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MMTV promoter–regulated caveolin-1 overexpression yields defective parenchymal epithelia in multiple exocrine organs of transgenic mice

Guang Yang[a], **Sanghee Park**[a], **Guangwen Cao**[b,1], **Alexei Goltsov**[a], **Chengzhen Ren**[a], **Luan D. Truong**[c], **Francesco DeMayo**[d], and **Timothy C. Thompson**[a,*]

[a]Department of Genitourinary Medical Oncology—Research, Unit 18-3, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009, USA, guangyang@mdanderson.org, shpark@mdanderson.org, agoltsov@mdanderson.org, czren@mdanderson.org, timthomp@mdanderson.org [b]Scott Department of Urology, Baylor College of Medicine, One Baylor Plaza, BCM381 , Houston, TX 77030, USA, gcao@smmu.edu.cn [c]Department of Pathology, The Methodist Hospital, 6565 Fannin Street, Houston, TX 77030, USA, ltuong@tmhs.org [d]Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza BCM130, Houston, TX 77030, USA, fdemayo@bcm.edu

Abstract

Caveolin-1 (Cav-1) is a major structural protein of caveolae, specialized plasma membrane invaginations that are involved in a cell-specific fashion in diverse cell activities such as molecular transport, cell adhesion, and signal transduction. In normal adult mammals, Cav-1 expression is abundant in mesenchyme-derived cells but relatively low in epithelial parenchyma. However, epithelial Cav-1 overexpression is associated with development and/or progression of many carcinomas. In this study, we generated and characterized a transgenic mouse model of Cav-1 overexpression under the control of a mouse mammary tumor virus (MMTV) long terminal repeat promoter, which is predominantly expressed in specific epithelial cells. The MMTVcav-1^{+} transgenic mice were fertile, and females bore litters of normal-size with no obvious developmental abnormalities. However, by age 11 months, the MMTVcav-1^{+} mice demonstrated overtly different phenotypes in multiple exocrine organs when compared with their nontransgenic MMTVcav-1^{-} littermates. Cav-1 overexpression in MMTVcav-1^{+} mice produced organ-specific abnormalities, including hypotrophy of mammary glandular epithelia, bronchiolar epithelial hyperplasia and atypia, mucous-cell hyperplasia in salivary glands, elongated hair follicles and dermal thickening in the skin, and reduced accumulation of enzymogen granules in pancreatic acinar cells. In addition, the MMTVcav-1^{+} transgenic mice tended to have a greater incidence of malignant tumors, including lung and liver carcinomas and lymphoma, than their MMTVcav-1^{-}

*Corresponding author. Tel.: (713) 792-9955; Fax: (713) 792-9956; timthomp@mdanderson.org.
[1]Department of Epidemiology, Second Military Medical University, 800 Xiangyin Rd., Shanghai 200433, China

Conflict of interest statement
The authors declare that there are no conflicts of interest.

littermates. Our results indicate that Cav-1 overexpression causes organ-specific, age-related epithelial disorders and suggest the potential for increased susceptibility to carcinogenesis.

Keywords

MMTV-promoter; Cav-1 overexpression; parenchymal epithelia; exocrine organs

Introduction

Caveolin-1 (Cav-1) is a 22-kD protein that functions as a principal structural component of caveolae in most mammalian cells. Cav-1 is involved in a cell- and context-specific fashion in diverse cell activities, such as molecular transport; cell adhesion; vesicular trafficking; cellular cholesterol, fatty acid and triglyceride homeostasis and signal transduction (Razani and Lisanti, 2001; Shaul and Anderson, 1998). In normal adult mammals, Cav-1 is highly expressed in mesenchyme-derived cells including smooth muscle cells, vascular endothelial cells, adipocytes, mammary gland myoepithelial cells, and myofibroblasts in the prostatic (Razani and Lisanti, 2001; Yang et al., 2008).

Early experiments that focused on the role of Cav-1 in growth control showed that Cav-1 levels are reduced in oncogene-transformed NIH-3T3 cells (Koleske et al., 1995), that targeted down-regulation of Cav-1 induces transformation of NIH-3T3 cells (Galbiati et al., 1998), and that enforced expression of Cav-1 suppresses the growth of fibroblasts and specific human breast cancer cell lines with myoepithelial cell features *in vitro* (Lee et al., 1998). To analyze the *in vivo* activities of Cav-1, multiple laboratories generated *Cav-1* gene homozygous knockout mice (Cao et al., 2003; Drab et al., 2001; Razani et al., 2001; Razani et al., 2002). In general, *Cav-1*$^{-/-}$ mice are viable and fertile but have a shorter life span than *Cav-1*$^{+/+}$ mice have (Cao et al., 2003; Yang et al., 2008) . These mice develop vascular dysfunction and thickened alveolar septa due to proliferation of endothelial cells and fibrosis (Cao et al., 2003; Drab et al., 2001; Razani et al., 2001). Male *Cav-1*$^{-/-}$ mice also developed hypercalciuria and urinary bladder stones (Cao et al., 2003). In the mammary gland, *Cav-1*$^{-/-}$ mice may develop epithelial hyperplasic lesions, potentially as a consequence of stromal cell abnormalities (Razani et al., 2001; Yang et al., 2008). Although the absence of Cav-1 has not been reported to increase the incidence of spontaneous malignancies, more hyperplastic lesions and tumors were observed in the skin of *Cav-1*$^{-/-}$ mice than in that of wild-type mice after application of dimethylbenzanthracene (Capozza et al., 2003). Further studies showed that loss of *Cav-1* gene expression can accelerate the development of hyperplastic and dysplastic mammary lesions and enhance tumorigenesis and metastasis in cancer-prone genetically engineered mice (Williams et al., 2003; Williams et al., 2004). These and other study results showing down-regulation of Cav-1 in human malignancies led to the notion that Cav-1 is a tumor-suppressor gene (Williams and Lisanti, 2005).

In contrast to studies that reported potential tumor-suppressor activities of Cav-1 , a recent study showed that *Cav-1*$^{-/-}$;TRAMP (transgenic mouse prostate) mice demonstrate significantly fewer primary tumors and lesions than *Cav-1*$^{+/+}$;TRAMP mice (Williams et al., 2005). An additional study reported that transgenic mice with targeted overexpression of Cav-1 in prostatic epithelial cells using the short probasin (PB) promoter (i.e., PBcav-1 mice) resulted in prostatic hyperplasia and atypia associated with pro-tumorigenic alterations in the local and metastatic tumor microenvironments (Watanabe et al., 2009). Further, Cav-1 secreted by prostatic epithelial cells in PBcav-1 mice created a local microenvironment that permitted tumor growth and increased serum Cav-1, which was associated with increased experimental prostate cancer lung metastatic activities. These results are consistent with those of numerous studies that have documented overexpression

of Cav-1 in prostate cancer and other malignant tissues (Shatz and Liscovitch, 2008; Thompson et al., 2009; Williams and Lisanti, 2005). Thus, the role of Cav-1 in tumorigenesis is complex and depends on the cell type and biological context. Our purposes in conducting this study were to generate a transgenic mouse model that overexpresses Cav-1 under the regulation of the MMTV long terminal–repeat promoter (MMTV-LTR), which is expressed in specific epithelial cells (Choi et al., 1987), and to characterize the pathologic effects of constitutive Cav-1 overexpression in such cells. Our histopathologic phenotyping demonstrated that MMTVcav-1^+ transgenic mice had defective parenchymal epithelia in multiple exocrine organs and appeared to be more susceptible than their MMTVcav-1^- littermates to the development of malignant tumors. These findings provided evidence of direct involvement of Cav-1 in the regulation of normal epithelial growth, differentiation, and function.

