in MM, plays a critical role in bone disease by protecting the bone from destruction (31). We observed that the expression of Spp1 was upregulated in Eμ c-Maf TG mice (Fig. 6).

Discussion

It is important to emphasize that v-maf is a classical oncogene that was identified in an avian transforming virus, AS42 (8). Large Maf proteins, such as c-Maf, Mafb and Mafa, can efficiently transform primary fibroblasts in vitro (9, 32–34). Previously, we generated c-Maf TG mice that overexpress c-Maf in the T-cell compartment, using the VA vector that contains the human CD2 promoter and locus control region. The V4 c-Maf TG mice developed T-cell lymphoma, thus providing evidence that c-Maf can function as an oncogene in T cells in vivo (17). Work over the last decade has provided evidence that c-MAF might play crucial roles in the pathogenesis of MM. It has been reported that the translocation and/or overexpression of c-MAF are identified frequently in human MM (6, 7, 23). Translocations that involve MAFA (23, 35) or MAFB [t(14;20)(q32;q12); refs. 35, 36], which encode other large MAF proteins, have also been identified. Altogether, translocations that involve a large MAF transcription factor are found in 8% to 10% of MMs: c-MAF is translocated in 5%, MAFB in 2%, and MAF4 in less than 1% of cases (23, 35). Although c-MAF translocations are observed in only 5% to 10% of MMs, c-MAF is overexpressed in 50% of cases (7, 23).

In this study, we demonstrated that aged c-Maf TG mice developed B-cell lymphomas with some clinical features that resembled those of human MM, namely, expansion of plasma cells, hyperglobulinemia, and renal involvement. Although we found that plasma cells (B220lowCD138þ) were increased in the spleen and bone marrow of the TG mice, the lymphoma cells in the spleens were B220 þ CD138 þ CD21 þ CD23 þ IgM−/IgD−. The phenotype of some Eμ c-Maf TG mice partially resembled that of types of B-cell lymphoma that contain plasmablasts and/or plasma cells rather than plasma cell dyscrasias, such as Waldenström’s macroglobulinemia (WM; also known as lymphoplasmacytic lymphoma) or MALT (mucosa-associated lymphoid tissue) lymphoma. WM is classified as an indolent form of B-cell lymphoma. Infiltration with
plasma cell (CD138)

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lymph nodes from real-time RT-PCR analysis. Total RNA was obtained from the enlarged expression profiles of

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