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 hyperglobulinemia. Quantitative RT-PCR analysis demonstrated that Ccnd2 and Ilg7, 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.

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 gammapathy 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 IgH 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 Vμ 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 Vμ promoter and Eμ enhancer. Construct-1 was injected into fertilized eggs from BDF1 mice to generate TG mice and then backcrossed 4 times into
For construct-2, the \( V_H \) promoter, \( E_m \) 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 \( E_m \) c-Maf transgene. The c-Maf cDNA was inserted into a vector that contained the \( V_H \) gene promoter (\( Vp \)), and an \( E_m \) enhancer (\( E_m \)), or one that contained \( Vp \), \( E_m \), and a \( 3'Ex \) enhancer (\( E_x \)). 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 \( E_m \) 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 \( E_m \) c-Maf TG mice (524, 99, and 68) and WT mice. C, immunofluorescence analysis of splenocyte subpopulations from \( E_m \) 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 \( E_m \) c-Maf TG mice. There were no apparent differences in the subpopulations of B cells, namely, follicular cells (B220\(^+\)CD23\(^{high}\)CD21\(^{low}\)) and marginal zone (MZ) B cells (B220\(^+\)CD23\(^{-}\)CD21\(^{high}\)). D, proliferative potential of B cells after treatment with LPS. Enriched splenic B220\(^+\) cell populations obtained from WT and \( E_m \) 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.

C57BL/6J. For construct-2, the \( V_H \) promoter, \( E_m \) 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.
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 IJH4 fragment, as described previously (20).

Isolation of mouse B220+ cells and reverse transcriptase PCR to analyze transgene expression

B220+ splenic B cells were isolated using magnetic microbeads from Miltenyi Biotec. Total RNA was prepared from the B220+ splenic cells of 10 weeks old TG or WT mice, and 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'-CTGCCGGCTTCAA-GAGGGTG-3' and 5'-TCCGGTTCACAATCACAT-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'-CAAACTTTGCTTTCCCTG-3' and 5'-CAAGGGCATATCCAAACA-3', which yielded a 250-bp product.

Western blot analysis

Nuclear extracts were prepared from the B220+ 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 B220, CD21, and IgM, and APC-labeled rat antibody against mouse B220 were obtained from BD Pharmingen.

Cell culture of B cells

B220+ cells (2 × 10^5) 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.

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'-CTGCCGGCTTCAA-GAGGGTG-3' and c-Maf reverse: 5'-GATCTCTGCTGAAGTTGC-3'; Ccnd2 forward: 5'-CCTCTTCTTACACTTGTGGTC-3' and Ccnd2 reverse: 5'-CTACCTCGCAGTCTTCT-3'; Hprt forward: 5'-CACACTGGAAGTCACCAGAAG-3' and Hprt reverse: 5'-CTACCCAGCAGGGTTCTCC-3'; Prdm1 forward: 5'-AACCGAGGCACTTTACC-3' and Prdm1 reverse: 5'-GTCAAAGGGCTTTCCTTC-3'; Pax5 forward: 5'-AAACAAGCCAAAAACCTTAC-3' and Pax5 reverse: 5'-CTGCTGTACTTTTGGCAATGTA-3'; Xbp1 forward: 5'-GGAGCAG-CAGTGGTGGATT-3' and Xbp1 reverse: 5'-CAGCTGGTCCATTCCAAAG-3'; Mafy forward: 5'-GATTCCCTTGGGCTTGGTGG-3' and Mafy reverse: 5'-GAGGGCGTGGAAGTGTG-3'; Itgb7 forward: 5'-GGTGTACAGGATTGTTCCAGA-3' and Itgb7 reverse: 5'-GCTGCACGTTCACTCAACAT-3'; Spp1 forward: 5'-AGGCAGGCGATTCTGTC-3' and Spp1 reverse: 5'-CCACGATGGCAGTGG-3'; Muc5 forward: 5'-TGGTACTTTTGGGCTTGGTGG-3' and Muc5 reverse: 5'-CAGCTGGTCCATTCCAAAG-3'; Pex5 forward: 5'-GTTGTCAGGATTGGTCAATGTA-3' and Pex5 reverse: 5'-CAACAAGGCAGCAACATGTA-3'.
forward: 5′-TGTTGTGATTGATGCCCCTGACTA-3′ and Hprt reverse: 5′-AGGCCATGTCGCCACAGGACTA-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 \( \mu \) 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 \( V_{\mu} \) gene promoter, an \( \mu \) enhancer sequence, and a \( 3′ E\)x enhancer sequence (Fig. 1A). We generated 2 constructs (the first did not contain the \( 3′ E\)x enhancer) and obtained 17 \( F_0 \) 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 \( EcoRI/HindIII \) 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 \( \mu \) 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 \( \mu \) 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\(^+\) CD23\(^{hi}\) CD21\(^{lo}\)) and marginal zone (MZ) B cells (B220\(^+\) CD23\(^{lo}\) CD21\(^{hi}\); Fig. 1C). Next, we analyzed the proliferative potential of WT and \( \mu \) c-Maf TG B cells in vitro. The proliferative potential of \( \mu \) 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 \( \mu \) c-Maf TG mice developed lymphoma

