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Wwox Hypomorphic Mice Display a Higher Incidence of B-Cell Lymphomas and Develop Testicular Atrophy

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Abstract

WWOX is a putative tumor suppressor gene encoded within common chromosomal fragile site region FRA16D, in chromosome band 16q23. Multiple studies have demonstrated that *WWOX* expression is often reduced or lost in various tumor types. *WWOX* tumor suppressor activity was suggested by re-expressing *WWOX* in breast, ovarian, and lung tumor cell lines leading to tumor growth inhibition in vivo. To determine whether loss of *Wwox* gene expression has a role in tumorigenesis, we generated a mouse strain containing a *Wwox* gene mutated by a gene-trap vector. Homozygous *Wwox* gene-trap mice (*Wwox*^{gt/gt}) had no detectable *Wwox* protein in most tissues examined, although, a low level could be detected in a minority of tissues. Because of these observations, we concluded that these mice are *Wwox* hypomorphs. Remarkably, *Wwox* hypomorphic mice are viable in contrast to the recently reported postnatal lethality of *Wwox* knockout mice. Testes from *Wwox*^{gt/gt} males had high numbers of atrophic seminiferous tubules and reduced fertility when compared with wild-type counterparts. We observed that the *Wwox*^{gt/gt} mice had a significantly shorter lifespan, and female hypomorphs had a higher incidence of spontaneous B-cell lymphomas. In conclusion, we describe a novel *Wwox* hypomorphic mouse model that overcomes postnatal lethality that was recently observed in *Wwox* knockout mice. Therefore, tumorigenesis studies using this model more closely recapitulates the loss of *WWOX* expression observed in human cancers. Importantly, our observation that *Wwox* hypomorphs had an increased incidence of B-cell lymphomas supports a role of *Wwox* as a tumor suppressor.

INTRODUCTION

The *WWOX* gene was first identified as a candidate tumor suppressor gene in human breast tumors. The coding region of *WWOX* spans over 1 MB and is located within the common chromosomal fragile site region, FRA16D, in chromosome band 16q23 (Bednarek et al., 2000; Ludes-Meyers et al., 2003). *WWOX* is highly conserved from worms to humans; in

mouse, it is located on chromosome 8 and similarity with the human counterpart is even observed in noncoding regions such as introns. Remarkably, the mouse *Wwox* gene colocalizes with the common chromosomal fragile site, Fra8E1 (Krummel et al., 2002). *WWOX* gene disruptions have been observed in many human malignancies of epithelial (Bednarek et al., 2000; Paige et al., 2001) and hematopoietic origins (Ishii and Furukawa, 2004). The loss of *WWOX* expression in human cancer occurs by multiple mechanisms including gene rearrangements such as chromosomal deletions and translocations consistent with chromosomal fragility (Krummel et al., 2000); transcriptional disruption due to aberrant splicing (Bednarek et al., 2001; Paige et al., 2001) and promoter methylation (Iliopoulos et al., 2005). *WWOX* tumor suppressive activity was first observed by inhibiting the growth of xenografts of human breast cancer cell lines (Bednarek et al., 2001; Ludes-Meyers et al., 2003). Several studies have correlated the loss of *WWOX* expression with clinical prognostic factors in breast and ovarian cancers (Guler et al., 2004; Nunez et al., 2005a,b).

WWOX protein consists of two functional motifs consisting of two WW domains involved in protein-protein interactions and a short-chain dehydrogenase (SDR) domain (Bednarek et al., 2000; Ludes-Meyers et al., 2003). Several potential protein partners have been identified and include regulators of cell growth and apoptosis such as p73, p53, LITAF/SIMPLE, SCOTIN, and EZRIN (Aqeilan et al., 2004; Ludes-Meyers et al., 2004). Overexpression of *WWOX* in some cell lines was reported to induce apoptosis (Aqeilan et al., 2004). Nevertheless, the physiological functions of *WWOX* remain unclear.