Materials and methods

MMTVcav-1 mice

The mouse Cav-1 (mcav-1) cDNA open reading frame was amplified by using PCR with the following oligonucleotides: a sense primer, 5′ GGGAAACCTCCTCAGAGCCT 3′, and an antisense primer, 5′ GATCAAGACAAACCATTCAT 3′. The mcav-1 fragment was then inserted into a TA-cloning vector. The restriction endonuclease EcoRI (New England Biolabs, Inc., Ipswich, MA, USA) was used to cut out the full-length mcav-1 cDNA from the TA-cloning vector, and it was then inserted into the MMTV-KbpA vector between the rabbit β-globin splice and the bovine growth hormone polyadenylation sequences (Fig. 1A). Expression of mCav-1 was driven by a 2.4-kb fragment of the MMTV LTR. The construct was linearized by application of NotI (New England Biolabs) and Asp718 (Roche). The fragment containing the MMTV promoter mcav-1 cDNA expression cassette was purified from low melting gel, and transgene DNA was then injected into fertilized oocytes from FVB mice.

To identify transgenic founder animals among the offspring, we screened DNA obtained from ear-punch tissue specimens by using PCR to amplify a 983-bp fragment with upstream 5′ GGATCCTGAGAACTTCAG 3′ and downstream 5′ ATCGTAGACAACAAGCGGTA 3′ primers specific for the transgene but not endogenous Cav-1. This resulted in confirmation of the transgenic expression of four independent transgenic lines. However, only one line, #5374, with relatively high transgene expression was chosen and propagated for the study in this report. 11–13 months old progeny of founder #5374 MMTVcav-1^+ mice and its wild-type littermates were used for histologic analysis or RNA extraction.

Mice were maintained under specific pathogen–free conditions in facilities accredited by the American Association of Accreditation of Laboratory Animal Care. All animal experimental procedures were conducted in accordance with the principles and procedures outlined in the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

Quantitative real-time RT-PCR

Total RNA was isolated from organs of MMTVcav-1^+ and MMTVcav-1^- mice by using a RiboPure RNA extraction kit (Ambion). RT reactions were carried out with a high-capacity cDNA archive kit (Applied Biosystem) according to the manufacturer’s protocol. PCR was performed as previously described (Ren et al., 2004; Ren et al., 2006) using the following Taqman probes and primers (Applied Biosystems): part #4352341E for mouse β-actin and transgene specific Cav-1 probe and primers: forward primer, 5′ TGGTTGTTGTGCTGTCTCATCA 3’; reverse primer, 5′ TGCAGGCTCTGAGGAGGTTT 3’; the probe, 5′ TTTGGCAAAGAATTC 3’. Real-time PCR was performed with a Step-

One real-time PCR system (Applied Biosystems) according to the manufacturer's instructions. The relative quantity of Cav-1 mRNA was determined by the ΔΔCT method as described by the manufacturer and normalized to β-actin RNA in the same cDNA preparation.

Protein extraction and western blot analysis

Formalin fixed, paraffin embedded tissue sections were used to extract protein with Qproteome FFPE tissue kit according to manufacturer protocol (QIAGEN GmbH, Germany). The protein concentration was measured using Micro BCA Protein assay kit (Thermo Scientific, USA). The proteins were separated using standard SDS-PAGE procedures. Western blot analysis of Cav-1 protein expression in the tissues of interest was performed using 1:100 dilution of Cav-1 antibody (# sc-894, Santa Cruz, Biotechnology). The levels of β-actin expression were used as a loading control.

Histopathologic and immunohistochemical analyses

At necropsy a careful observation was made for gross changes, then selected organs were removed by dissection and weighed. Tissue samples were fresh frozen in OCT (Optimal Cutting Temperature compound; Tissue-Tek, Sakura Finetek) or fixed in 10% buffered formalin and embedded in paraffin for sectioning. Sections (4–5 μm) were stained with hematoxylin and eosin (H&E) according to standard protocols and evaluated histologically.

Immunohistochemical analysis using standard ABC detection was done essentially as previously described (Yang et al., 1999). Antibodies used included rabbit polyclonal anti–caveolin-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and proliferative cell nuclear antigen (PCNA) (PC-10, Dako, Carpinteria, CA, USA). Some sections which were incubated in normal rabbit or mouse serum, replacing the primary antibodies, were used as controls. The TUNEL technique as previously described was used to label apoptotic cells (Yang et al., 1997).

Morphologic parameters of various organs were assessed and immunohistochemical quantitation of PCNA-positive and apoptotic bodies were conducted on randomly selected fields (measuring 0.198 μm^2 each) for each specimen according to morphometric criteria using the Eclipse 90i automated image analysis system (Nikon Instruments, Inc., Melville, NY, USA) with NIS-Elements software (version AR 3.0; Nikon).

Statistical analyses

All values are expressed as means ± SEM. Statistical analyses for body weight and organ wet weights were carried out using the two-tailed Student's unpaired *t* test. Differences were considered statistically significant when $P < 0.05$. Comparisons in tumor incidence rates were performed using Chi-square test. All analyses were performed using Statview 5.0 software (SAS Institute, Cary, NC, USA).

Results

Generation of MMTVcav-1$^+$ transgenic mice

A construct encoding mcav-1 cDNA under the control of the MMTV-LTR (Fig. 1A) was microinjected into fertilized oocytes from FVB mice. The confirmation of the transgenic expression resulted in four founder lines. RNA from various organs was isolated from each of the four lines and was subjected to the quantitative RT-PCR analysis with the Cav-1 transgene–specific primers. Among the 4 transgenic founder lines of mice (#5362, #5374, #5381, #5383), only one line, #5374, robustly expressed the Cav-1 transgene in all the organs analyzed and thus was chosen for further analyses (Figs. 1B, C). From the #5374

founder, a cohort of transgenic mice (MMTVcav-1$^+$) and their nontransgenic Cav-1 wild-type (MMTVcav-1$^-$) littermates were generated. The transgenic MMTVcav-1$^+$ mice appeared normal, and their growth pattern and body weight were indistinguishable from those of their nontransgenic littermates. Reproductive activities, including the number of pups per litter and lactation, appeared normal. An interesting observation was that the average wet weights of the liver in male MMTVcav-1$^+$ mice were statistically significantly higher than those of their nontransgenic counterparts ($P = 0.001$, Student's t test) although their average body weights were similar at adult age (Fig. 2).

Cav-1 overexpression leads to benign histopathologic phenotypes in transgenic MMTVcav-1$^+$ mice

Quantitative real-time RT-PCR (qRT-PCR) analysis of multiple organs demonstrated that MMTVcav-1 transgene expression in testis and pancreas was highest when it was normalized to transgene levels in the bladder, which were the lowest levels found in the panel of organs analyzed in male mouse (Fig. 1B). In female mouse, the mammary gland, spleen, skin, pancreas, and salivary gland expressed relatively high MMTVcav-1 transgene levels when they were normalized to those in the uterus, which demonstrated the lowest transgene levels among the organs analyzed (Fig. 1C). Histopathologic analyses revealed defective morphologic features in multiple organs, including the mammary glands, salivary glands, pancreas, skin, and lungs of the MMTVcav-1$^+$ mice.

In the mammary glands of 11-month-old virgin female transgenic mice, epithelial ductal tree and ductal side-branching activities and the number of glandular epithelial components were significantly reduced relative to those in the nontransgenic littermates (Figs. 3A, B). On immunostaining, Cav-1 was present predominantly in the adipocytes and myoepithelial cells of the ductal and glandular epithelia in the mammary glands of both transgenic and nontransgenic mice (Figs. 3C, D). However, the Cav-1 immunoreactivity in the cytoplasm of ductal and glandular epithelial cells was greater in the MMTVcav-1$^+$ mice than in the MMTVcav-1$^-$ mice. Cellular proliferation and apoptotic activities in the ductal and glandular mammary epithelia were similar in the MMTVcav-1$^+$ and MMTVcav-1$^-$ mice, as analyzed by PCNA immunostaining and TUNEL staining, respectively (data not shown).