The \( \mu \) 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 \( \mu \) 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 \( \mu \) c-Maf TG mice were of B-cell origin

\( \mu \) 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 \( \mu \) 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\(^+\)CD23\(^-\)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\(^{hi}\)CD138\(^+\)) was increased in the TG mice (Fig. 2C).

Hypergammaglobulinemia in aged \( \mu \) c-Maf TG mice

Approximately 50% (7/14) of the \( \mu \) 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 \( \mu \) 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). P < 0.005. In addition, the mean amount of total IgM in the TG mice was 1,083 ± 223 (mg/dL), whereas in the control mice this value was 564 ± 92 (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 (B220<low>CD138<+>) 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).

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Figure 2. Eμ c-Maf TG mice developed B-cell lymphoma. A, cumulative lymphoma-free survival rate curves of Eμ c-Maf TG and WT mice. Statistically significant differences (P < 0.05) were detected between the two groups. B, the number of plasma cell-like lymphoma cells in the spleens of Eμ c-Maf TG mice was increased compared with that in the controls (WT). Disruption of the normal follicles and accumulation of lymphoma cells were observed in spleens from the TG mice (H&E). C, the B-cell lymphoma cells (B220<+>) also expressed CD138, as shown by immunohistochemical staining of B220 and CD138. Immunofluorescence showed that plasma cells (CD138<+>, red) that had infiltrated into the spleens of Eμ c-Maf TG mice also expressed c-Maf (green), but this was not observed in the spleens of WT mice. Flow cytometry revealed that the number of B220<+>CD138<+> cells was increased in the spleens of Eμ c-Maf TG mice that carried lymphoma. The number of mature plasma cells (B220<+>CD138<+>) in the spleen was also increased. D, B220<+>CD138<+> cells in the spleens of Eμ c-Maf TG mice were characterized as B220<+>CD138<+>-CD21<+>CD23<+>IgM<+>IgD<+> by flow cytometry.
Autonomous proliferation in nude mice of lymphoma cells from Em c-Maf TG mice

To ascertain whether the infiltrating tumor cells in Em 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 (10⁶ 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 Em 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 Em 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 Eμ 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 Eμ c-Maf/TG mice. The level of c-Maf expression in the lymphoma-infiltrated lymph nodes of Eμ c-Maf/TG 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 Eμ 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 Eμ 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

Figure 4. Renal involvement in Eμ c-Maf TG mice. A, renal tissue sections from 60 weeks old Eμ c-Maf TG mice with lymphoma and WT mice were stained with PAS. Left, renal tubules; right, glomeruli. B, glomerular immunoglobulin deposition. Serial frozen sections of renal tissue from WT and Eμ c-Maf TG mice were analyzed by immunofluorescent staining using specific antibodies against mouse immunoglobulin α and λ轻 chains, as well as antibodies against IgG, IgM, and IgA heavy chains.
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 E\textsubscript{em} c-Maf TG mice. We also analyzed Spp1 (osteopontin) expression, because we could not find any obvious osteolytic lesions in the E\textsubscript{em} 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 E\textsubscript{em} 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 VA 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 MAF\textsubscript{A} (23, 35) or MAF\textsubscript{B} [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%, MAF\textsubscript{B} in 2%, and MAF\textsubscript{A} 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 (B220\textsubscript{low}CD138\textsuperscript{+}) were increased in the spleen and bone marrow of the TG mice, the lymphoma cells in the spleens were B220\textsuperscript{+}CD138\textsuperscript{+}CD21\textsuperscript{+}CD23\textsuperscript{+}IgM\textsuperscript{+}/C0. The phenotype of some E\textsubscript{em} 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