We have utilized the gene-trap technology to generate a *Wwox* hypomorphic mouse that has either dramatically reduced or complete loss of *Wwox* expression depending on the tissue examined. Recently, it was reported that *Wwox* knockout mice die postnatally and a few of these animals developed osteosarcoma-like lesions (Aqeilan et al., 2007); however, tumorigenesis studies could only be performed in *Wwox* heterozygotes. Importantly, the *Wwox* hypomorphic mice described here are viable making them ideal for long-term tumorigenesis studies. Using this model, we demonstrate that the disruption of *Wwox* expression resulted in defects affecting male testis and increased the development of B-cell lymphomas in females.

MATERIALS AND METHODS

Hypomorph Generation

The ES cell line, XG218, was obtained from BayGenomics (<http://baygenomics.ucsf.edu/>) and has a gene-trap inserted in *Wwox* intron 4. Chimera production was performed at the University of M.D. Anderson genetically engineered mouse facility. Housing and breeding of mice and all experiments performed with mice are in accordance with the NIH guidelines and regulations in AALAC accredited facilities.

Analysis of *WWOX* mRNA

Embryos were dissected from pregnant females and the yolk sac and embryo were separated. Whole embryos were dissolved in TRIzol reagent and RNA was extracted. cDNA

was synthesized using random hexamer primers and used for multiplex PCR using primers A: 5'-GGATCCAAGATTGGCATTACCGTGG-3', B: 5'-ATTCAG GCTGCGCAACTGTTGGG-3' and C: 5'-AAAGG ACTTGGCAGTCTCGAACC-3' (Fig. 1A). Real-time PCR was done using TaqMan expression assay mM00497967_m1 (Applied Biosystems) spanning *Wwox* exons 4 and 5. All assays were done in duplicate with RNA from tissues of three individual animals.

Genotyping

DNA extracted from yolk sacs and tails was digested with EcoRV and analyzed by Southern blotting using a *Wwox* intron 4 probe made using PCR with primers 5'-CAGTTTAAAGAGCATCCACCCACTGTTGGC-3', 5'-CCTAGTCGTGA GGCGAGTTTGGATAGG-3' and C57Bl/6 DNA as template.

Protein Expression

Protein and RNA from embryos were obtained using TRIzol. Protein was extracted from frozen adult tissues using 1% NP40 lysis buffer and western blotting done as described (Ludes-Meyers et al., 2004). Affinity purified anti-WWOX rabbit polyclonal antibodies prepared by our laboratory were raised against WWOX amino acid residues 16–93 that are identical between human and mouse. This antibody has been extensively characterized for its excellent specificity as shown previously (Nunez et al., 2005a,b, 2006). Anti-actin (Sigma, A1978) was used as a protein-loading control.

Long-Term Survival Analysis

The experimental cohorts used for the survival analysis consisted of 15 *Wwox*^{+/+} versus 20 *Wwox*^{gt/gt} female and 20 *Wwox*^{+/+} versus 19 *Wwox*^{gt/gt} male mice. Mice were allowed to live out their life span or until 104 weeks of age at which time they were euthanized and necropsies were performed. Mice were also euthanized when moribund or when showing signs of chronic disease.

Histological Analysis

Tissues from necropsies were fixed in formalin and embedded in paraffin. Tissue sections were stained with H&E or processed for immunohistochemistry. Antibodies used anti-CD3 (Serotec, MCA1477T) and anti-CD45R/B220 (Serotec, MCA1258G). Histological analyses were performed by two pathologists without prior knowledge of genotypes. Percentages of atrophic seminiferous tubules were determined using testicle image scans (average of 227 tubules/testicle analyzed).

RESULTS

Generation of WWOX Gene-Trapped Mutant Mice

We utilized gene-trap technology to determine whether the reduction of *Wwox* gene expression affects mouse development and tumorigenesis. ES cell clone XG218 (BayGenomics, San Francisco, CA) harbors a gene-trap inserted into *Wwox* intron 4 resulting in a *Wwox* splice variant containing *Wwox* exons 1–4 spliced in-frame with the β-

geo gene (Fig. 1). The resultant *Wwox*- β -geo fusion protein contains both *Wwox* WW domains but lacks the oxidoreductase domain thus ablating enzymatic activity.