Salivary glands from MMTVcav-1$^+$ mice also exhibited more morphologic abnormalities than did those from MMTVcav-1$^-$ mice. In the submandibular glands of MMTVcav-1$^+$ mice, mucous epithelial cells demonstrated hyperplasic or hypertrophic changes. As a result, the mucous acini appeared proportionally larger in the MMTVcav-1$^+$ than in the MMTVcav-1$^-$ mice (Figs. 4B and A, respectively). An interesting observation was that these abnormalities were more evident in female mice. Ductal epithelial hyperplasia and/or atypia in the submandibular glands was apparent in 4 of the 11 (36%) MMTVcav-1$^+$ females analyzed but in none of the 10 males analyzed (Fig. 4C). In addition, the mucous epithelial cells in MMTVcav-1$^+$ mice exhibited substantially greater Cav-1 immunostaining than did those in MMTVcav-1$^-$ mice, in which Cav-1 immunostaining was mainly localized in the stromal cells (Figs. 4D, E). In the sublingual glands, the morphologic features appeared similar in the MMTVcav-1$^+$ and MMTVcav-1$^-$ mice (data not shown). Total Cav-1 protein levels in the salivary glands of the transgenic mice were higher than those in their nontransgenic littermates, as shown by western blotting (Fig. 4F).

In the exocrine pancreas of MMTVcav-1$^+$ mice, the acinar cells had less accumulation of eosinophilic zymogen granules in their apical cytoplasm and greater basophilic basal cytoplasm than their MMTVcav-1$^-$ littermates had (Figs. 5A–D). Moreover, the pancreatic ductules in MMTVcav-1$^+$ mice were dilated and filled with an eosinophilic secretion. Further, punctuate Cav-1 immunostaining was apparent in the cytoplasm of the acinar cells and in the epithelia of pancreatic ductules of MMTVcav-1$^+$ mice (Fig. 5F). But in

MMTVcav-1^{-} mice Cav-1 immunostaining was mainly localized to stromal and endothelial cells (Fig. 5E). These phenotypic differences were observed in both sexes (Fig. 5). Additionally, the size and number of the endocrine islets labeled by cav-1 immunostaining of the pancreatic tissues were similar in MMTVcav-1^{+} and MMTVcav-1^{-} mice (data not shown). Finally, immunoblotting analysis showed that the total Cav-1 protein levels in pancreatic tissues of MMTVcav-1^{+} transgenic mice were higher than those in their nontransgenic littermates (Fig. 5G).

The general structural organization of skin tissues in the MMTVcav-1^{+} and MMTVcav-1^{-} mice appeared similar, and there were no obvious differences in the epidermis and sebaceous glands. However, when hematoxylin and eosin stained skin sections obtained from the same anatomical area and cut transversely at a similar plane were analyzed, length of the hair follicles in the telogen phase, and thickness of the dermis in the MMTVcav-1^{+} mice were greater than those in MMTVcav-1^{-} mice (Figs. 6A–D). These differences between MMTVcav-1^{+} and MMTVcav-1^{-} mice appeared to be more prominent in male than in female mice. There were high levels of Cav-1 immunostaining in the epidermis and hair follicles, but there were no obvious differences in Cav-1 immunostaining patterns between the MMTVcav-1^{+} and MMTVcav-1^{-} mouse skin tissues (Figs. 6E, F).

Finally, in the lung tissues, atypia and hyperplastic epithelial lesions were observed in the intrapulmonary bronchi and bronchioles in 7 of 14 (50%) female and 2 of 13 (15%) of the MMTVcav-1^{+} male mice, whereas such lesions were seen in only 1 of 18 (6%) of the MMTVcav-1^{-} mice of both sexes. The pulmonary epithelial atypia and/or hyperplasia in MMTVcav-1^{+} mice were characterized by increased infoldings and the presence of cells with nuclear irregularity, such as enlarged, pale nuclei with prominent nucleoli (Fig. 7). In lung tissues of both MMTVcav-1^{+} and MMTVcav-1^{-} mice, Cav-1 was highly expressed in the interalveolar septa, as demonstrated by immunostaining; however, the bronchiolar epithelia appeared to have greater Cav-1 immunostaining in MMTVcav-1^{+} mice than that in the MMTVcav-1^{-} mice (Figs. 7F and E, respectively). Moreover, the total Cav-1 protein levels in the lungs of the transgenic mice were higher than those in their nontransgenic littermates, as shown by western blotting (Fig. 7G).

Incidence of malignancies in the MMTVcav-1^{+} transgenic mice

Cohorts of 47 MMTVcav-1^{+} mice and 29 MMTVcav-1^{-} littermates, 11–13 months old, were evaluated for the occurrence of malignant tumors. Eight of the 47 (17%) MMTVcav-1^{+} mice developed malignancies (Table 1). The spectrum of tumors (Fig. 8) included 4 lung carcinomas, 3 lymphomas, and 1 liver carcinoma. In contrast, only 1 of the 29 (3%) MMTVcav-1^{-} mice developed a malignancy (lymphoma). Thus, the incidence of malignancy in the transgenic mice was about 5 times higher than that in the MMTVcav-1^{+} mice (17% vs. 3%). This difference approached but did not achieve the level of statistical significance ($P = 0.0752$, X^2 test). In 2 of the 4 lung carcinomas, Cav-1 immunostaining labeled the cancer cells (Fig. 8B).

Discussion

In this study, we successfully generated a transgenic mouse model based on the Cav-1 overexpression driven by the MMTV-LTR promoter and used it to characterize the pathologic effects of constitutive Cav-1 overexpression on specific epithelial cells. Those effects were overt benign phenotypic changes: hypotrophy of the glandular epithelia of mammary glands, mucous cell hyperplasia in salivary glands, reduced accumulation of zymogen granules in pancreatic acinar cells, elongated hair follicles and thicker skin dermis, and epithelial atypia and hyperplasia of the pulmonary bronchioles. This broad range of

lesions in multiple cell types and organs may reflect the diverse functions of Cav-1 (Liu et al., 2002).

Although Cav-1 is an integral membrane protein, it is also present in the secretory pathway of many exocrine cells (Liu et al., 1999). Our results showed that its overexpression induces disorders in specific exocrine cells (i.e. glandular epithelial cells of the mammary gland, mucous cells in the submandibular gland, and acinar cells in the exocrine pancreas). Secretion of Cav-1 from pancreatic acinar cells has been found in a complex with lipids (Thomas et al., 2004). Our observations are thus consistent with a role for Cav-1 in regulation of exocytosis (Liu et al., 1999).

Some of the phenotypic changes we observed, such as hypotrophy of the glandular epithelia of mammary glands, may be related to the aging process. The transgenic female mice appeared to conceive normally, deliver healthy, normal pups, and have normal lactation. However, 11-month-old virgin transgenic females had fewer epithelial cells in the mammary gland than the nontransgenic female mice had. This hypotrophic effect is in sharp contrast to but still somewhat consistent with the occurrence of hyperplastic mammary lesions observed in Cav-$1^{-/-}$ mice (Razani et al., 2001; Williams et al., 2003; Williams et al., 2005; Yang et al., 2008). The mechanism(s) that underlie mammary gland hypotrophy remain(s) to be elucidated. It has been reported that elevated Cav-1 expression may induce cell senescence in cultured mouse embryonic fibroblasts (Volonte et al., 2002). Additionally, degenerating neurons in Alzheimer's diseases had higher Cav-1 levels than normal cells (Gaudreault et al., 2004), and nucleus pulposus cells from degenerated spinal disks in humans exhibited elevated Cav-1 levels and a positive correlation between Cav-1 expression and p16INK4a, a cell-senescence marker (Heathfield et al., 2008). Although Cav-1 overexpression has not been shown to contribute directly to the degenerative processes, it is possible that Cav-1 overexpression accelerates degeneration changes associated with the aging process in the transgenic mammary gland.