Figure 5. Autonomous proliferation of c-Maf-induced lymphoma cells. A, mononuclear cells that had been isolated from the spleens of E\textsubscript{em} c-Maf TG mice and transplanted into nude mice infiltrated the spleens of the recipient mice. Enlarged liver and spleen were observed in the recipient mouse. B, Southern blot analyses of lymphoma cells from the spleens of E\textsubscript{em} c-Maf TG mice using a JH4 probe after digestion with EcoRI. Lane 1 (DNA from tail of a WT mouse), lane 2 (DNA from the tail of an E\textsubscript{em} c-Maf TG mouse), and lanes 3 to 6 (DNA from the enlarged spleens from E\textsubscript{em} c-Maf TG mice) show the presence of a DNA fragment corresponding to the germ line JH4 gene (solid arrow). Lanes 3 to 6 show DNA fragments that corresponded to rearrangements of the Igh gene (asterisk). Lanes 2 to 6 show a DNA fragment that corresponded to the Igh transgene (hollow arrow). C, transplanted tumor cells showed a plasma cell-like appearance in the spleens of the recipients, as shown by H&E staining.
Mouse Model of the t(14;16)(q32;q23) Translocation

Figure 6. Analysis of c-Maf target genes by quantitative RT-PCR. The expression profiles of Maf (c-Maf) and its target genes were examined by real-time RT-PCR analysis. Total RNA was obtained from the enlarged lymph nodes from Eμ c-Maf TG mice. All of the data are presented as the mean ± SE. □, WT; mRNA from B220− B cells from the spleens of 50 weeks old WT mice; ■, TG; mRNA from the lymph nodes of mice from Eμ c-Maf TG line S24. *, P < 0.05.

clonal lymphoplasmacytic cells, predominantly in the bone marrow, and an IgM monoclonal gammopathy are diagnostic findings of WM (37). MALT lymphoma is a distinct subtype of MZ B-cell lymphomas. The large number of plasma cells among MALT lymphoma cells is a well-known phenomenon known as plasmacytic differentiation (38). With the demonstration that the tumors express both mature B-cell (B220−) and plasma cell (CD138−) markers, it should be noted that they fall within a spectrum of mouse plasma cell–related tumors (anaplastic, plasmablastic, and plasmacytic plasmacytoma) described previously (39, 40, 41). The Eμ c-Maf TG mouse is a murine model of the human MM t(14;16)(q32;q23) chromosomal translocation. Primary early-onset reciprocal chromosomal translocations in MM occur most frequently at IGH on 14q32. Meanwhile, secondary late-onset translocations and gene mutations have been implicated in MM progression (42). The fact that Eμ c-Maf TG mice displayed immature phenotypes rather than classical MM might suggest that c-MAF translocation alone is not sufficient for the development of classical MM. Experimental efforts to generate mouse models of MM have typically involved the targeted expression of an oncogene in the B-cell compartment by TG approaches (28). These strategies have generally yielded B-cell malignancies that display immature phenotypes or plasmacytomas rather than classical MM (28). Given that c-MAF is known to be translocated and/or overexpressed in human MM, the Eμ c-Maf TG mouse may offer an ideal system to study the pathogenesis of human MM. Aged Eμ c-Maf TG mice developed B-cell lymphomas rather than plasma cell dyscrasias. In the majority of cases, TG mice that express an oncogene or proto-oncogene specifically in the B-cell compartment develop plasmacytoma and not classical MM. This is the case for c-abl (43), Il-6 (44), and Bcl-2/c-Myc (45) mice; however, Eμ Xbp1s (28) TG mice are the exception. Recently, it was reported that TG mice in which the c-Myc gene was subject to conditional Aid (activation-induced deaminase)-dependent activation developed the MM/MGUS phenotype (46). We do not understand the underlying differences in the promotion of tumor formation between mice and humans.

Further studies will be needed to define the role of c-Maf in the development of both human and mouse MM. Clarification of the mechanism by which c-Maf contributes to oncogenesis may ultimately facilitate the discovery of more specific therapies to prevent the progression of MM.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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