To determine whether the gene-trap insertion affects *Wwox* expression in mouse tissues, we analyzed the RNA and protein from 10.5 dpc F2 embryos (Fig. 1B). RT-PCR analysis demonstrated that only embryos with trapped *Wwox* alleles had the expected *Wwox*- β -geo splice variant. Moreover, wild-type *Wwox* transcripts were undetectable in embryos homozygous for a trapped *Wwox* allele (Fig. 1B). Real-time PCR was used to quantitate the relative levels of *Wwox* expression in embryos of each genotype and as expected *Wwox*^{+/gt} embryos had a 50% reduction of *Wwox* mRNA compared with wild-type while *Wwox*^{gt/gt} embryos had a reduction by more than 95% (Fig. 1C). *Wwox* protein expression was consistent with the observed mRNA expression levels (Fig. 1B). These results confirmed that the gene-trap is in *Wwox* intron 4 and results in the production of the expected *Wwox*- β -geo splice variant and, importantly, leads to a highly significant reduction of *Wwox* mRNA and protein expression.

In spite of the dramatic reduction of *Wwox* expression by the gene-trap, we observed normal Mendelian ratios in the F2 generation (Supplementary Table 1) and F2 mice remained viable for up to 2 years of age. This is in contrast to the recent observation that *Wwox* knockout mice die by 4 weeks of age (Aqeilan et al., 2007). To demonstrate that *Wwox* expression remained low in adult *Wwox*^{gt/gt} mice, we analyzed protein and mRNA in several adult tissues. *Wwox* protein was undetectable in most tissues of *Wwox*^{gt/gt} mice (Fig. 1D); however, low levels of *Wwox* could consistently be detected in testes. *Wwox* mRNA was significantly reduced in adult tissues as well. Real time PCR showed *Wwox* mRNA reduced by 85–98% of wild-type depending on the tissue examined (Fig. 1E). However, despite the dramatically reduced mRNA levels, a small fraction of full-length *Wwox* transcripts can be produced by splicing around the gene-trap as observed with other trapped alleles (Voss et al., 1998). This will result in the production of small amounts of functional *Wwox* protein (e.g., testes). Therefore, we conclude that *Wwox*^{gt/gt} mice are viable hypomorphs.

Testicular Defects in *Wwox*^{gt/gt} Male Mice

We observed that the testes of young and, more prominently, old *Wwox*^{gt/gt} male mice had severe degeneration of numerous seminiferous tubules. We determined that *Wwox* is strongly expressed in Leydig cells and moderately expressed within cell layers of seminiferous tubules (Fig. 2A) with staining intensity decreasing with differentiation. Furthermore, spermatozoa, cells devoid of cytoplasm, are negative for *Wwox* expression confirming our original observations that *Wwox* is exclusively expressed in cytoplasm (Bednarek et al., 2001). The percentage of atrophic seminiferous tubules per testis was determined in *Wwox*^{+/+} and *Wwox*^{gt/gt} mice at ages of 3 months ($n = 8$ and $n = 10$, respectively) and 15 months ($n = 4$ and $n = 4$, respectively) (Fig. 2G). The degenerative lesions consisted of seminiferous tubules devoid of mature spermatocytes with incipient atrophy (Figs. 2C, 2E, and 2F) and associated with Leydig cells hyperplasia. Testes from *Wwox*^{gt/gt} mice displayed higher percentages of degenerative tubules than WT in both young and old animals (Fig. 2G).