We also found differences between the sexes for some of the phenotypic alterations. Significantly higher liver wet weights and skin phenotypic changes were found predominantly in males; and the proportion of mucous acini in the submandibular glands, and atypia and hyperplastic epithelial lesions in the intrapulmonary bronchi and bronchioles were found preferentially in females. These sexual differences may reflect, in part, the effects of sex hormone activities that have been shown under some conditions to be related to Cav-1 expression (Li et al., 2003; Lu et al., 2001; Mercier et al., 2009). Alternatively, the differences may result from different levels of transgenic activation through the MMTV promoter, which is regulated by steroid sex hormones (Otten et al., 1988).

Our analysis also revealed relatively elongated hair follicles and thickened dermis in the transgenic mice. These results are intriguing in light of the finding that Cav-1 expression in the human skin is preferentially localized to the bulge region of hair follicles (Selleri et al., 2005), where the stem cells reside (Cotsarelis et al., 1990). It has been proposed that Cav-1 is involved in regulation of follicle growth (Selleri et al., 2005) and has been reported that increased Cav-1 expression in skin may lead to increased procollagen synthesis in the dermis (Kim et al., 2008). Thus, it is conceivable that Cav-1 overexpression contributes to longer hair follicles and thickened dermis but further experiments are needed to confirm a possible mechanistic role for Cav-1 in hair growth.

The 5-fold greater incidence rate of malignant tumors we observed in the transgenic mice was interesting, but the difference was not statistically significant. The roles of Cav-1 in cellular growth, cell transformation, and malignant progression are complex, however, and a substantial body of work now clearly indicates that it can have either growth-suppressive or

oncogenic properties, depending on the cell type. In addition to experimental studies using *in vitro* and *in vivo* models that show cell type–specific growth-suppressive or oncogenic activities, numerous correlative studies using clinical tissue samples have corroborated this functional dichotomy

Some reports have documented down-regulation of Cav-1 in various malignant human tissues, including osteosarcoma (Cantiani et al., 2007), fibrosarcoma (Wiechen et al., 2001), colon cancer (Bender et al., 2000), follicular thyroid cancer (Aldred et al., 2003), ovarian cancer (Davidson et al., 2001; Wiechen et al., 2001), mucoepidermoid carcinoma of the salivary gland (Shi et al., 2007), lung adenocarcinoma (Kato et al., 2004; Wikman et al., 2004), and relatively small, estrogen receptor–positive breast cancer (Sagara et al., 2004). It is remarkable that many of these malignancies are of stromal cell origin. Overall, those findings concur with the notion that Cav-1 is a tumor-suppressor protein. A recent novel and somewhat surprising observation is relatively reduced concentrations of Cav-1 in human cancer–associated fibroblasts from breast cancers and prostate cancer (Di Vizio et al., 2009; Mercier et al., 2008).

In contrast to those correlative studies that show Cav-1 down-regulation in malignant cells, numerous other studies have documented Cav-1 overexpression in multiple cancers, including prostate cancer (Di Vizio et al., 2008; Goto et al., 2008; Karam et al., 2007; Satoh et al., 2003; Yang et al., 1998; Yang et al., 1999), esophageal squamous carcinoma (Hu et al., 2001; Kato et al., 2002), oral carcinoma (Hung et al., 2003), papillary carcinoma of the thyroid (Ito et al., 2002), pancreatic cancer (Suzuoki et al., 2002; Terris et al., 2002), renal carcinoma (Carrion et al., 2003; Horiguchi et al., 2004; Joo et al., 2004), bladder cancer (Rajjayabun et al., 2001; Sanchez-Carbayo et al., 2002), metastatic lung cancer (Ho et al., 2002), squamous carcinoma of the lung (Yoo et al., 2003), Ewing sarcoma (Tirado et al., 2006), and basal-like breast carcinoma (Elsheikh et al., 2008; Garcia et al., 2007). The results of these studies have been consistent with Cav-1–mediated oncogenic activities in human malignancies.

We find it notable that our results from this study demonstrated both hypotrophy of glandular epithelia of the mammary glands and hyperplasia of lung and salivary gland epithelial cells in MMTVcav-1 transgenic mice, findings that are consistent with both cell type– and context-dependent Cav-1 growth-related activities.

In summary, we generated a transgenic mouse model that constitutively overexpresses Cav-1 under the regulation of MMTV promoter, i.e. MMTVcav-1 transgenic mice. The transgenic mice displayed defective morphologic features, mainly in epithelial cells of multiple exocrine organs. Our results indicate that Cav-1 overexpression causes organ-specific, age-related epithelial disorders and suggest the potential for increased susceptibility to carcinogenesis. MMTVcav-1 transgenic mice should serve as a valuable tool for further characterization of the biologic functions of Cav-1 and its pathologic role in tumor progression.

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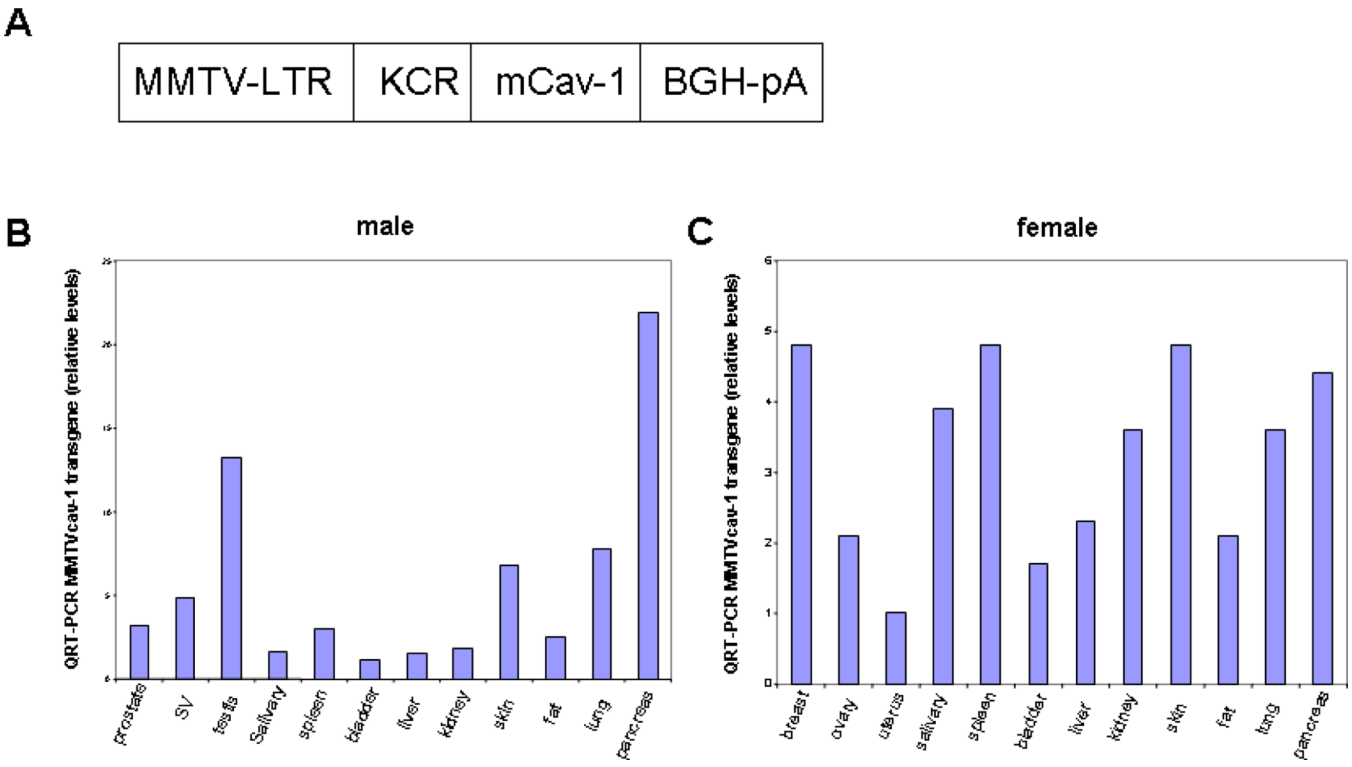

Fig. 1.

