Critical Function for ADAM9 in Mouse Prostate Cancer

Lucie Peduto, Victor E. Reuter, David R. Shaffer, Howard I. Scher, and Carl P. Blobel

Abstract

ADAM9 is a membrane-anchored metalloprotease that is markedly up-regulated in several human carcinomas. Here, we show that ADAM9 is similarly up-regulated in mouse models for prostate, breast, and intestinal carcinoma. To assess whether ADAM9 is critical for the pathogenesis of prostate carcinoma, one of the most common cancers in men, we evaluated how loss of ADAM9 affects tumorigenesis in W1/6 mice, a mouse model for this disease. In the absence of ADAM9, most tumors in 50-week-old W1/6 mice were well differentiated, whereas littermate controls expressing wild-type ADAM9 had predominantly poorly differentiated, and in some cases significantly larger, tumors. Moreover, gain-of-function experiments in which ADAM9 was overexpressed in mouse prostate epithelium resulted in significant abnormalities, including epithelial hyperplasia at 4 to 6 months of age, and prostatic intraepithelial neoplasia after 1 year. A potential underlying mechanism for the role of ADAM9 in prostate cancer emerged from cell-based assays: ADAM9 can cleave and release epidermal growth factor and FGFR2iiib from cells, both of which have pivotal functions in the pathogenesis of this disease. Taken together, these results suggest that ADAM9 contributes to the pathogenesis of prostate cancer and potentially also other carcinomas, raising the possibility that ADAM9 might be a good target for anticancer drugs.

Introduction

Cell-to-cell communication is vital for development and adult tissue homeostasis. However, dysregulated or inappropriate cell-to-cell communication can contribute to the pathogenesis of diseases such as cancer. Several key components of the cell-to-cell signaling machinery, including all ligands of the epidermal growth factor receptor (EGFR) as well as other growth factors, cytokines, and their receptors, are synthesized as membrane-anchored metalloproteases whose function can be modulated by proteolytic cleavage and release from the cell (1). The enzymes that are responsible for this process, which is called protein ectodomain shedding, are frequently members of the ADAM (a disintegrin and metalloproteinase) family of membrane-anchored metalloproteases (1, 2). Protein ectodomain shedding functions as a molecular signaling switch that can activate or inactivate the released substrate protein and has emerged as a particularly important regulator of the function of EGFR ligands (1).

The ability of ADAMs to affect the function of growth factors and receptors raises questions about whether dysregulation of ADAMs might contribute to tumorigenesis. Recently, overexpression of ADAM9 has been reported in several human carcinomas, including breast (3), liver (4, 5), pancreas (6), and gastric cancer (7). The goal of this study was to address whether ADAM9 might play a causative role in tumor development. In situ mRNA analysis showed that ADAM9 is highly expressed in epithelial cells of mouse models for prostate, breast, and gastrointestinal carcinoma. More detailed analysis of prostate tumors revealed high levels of ADAM9 mRNA in well-differentiated carcinomas, but only low or undetectable levels in poorly differentiated carcinomas. This prompted an evaluation of how loss of ADAM9 function affects tumorigenesis in the W1/6 mouse model for prostate cancer (8), and how gain of function through transgenic overexpression of ADAM9 affects epithelial cells in the mouse prostate. The loss-of-function experiments suggest that ADAM9 is critical for tumor progression past the well-differentiated state, whereas gain-of-function experiments provided additional evidence for a causal role of ADAM9 in tumorigenesis. In cell-based assays, ADAM9 cleaves and releases membrane proteins with known roles in prostate epithelial proliferation and transformation, FGFR2iiib and EGF, establishing potential mechanisms for its role in carcinogenesis.

Materials and Methods

Materials. PCR reagents were from Promega (Madison, WI), restriction enzymes from NEB (Beverly, MA), goat anti-mouse ADAM9 ectodomain antibodies from R&D Systems (Minneapolis, MN), and rabbit antisnapsin-phosphorylation antibodies from Zymed Laboratories (San Francisco, CA).