Wwox^{gt/gt} male mice have poorer reproductive abilities. Typically, when WT mating pairs are housed together for 6 weeks females will have three consecutive litters. However, under these conditions, *Wwox*^{gt/gt} mating pairs had an average of only 1.3 litters (Supplementary Table 2). To determine whether female or male *Wwox*^{gt/gt} mice had impaired reproductive ability, we performed mating studies using known fertile WT males and females paired with virgin *Wwox*^{gt/gt} partners. *Wwox*^{gt/gt} females had an average of 2.5 litters when paired with WT males but WT females had an average of only 1 litter when paired with *Wwox*^{gt/gt} males (Supplementary Table 2). We conclude that the disruption of *Wwox* gene expression leads to premature testicular degeneration in *Wwox*^{gt/gt} males resulting in a reduction of fertility.

***Wwox*^{gt/gt} Mice Displayed Increased Tumorigenesis**

To determine whether *Wwox* functions as a tumor suppressor in this mouse model, we performed a 2-year aging experiment comparing the survival of *Wwox*^{+/+} and *Wwox*^{gt/gt} mice. *Wwox*^{gt/gt} mice had an increased death rate beginning at 3–4 months of age. During the first 18 months of the study, 23% of the *Wwox*^{gt/gt} mice had died compared with 0% of WT mice (Fig. 3). The decrease in survival was similar for both the male and female cohorts (26.3% vs. 20%, respectively). Histopathological analysis of mice that died at an early age did not reveal a definable cause of death. The final results of the 2-year study demonstrated that the overall survival of *Wwox*^{gt/gt} mice was significantly decreased compared with their WT counterparts ($P = 0.0188$, Fig. 3).

We observed a significant increase in the incidence of malignant neoplasias in female *Wwox*^{gt/gt} mice (9 of 14 mice) when compared with female WT mice (3 of 15 mice, $\chi^2 = 5.85$, $P = 0.015$). The majority of tumors that developed in *Wwox*^{gt/gt} females consisted of B-cell lymphomas, i.e., CD45R/B220 positive lymphomas (Table 1, Fig. 4, $\chi^2 = 6.15$, $P = 0.013$). Most female mice displayed diffuse adenopathies and splenomegaly. In addition, the lymphomas that developed in the *Wwox*^{gt/gt} mice appeared more invasive than the few lymphomas observed in WT females having a high degree of infiltration of multiple organs such as pancreas and mesenteric lymph nodes in particular, as well as kidneys and liver. In quite a few instances, the pattern of lymphoma development was similar to that observed in plasmacytomas. On the other hand, tumor incidence in male mice was similar between the two genotypes. Male mice displayed a higher incidence of lung adenomas compared with females, which is characteristic of their genetic background.

Interestingly, some female animals showed the development of multiple neoplasias, such as the lesions illustrated in Figure 4. For example, a single female mouse had a B-cell lymphoma, infiltrating kidney (Fig. 4A), a lung adenocarcinoma (Fig. 4B), mammary gland hyperplasia, adenosis, and some areas displaying mammary intraepithelial neoplasias (MIN) (Fig. 4C). Similarly, the mouse whose neoplastic lesions are shown in Figures 4D–4F displayed a very aggressive B-cell lymphoma infiltrating the pancreas (Fig. 4D) and multiple MIN lesions could be observed (Fig. 4E). The lesions shown in Figures 4G and 4H again displayed CD45R/B220 positive lymphoma cells infiltrating the pancreas (Fig. 4G) and a MALT type B-cell lymphoma affecting the Peyer's patch of the small intestine (Fig. 4H). These results demonstrate that the disruption of *Wwox* gene expression increases the incidence of tumorigenesis in aging mice.

DISCUSSION

We have generated a *Wwox* hypomorphic mouse strain using gene-trap mutagenesis that leads to premature testicular degeneration and an increase in the spontaneous development of B-cell lymphomas in female mice. We demonstrated that the *Wwox* hypomorphic mice are viable and fertile despite the dramatically reduced *Wwox* expression. Histopathological analyses led to the observation that the testes of *Wwox*^{gt/gt} males had a higher degree of degenerated and atrophic seminiferous tubules and decreased fertility compared to their WT counterparts. In addition, long-term survival analyses demonstrated that *Wwox*^{gt/gt} mice had decreased survival compared with WT mice. Most importantly, histopathological analyses of mice that died during the study and at the 2-year final time point revealed that *Wwox*^{gt/gt} females developed significantly more malignancies than WT females.