Caveolin-1 (Cav-1) expression in MMTVcav-1^{+} transgenic mice. (A) Schematic diagram of the MMTVcav-1^{+} transgene construct. (B, C) Expression of MMTVcav-1 transgene in various organs of 11-month-old male (B) and female (C).

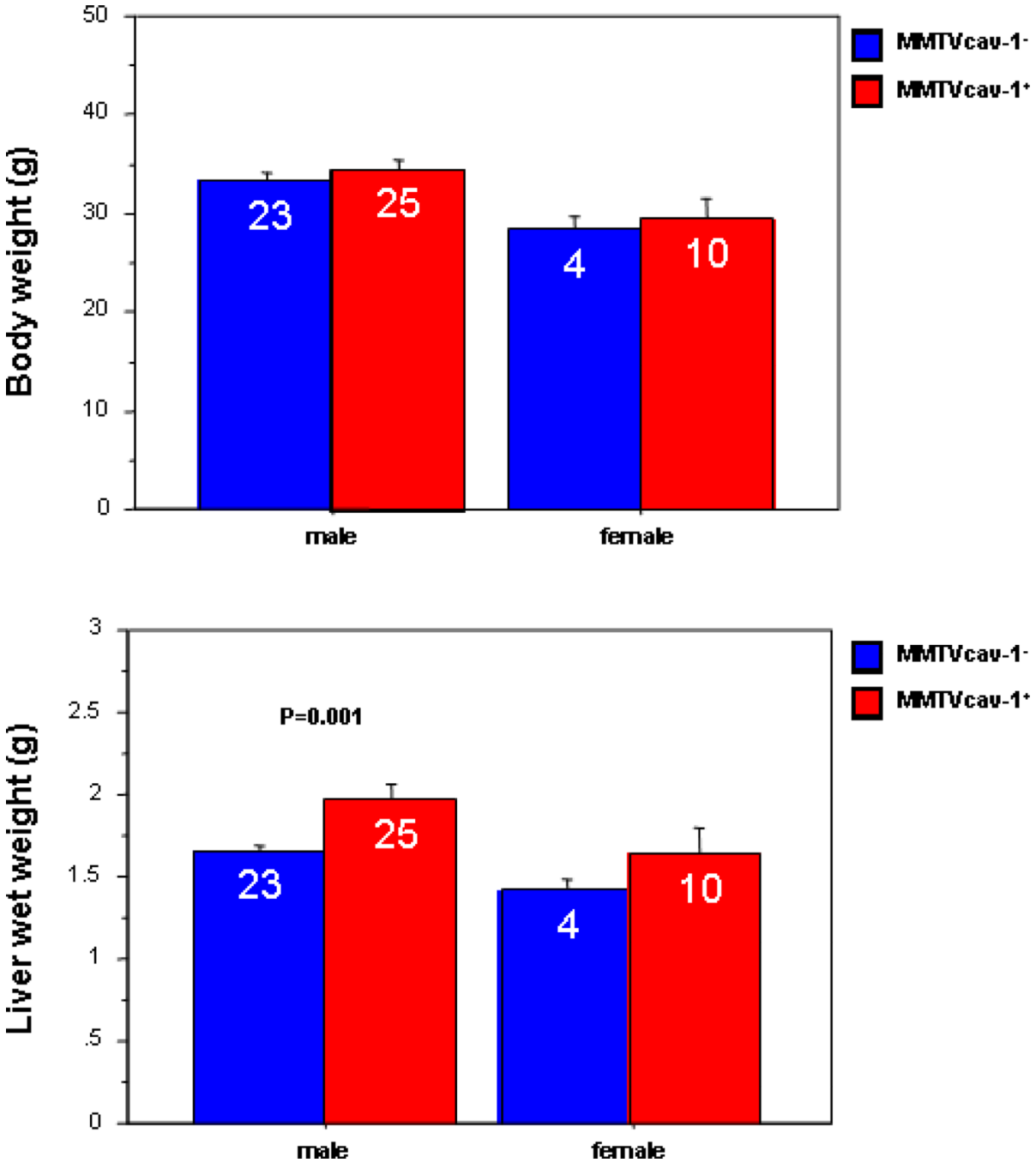

Fig. 2.

Body weight and liver wet weight comparisons of the MMTVcav-1^{+} transgenic mice and their nontransgenic littermates. Values within the bars indicate the numbers of animals examined. Error bars represent the standard error.

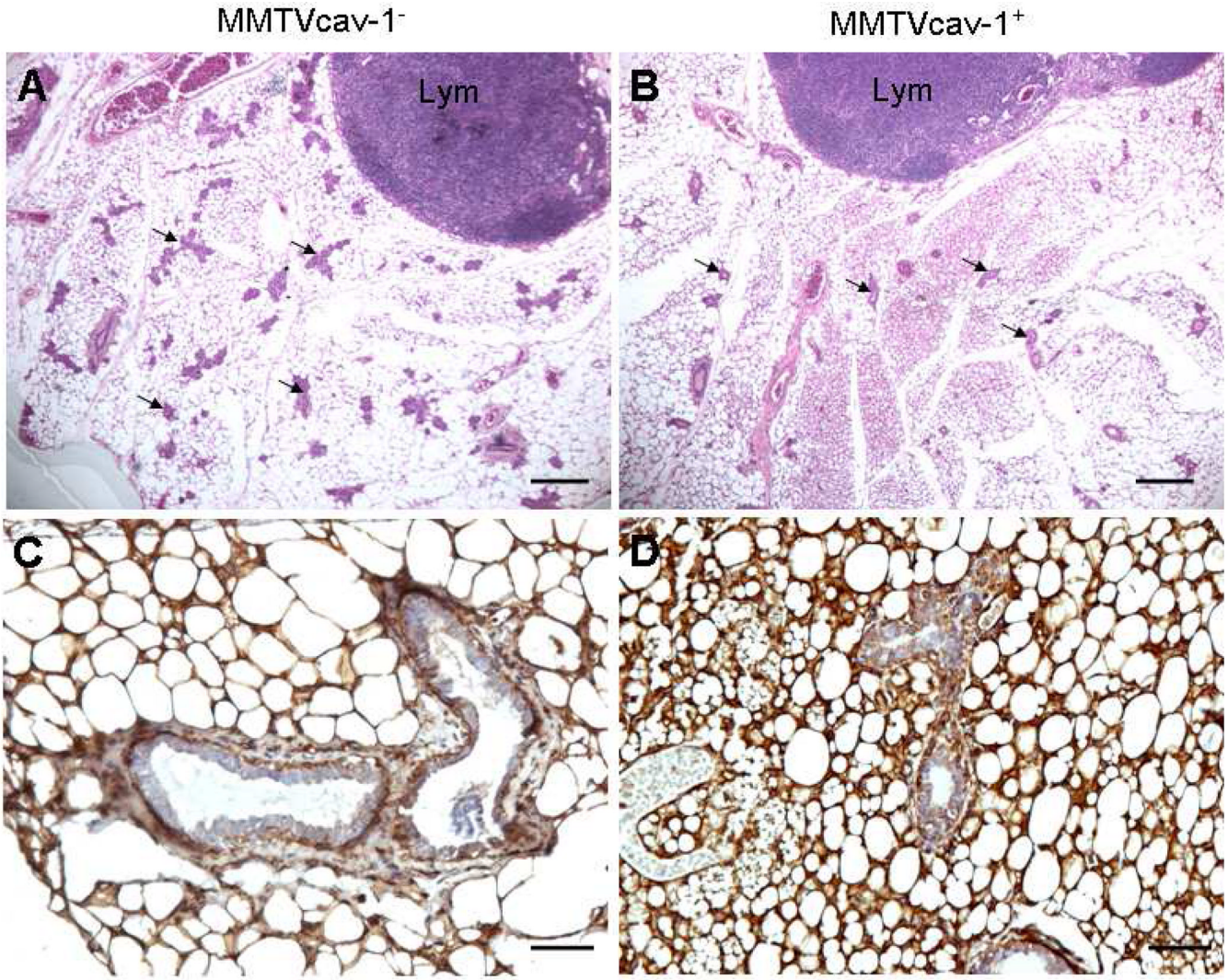

Fig. 3.