In situ hybridization. Tumor sections from three mouse carcinoma models were used for ADAM9 expression analysis by mRNA in situ hybridization as previously described (9, 10). In the prostate cancer model (called W1/6 hereafter), the SV40 large T antigen is expressed in glandular epithelial cells of the prostate under control of the probasin promoter (8). In the breast cancer model, expression of the polyoma virus middle T antigen in mammary epithelium is driven by the murine mammary tumor virus promoter (MMTV-PyMT; ref. 11), and in the intestinal cancer model a germ line mutation in the adenosinatosis polyposis coli gene (Apc) leads to multiple intestinal neoplasias (Min; ref. 12).

PB-ADAM9 transgenic mice. The complete mouse ADAM9 cDNA coding sequence (bp 14-2,551) (ref. 10) was amplified by PCR with primers that introduced a 5¢ EcoRV site and a 3¢ NotI site, inserted into the SK-PB plasmid, which contains a rat probasin (PB) minimal promoter (kindly provided by Dr. N.M. Greenberg, Baylor College of Medicine, Houston, TX) and sequenced to rule out mutations. Transgenic mice were generated by pronuclear microinjection of QiaexII (Qiagen, Valencia, CA) purified PB-ADAM9 cDNA into fertilized C57Bl/6/J × CBA-E2 eggs. Founder animals were identified by PCR on genomic DNA from tail biopsies with ADAM9 primers that only amplify transgenic ADAM9 cDNA, but not genomic DNA (forward primer, bp 734-747: 5¢-CAGTGAGCGAGTATTGTTCCG-3¢; reverse primer, bp 1,489-1,513: 5¢-CTGGCAATCTTTACAACAGTCGCC-3¢). All histopathologic comparisons were between pairs of heterozygous transgensics and nontransgenic littermate controls. All animal experiments...
were approved by the Institutional Animal Care and Use Committees of the Memorial Sloan-Kettering Cancer Center and the Hospital for Special Surgery.

**Generation of \(W^{10}/Adam9^+/-\) mice.** Transgenic \(W^{10}\) females (8) were crossed with \(Adam9^+/-\) males (mixed SV129-C57BL/6 background) to generate F1 \(W^{10}/Adam9^+/-\) mice and nontransgenic \(Adam9^+/-\) littermates. For tumor studies, \(W^{10}/Adam9^+/-\) mice were bred with \(Adam9^+/+\) mice to obtain the following genotypes: \(W^{10}/Adam9^+/-\), \(W^{10}/Adam9^+/+, W^{10}/\) \(Adam9^+/-\) and \(W^{10}/Adam9^+/-\). All offspring were genotyped by PCR on genomic DNA to detect the \(W^{10}\) transgene as described (8), and by Southern blot to detect the targeted \(Adam9\) allele (13). Male littermates containing the \(W^{10}\) transgene that were either \(Adam9^+/-\) and \(Adam9^+/+\) were saved and analyzed at 50 weeks of age.

**Histopathologic analysis and immunostaining.** Transgenic PB-ADAM9 mice and littermate controls from three different lines were sacrificed at 4, 6, and 12 months of age, and \(W^{10}/Adam9^+/-\), \(W^{10}/Adam9^+/+\), and \(W^{10}/Adam9^+/-\) were sacrificed at 50 weeks of age. The urogenital tract was removed en bloc and fat and connective tissue were trimmed off. Individual prostate lobes were isolated and fixed overnight with 4% paraformaldehyde for histologic analysis. A portion of the urogenital tracts of 4-month-old PB-ADAM9 mice were embedded in OCT Tissue-Tex (Sakura Miles, Elkhardt, IN), frozen, cryosectioned, and subjected to immunofluorescent staining for ADAM9. Antisynaptophysin immunostaining was done on paraformaldehyde-fixed sections using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA).