It is surprising that the *Wwox* hypomorphic mice have such high survival rates given the recent report that *Wwox* knockout mice die by 4 weeks of age (Aqeilan et al., 2007). This may be due to low levels of functional *Wwox* protein encoded by full-length *Wwox* mRNA produced by splicing around the gene-trap in tissues critical for survival. Real-time PCR detected consistent yet low levels of full-length *Wwox* transcripts in several tissues from hypomorphic mice. Furthermore, testes from wild-type mice had very high expression levels and testes from hypomorphs had the highest levels of full-length transcript and consequently had detectable levels of *Wwox* protein. Also, there were differences in the types of neoplasias observed in hypomorphic mice compared with knockout mice. A subset (4 of 13) of knockout mice developed bone lesions at an early age while we did not find evidence of bone abnormalities. In contrast to the observations of Aqeilan et al. (2007), we and others have observed abundant *WWOX* expression in both human and mouse liver using northern blotting, RT-PCR, and western blotting (Iida et al., 2005; Nunez et al., 2006; Park et al., 2004). Furthermore, *WWOX* was determined to be the 12th most highly conserved gene expressed in the liver over the period of 70 million years of primate evolution (Gilad et al., 2006).

The differences in phenotypes may be due, in part, to strain backgrounds. *Wwox* hypomorphs were generated from 129/Ola ES cells compared with 129/Svj ES cells used by Aqeilan et al. (2007). Threadgill et al. (1997) concluded that the 129/Svj substrain is a contaminated inbred strain significantly different from other 129 substrains, including 129/Ola and has given rise to discrepancies when comparing the phenotypes of genetically modified mice. However, both *Wwox* hypomorphic mice and *Wwox* knockout mice had a higher incidence of B-cell lymphomas confirming *Wwox* as a tumor suppressor gene.

Testicular defects similar to those we observed in *Wwox*^{gt/gt} have been observed in other transgenic mouse strains. Successful gametogenesis depends on balancing the regulation of cell division and apoptosis of gamete precursors. Disruption of this balance leads to gonadal atrophy and possibly infertility. *WWOX* is involved in regulating apoptosis by inhibiting p73 transcriptional activity (Aqeilan et al., 2004). p73 is expressed in all testicular cells; however, normal nuclear staining was only observed in the later stages of spermatogenesis. Interestingly, in Leydig cells and spermatogonia, p73 is located only in the cytoplasm, whereas, translocation to the nucleus was observed at later stages of spermatogenesis

(Hamer et al., 2001). It is possible that WWOX is required for the cytoplasmic localization of p73 and that p73 dysregulation plays a role in the observed testicular phenotype in *Wwox^{gt/gt}* mice.

Testicular development is also highly dependent on paracrine and endocrine hormonal regulation. WWOX is highly expressed in Leydig cells (Fig. 2A, Nunez et al., 2006; Watanabe et al., 2003) as well as in the pituitary gland (Nunez et al., 2006). Leydig cells are the main source of testosterone that is required for spermatogenesis. Since the WWOX SDR domain has a high degree of similarity to sex steroid oxidoreductases, it is possible that the steroid hormone metabolism is altered in *Wwox^{gt/gt}* mice. In addition, the pituitary gland produces luteinizing and follicle-stimulating hormones that regulate Leydig cell and Sertoli cell functions, respectively, required for successful spermatogenesis. Therefore, *Wwox* may play a role in multiple steps of testicular function and regulation of differentiation.