Mammary glands in 11-month-old virgin female MMTVcav-1^{-} (A, C) and MMTVcav-1^{+} (B, D) mice on H&E-stained (A, B) and Cav-1 immunostained (C, D) sections. The numbers of the ductal and/or glandular components (some of them indicated by arrows) were considerably lower in the transgenic mice (B) than in their nontransgenic littermate controls (A). Lym: lymphoid nodule. Scale bars: 500 μm (A, B), 100 μm (C, D).

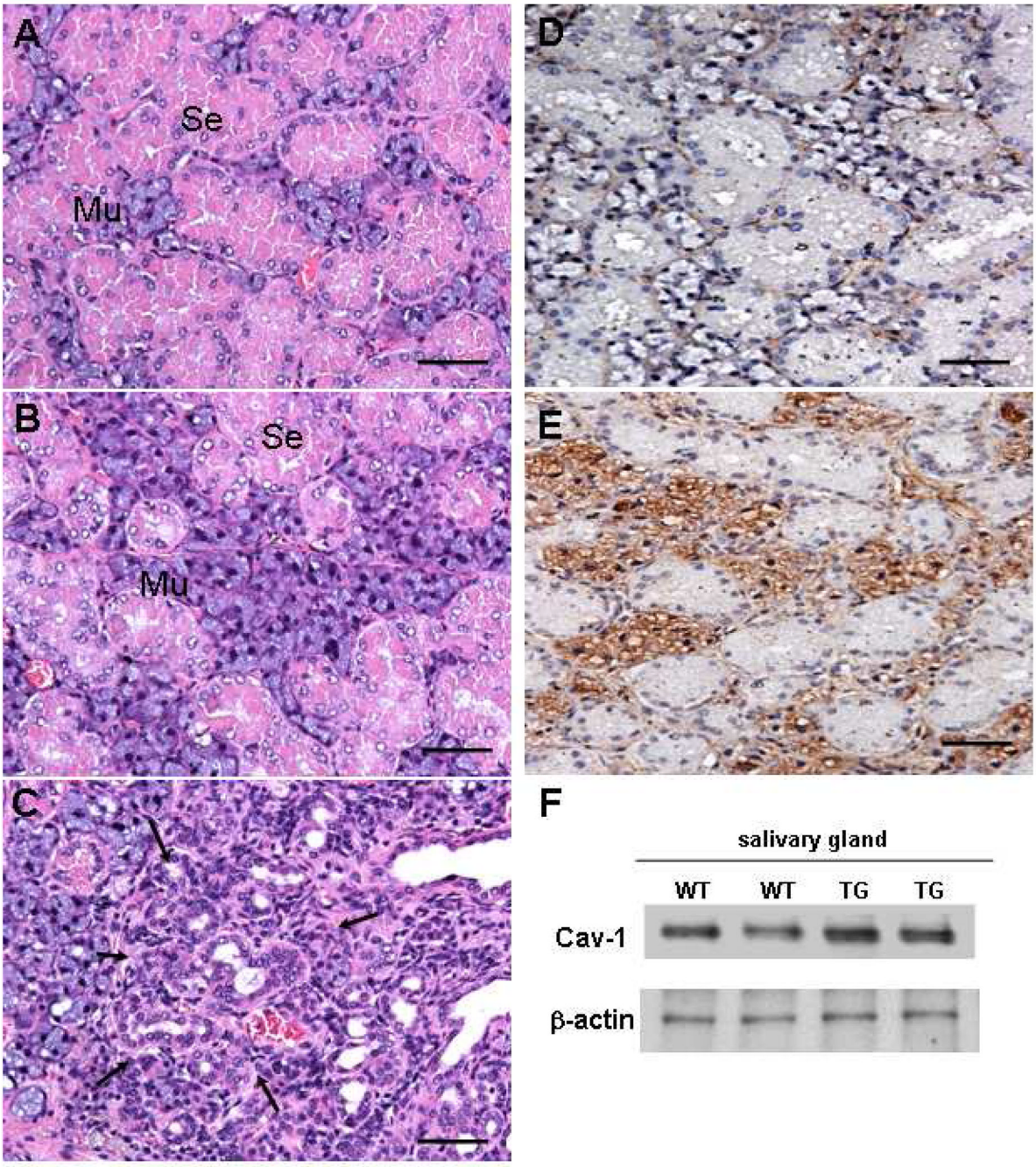

Fig. 4.

H&E-stained sections from the submandibular salivary glands of 12-month-old female MMTVcav-1^{-} (A) and MMTVcav-1^{+} mice (B, C). Cav-1 immunostaining in MMTVcav-1^{-} (D) and MMTVcav-1^{+} (E). Cav-1 western blotting of salivary glands from MMTVcav-1^{+} and MMTVcav-1^{-} mice (F). Each lane represents individual mouse. Scale bars: 100 μm. Se :serous ,Mu :mucous glandular cells. Arrows in C outline the area with ductal hyperplasia and/or atypia.

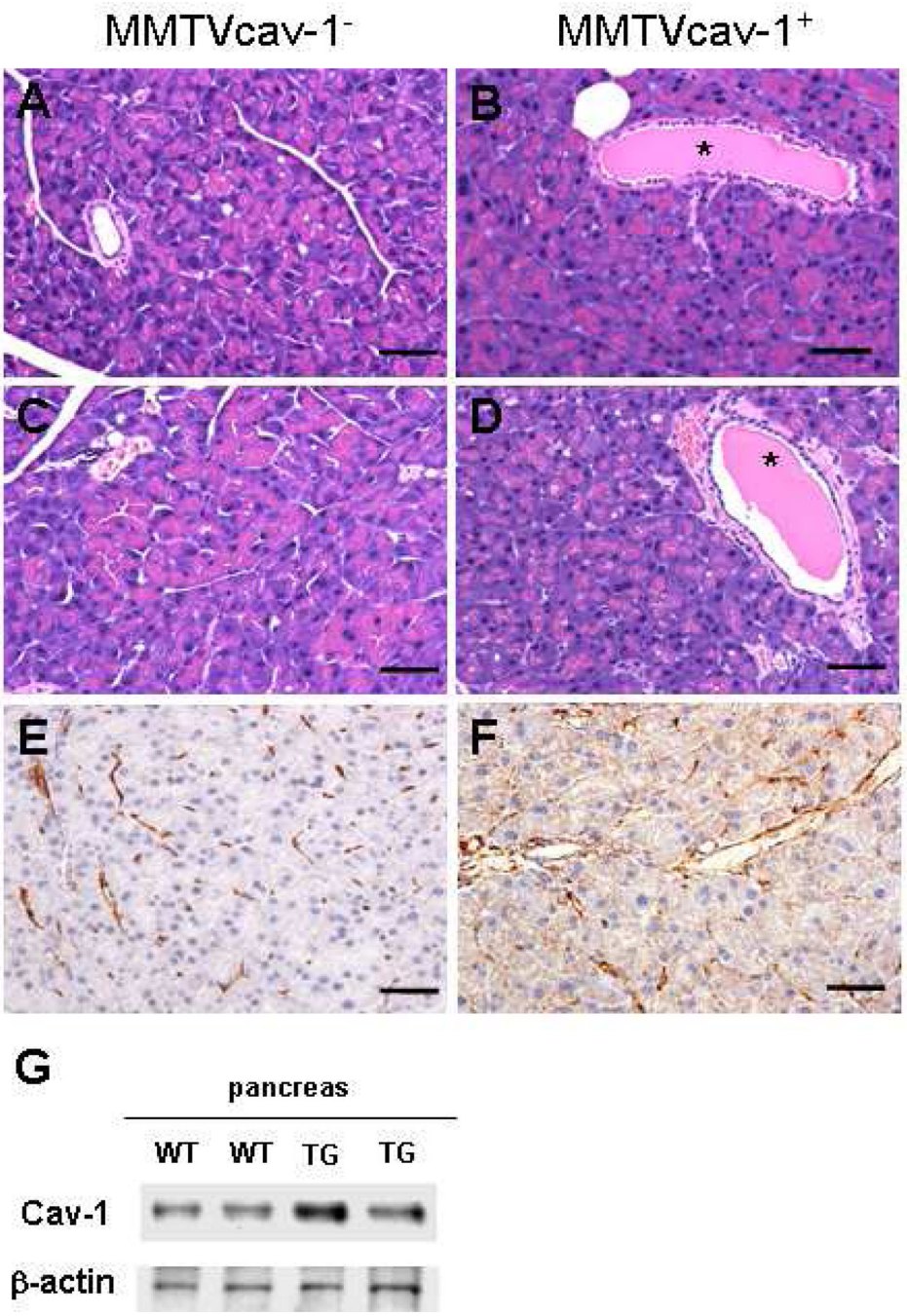

Fig. 5.

Histologic features of the pancreas of 12-month-old MMTVcav-1^{-} (A, C, E) and MMTVcav-1^{+} (B, D, F): male (A, B) and female (C, D) mice on H&E-stained sections. The MMTVcav-1^{+} exocrine pancreas comprised acini with relatively decreased accumulation of eosinophilic zymogen granules in the apical cytoplasm of acinar cells. Greater Cav-1 immunostaining was found in the cytoplasm of pancreatic acinar cells of MMTVcav-1^{+} mice (F) than in that of the MMTVcav-1^{-} mice (E), in which Cav-1 immunostaining was mainly localized in the stromal and endothelial cells. Scale bars: 100 μm. (G). Cav-1 western blot of pancreatic tissues from MMTVcav-1^{+} and MMTVcav-1^{-} mice. Each lane represents individual mouse. * denote dilated intralobular ducts full of secretion.

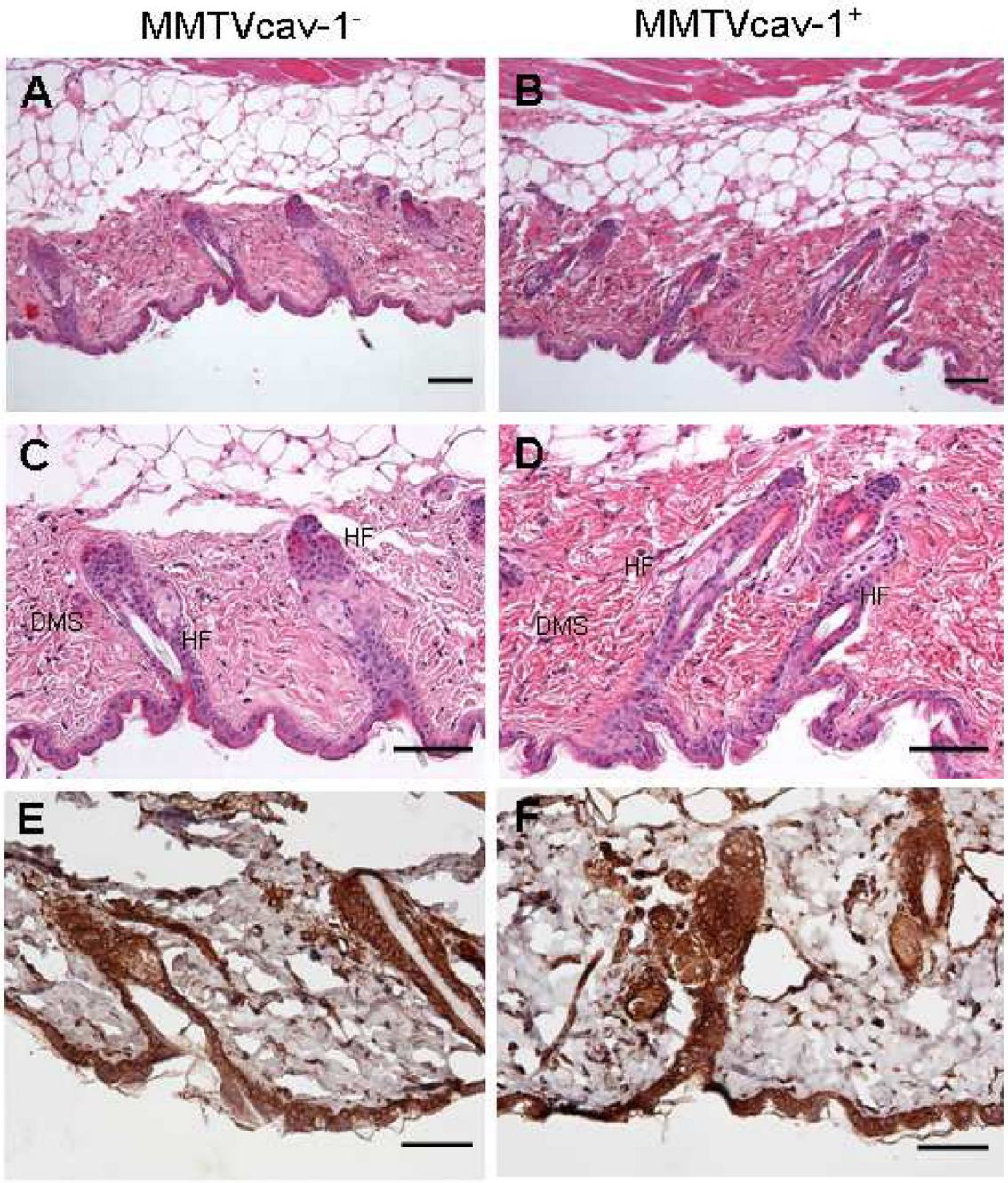

Fig. 6.

H&E-stained skin from MMTVcav-1^{-} (A and C) or MMTVcav-1^{+} (B and D) mice. The skin samples with hair follicles at the telogen phase were obtained from the same anatomic areas and cut transversely at a similar body plane for morphological comparisons. MMTVcav-1^{+} mouse skin (B, D) had longer hair follicles (HF) and a thicker dermis (Dms) than those in MMTVcav-1^{-} mouse skin (A, C). The epithelia of the epidermis and hair follicles and the fibroblasts and smooth muscle cells of the dermis of both MMTVcav-1^{-} (E) and MMTVcav-1^{+} (F) mice were strongly labeled by Cav-1 antibody. Scale bars: 100 μm.