B-cell lymphomagenesis in mouse models can be caused by either increased B-cell proliferation (e.g., Eμ-myc transgenic) or inhibition of B-cell apoptosis (e.g. bcl-2-immunoglobulin transgenic). Since *Wwox* has been shown to be involved in regulating the apoptotic response to various stimuli, we hypothesize that the increase in B-cell lymphoma development may be due to the increased survival of B cells resulting in an expansion of the B-cell population over the lifetime of the mouse. Importantly, dysregulation of WWOX gene expression has been previously described in human hematopoietic malignancies (Ishii et al., 2003). They observed that 51% of primary hematopoietic neoplasias had either altered or lost WWOX expression at both the mRNA and protein levels. We also found the cases of nonperitoneal plasmacytoma and GALT B-cell tumors that are rarely observed in inbred mice. Plasmacytoma is similar to human multiple myeloma (MM), a B-cell neoplasm. There is not a true mouse model equivalent to human MM. Nevertheless, it is interesting to note that one of the nonrandom genomic rearrangements reported in human MM is the presence of translocations affecting chromosome 16 and more specifically WWOX (Krummel et al., 2000). One such translocation is t(14;16)(q32;q23) found in 5–10% of MM patients that results in the disruption of the IgH locus at 14q32 and the WWOX gene at 16q23. More recently, using expression arrays, it was found that WWOX was significantly underexpressed in human MM even in the absence of 16q deletions (Walker et al., 2006; Zhan et al., 2002). Therefore, it is likely that the disruption of WWOX expression, by multiple mechanisms, is important in the development of human MM.

Our findings that B-cell lymphoma development occurs spontaneously when *Wwox* expression is drastically reduced strongly supports the hypothesis that the disruption of *Wwox* expression, in itself, is cancer predisposing. It is worth noting that the tumors we observed typically formed over a 2-year time span. During this long latency period spontaneous secondary changes likely occurred that contributed to tumorigenesis. These results are consistent with a model in which *Wwox* acts as a caretaker gene, whose absence does not directly initiate tumorigenesis but rather facilitates or alters susceptibility for tumor development. Further studies are needed to determine whether *Wwox^{gt/gt}* mice could serve as a surrogate model for the study of human B-cell lymphomas and B-cell neoplasias in general.

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Figure 1.

Gene-trap insertion into *Wwox* intron 4 disrupts *Wwox* expression. **A:** Diagram representing the *Wwox* gene-trapped (*Wwox*^{gt}) mutant allele and resulting mutant mRNA transcript. The numbered black boxes represent *Wwox* exons, and the open box represents the gene-trap affecting *Wwox* intron 4. The arrows show the location of the primers used for multiplex RT-PCR analyses. **B:** (Top panel) Southern blot genotype analysis of embryo yolk sac DNA with the *Wwox* intron 4 specific probe. Arrows point to the gene-trap mutant (gt) and wild-type (wt) bands. The genotype of each embryo is given at the top of each lane. wt = *Wwox*^{+/+}; ht = *Wwox*^{+/gt}, and hm = *Wwox*^{gt/gt}. (middle panel) RT-PCR analysis of embryo RNA. Agarose gel analysis of multiplex RT-PCR products using primers A, B, and C shown in (A). Total embryonic RNA from each embryo was used as the template. Note the appearance of the *Wwox* gene-trap specific band only in *Wwox*^{+/gt} and *Wwox*^{gt/gt} embryos. (Bottom panel) Western-blot analysis of total protein lysates from each embryo using anti-WWOX antibody. No *Wwox* protein was detected in *Wwox*^{gt/gt} embryos. **C:** Real-time PCR analysis of embryo RNA. Total RNA from two embryos of each genotype was analyzed using a TaqMan expression assay for *Wwox* exons 4–5. Each assay was done in triplicate. The average expression level $\pm$ SEM is represented for each genotype. **D:** Western-blot analysis of *Wwox* protein expression in adult tissues. (Top panel) *Wwox* expression in kidney, spleen, and thymus. (Bottom panel) *Wwox* expression in liver and testes. Total

protein lysates were prepared from tissues of two individual animals of the indicated genotype. Equal amounts of total protein for immunoblot detection of Wwox protein using anti-Wwox antibody were loaded. Antiactin was used as a loading control. Wwox protein is undetectable in adult tissues, with the notable exception of testes. E: Real-time PCR to measure the abundance of wild-type *Wwox* mRNA in adult tissues of *Wwox*^{+/+} and *Wwox*^{gt/gt} mice. *Wwox* mRNA was detected using a TaqMan expression assay specific for *Wwox* exons 4–5 boundary and normalized to Gapdh. Wwox expression for each tissue is plotted as the mean of three animals in arbitrary units (error bars represent $\pm$ SEM).