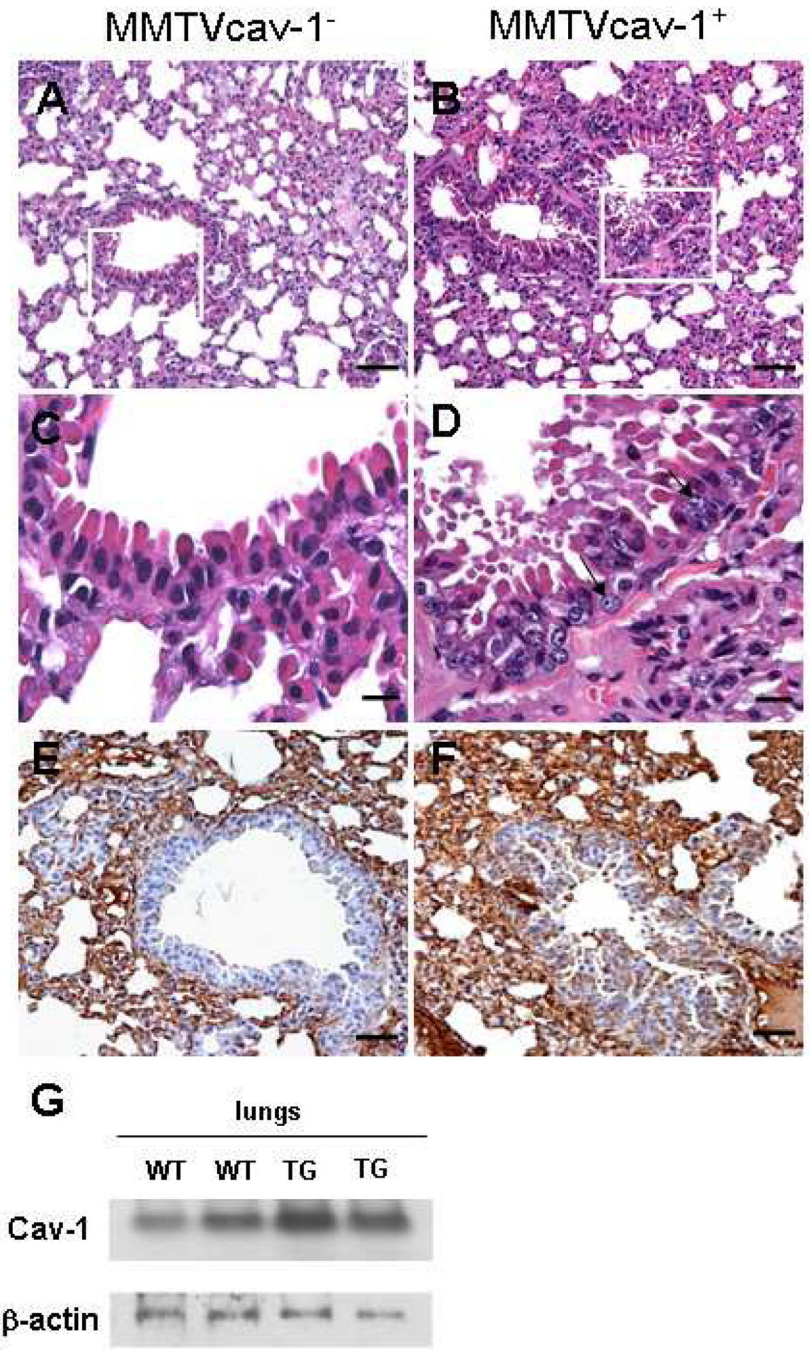

Fig. 7.

Histologic features of the lung from 12-month-old male MMTVcav-1^{-} (A) and MMTVcav-1^{+} mice (B). C and D are the magnified regions outlined by the frames in A and B, respectively. Arrows in D indicate atypical nuclei. Cav-1 immunostaining was predominantly localized to alveolar septa in both MMTVcav-1^{-} (E) and MMTVcav-1^{+} mice (F). The bronchiolar epithelia of MMTVcav-1^{+} mice (F) had greater Cav-1 immunostaining than the MMTVcav-1^{-} mice (E) had. Scale bars: 100 μm (A, B, E, F), 30 μm (C, D). (G). Cav-1 western blot of lung tissues from MMTVcav-1^{+} and MMTVcav-1^{-} mice. Each lane represents individual mouse.

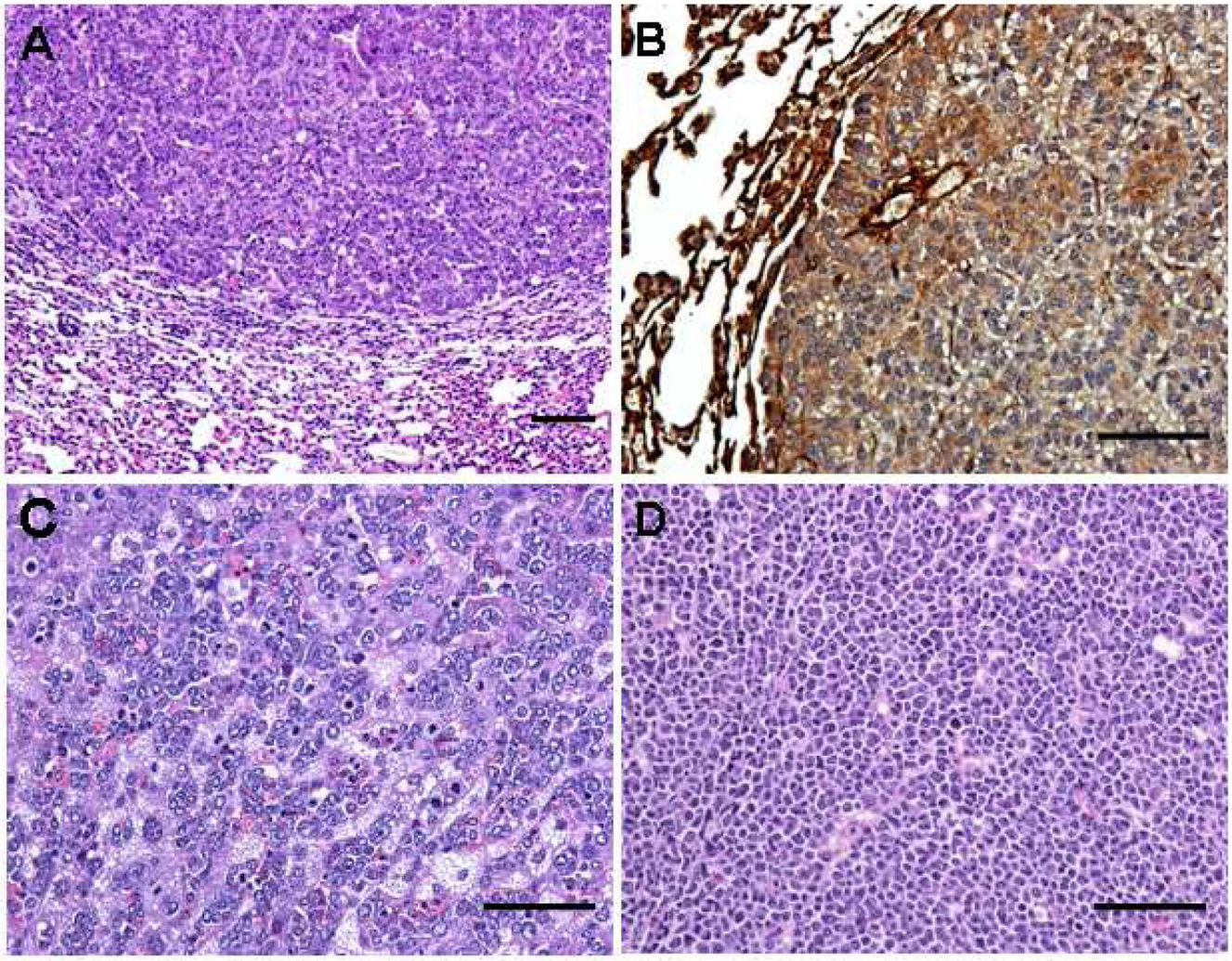

Fig. 8.

Histologic features of malignant tumors that developed in MMTVcav-1^{+} mice. (A) Lung carcinoma. (B) Cav-1 immunostaining of lung carcinoma. (C) Liver carcinoma. (D) Lymphoma. Scale bars: 100 μm.

Table 1

Malignant tumors arising in transgenic mice[a]

	MMTVcav-1$^-$		MMTVcav-1$^+$	
Type	**n=29**	**%**	**n=47**	**%**
Lung carcinoma	0		4	8.5
Lymphoma	1	3.4	3	7.9
Liver carcinoma	0		1	1.8
total	**1**	**3.4**	**8**	**17.0**

[a]MMTVcav-1$^+$ mice and their MMTVcav-1$^-$ littermates aged 11–13 months were examined for malignancies. n denotes the number of animals. The percentage indicates tumor incidence rate.