Figure 2.

Histological analyses of mouse testis. A: *Wwox* expression in the testis of wild-type adult mouse as detected by means of immunohistochemistry using anti-*Wwox* antibody. B and D: *Wwox*^{+/+} and (C and E) *Wwox*^{gt/gt} of the indicated age. Small arrowheads point to Leydig cells hyperplasia. Large arrows point to atrophic seminiferous tubules. F: Atrophic tubule at a higher magnification. G: Dot plot summarizing the quantitation of testes degeneration. *Wwox*^{gt/gt} males had a significantly higher percentage of degenerate tubules than *Wwox*^{+/+} males in young (3–8 months, $P < 0.0001$) and old (15–20 months, $P < 0.005$) mice. Statistical analysis was done using the Wilcoxon rank-sum test. Horizontal bars represent the mean and the number of males in each group is in parenthesis.

Figure 3. Kaplan-Meier survival curves comparing the 2-year survival of $Wwox^{+/+}$ ($n = 35$) and $Wwox^{gt/gt}$ ($n = 39$) mice. $Wwox^{gt/gt}$ mice had a significantly shorter survival ($P = 0.0188$, Breslow test).

Figure 4.

Histopathology of tumors from three individual mice. A: Anti-CD45R/B220 stained B-cell lymphoma infiltrating the kidney. H&E stained (B) lung adenocarcinoma and (C) mammary gland with MIN lesions and hyperplasia from the same mouse. Anti-CD45R/B220 stained (D) pancreas and (F) spleen. The pancreas is highly infiltrated with B cells, and the spleen morphology is completely disorganized. E: H&E stained mammary gland with MIN (arrow). Anti-CD45R/B220 stained (G) pancreas and (H) small intestine from a mouse with MALT-type B-cell lymphoma. I: H&E stained section of lung contains a substantial lymphoma cells infiltrate.

TABLE 1

Neoplastic Lesions Observed

Wwox^{gt/gt}	Wwox^{+/+}
Female mice	
Plasmacytoma (80 weeks) ^a	Lymphoma
B-cell lymphoblastic lymphoma-MIN (96 weeks)	Osteosarcoma
Small B-cell lymphoma (98 weeks)	Leukemia-vaginal Ca
B-cell lymphoma-lung adenoca-MIN ^b	Meningioma
MALT type B-cell lymphoma	
Small B-cell lymphoma	
B-cell lymphoma	
B-cell lymphoma	
B-cell lymphoma	
Malignancies = 9/14(64.3%)	Malignancies = 3/15(20%) ^c
Male mice	
Lymphoma-epididymal adenoma (35 weeks)	Lung adenoma (89 weeks)
Harderian adenoma (86 weeks)	Lung adenoma (89 weeks)
Lymphoma-lung adenoma (91 weeks)	Lung adenoca-Leydig cell tumor
Lung adenoca-lymphoma	Lymphoma
Lymphoma	Lymphoma
Lung adenoma-epididymal leiomyoma	Lymphoma
Leydig cell tumor	Lymphoma
Lung adenoma	
Lung adenoma	
Seminal vesicle fibroma	
Malignancies = 5/14(35.7%)	Malignancies = 5/18(27.8%) ^d

^aThe number in parenthesis indicates age at time of death.

^bWhen no age is indicated the animal survived to the end of the study.

^c $P = 0.015$.

^d $P = 0.23$.