**Cell-based protein ectodomain shedding assays.** Expression plasmids for alkaline phosphatase–tagged EGF ligands and the ectodomain shedding assay have been described previously (14, 15). The FGF2R3iiB cDNA was amplified by PCR using full-length human FGF2R3iiB cDNA as template (kindly provided by D.M. Ornitz, Washington University, St. Louis, MO) and ligated into the pAPtag5 expression plasmid (Genehunter, Nashville, TN). The AP-FGF2R3iiB deletion mutant \(\lambda_{360-1500}\) was generated by overlapping PCR. All expression constructs were sequenced to rule out undesired mutations. Expression constructs for ADAM9 and the catalytically inactive E–A mutant were described previously (10, 16).

**Results**

**Up-regulation of ADAM9 in mouse models for prostate, breast, and intestinal cancer.** To examine whether ADAM9 is up-regulated in mouse tumor models, we determined its expression by mRNA in situ hybridization at different stages of the \(W^{10}\) mouse model for prostate cancer, in which the SV40 large T antigen is expressed in prostate epithelium under control of the probasin promoter (8). Several histologically well defined states of prostate cancer are observed in mouse models for prostate cancer, including high-grade prostatic intraepithelial neoplasia, well, moderately, and poorly differentiated carcinoma (17). Whereas ADAM9 was only weakly expressed in prostates from wild-type (WT) mice (Fig. 1A–C) or in \(W^{10}\) mice with prostatic intraepithelial neoplasia (data not shown), we observed high expression of ADAM9 in epithelial cells of well-differentiated tumors (Fig. 1D–F), characterized by densely packed, well-formed glands. This pronounced expression of ADAM9 was no longer seen at more advanced stages of prostate cancer, such as in poorly differentiated tumors (Fig. 1G–I) or in metastatic carcinoma (Fig. 1J–L). The expression pattern of mouse ADAM9 at different stages of the \(W^{10}\) prostate cancer model is consistent with a gene chip analysis of ADAM9 expression at different states of human prostate cancer, which revealed a significantly increased expression of ADAM9 in nontreated primary prostate tumors compared with nonneoplastic prostate, whereas no significant increase was seen in metastatic prostate cancer compared with nonneoplastic prostate.

When we examined ADAM9 expression in a second mouse carcinoma model—breast tumors from 4-month-old MMTV-PyMT mice—we found very high ADAM9 expression in tumor cells (an acinus distended by tumor cells is shown in Fig. 1M–O), whereas little expression of ADAM9 was evident in the surrounding mammary tissue, or in high grade, poorly differentiated sections of solid tumors (data not shown). ADAM9 is expressed in a region that is similar to one that was previously described as early carcinoma resembling human ductal carcinoma in situ with early invasion in tumor sections from MMTV-PyMT mice (18). In a third mouse carcinoma model—intestinal tumors from Apc/Min/+ mice—ADAM9 was highly expressed in adenomas with strong epithelial proliferation, whereas little ADAM9 was expressed in adjacent apparently normal villi (Fig. 1P–R). Thus, ADAM9 expression was strongly up-regulated in epithelial cells of three different mouse carcinoma models.

**More well-differentiated tumors are seen in \(W^{10}\) mice lacking ADAM9 compared with \(W^{10}\) littermate controls.** To determine whether loss of ADAM9 affects development of prostate cancer or its progression, we crossed \(Adam9^-/-\) with \(W^{10}\) mice, a model for prostate cancer (8). Through appropriate matings, we generated \(W^{10}\) mice lacking ADAM9 as well as littermate controls carrying one or both WT alleles of ADAM9 (see Materials and Methods). At 50 weeks of age, prostates of all mice were harvested and weighed in a double-blinded manner. We found that 5 of 22 \(W^{10}\) mice with one or both WT alleles of ADAM9 (\(W^{10}/Adam9^+/+\) or \(W^{10}/Adam9^+/-\)) had tumors weighing >200 mg at 50 weeks, whereas all tumors in the 18 \(W^{10}/Adam9^-/-\) mice analyzed weighed <200 mg (\(P < 0.05\), t test; Fig. 2A).

To determine whether ADAM9, which is highly expressed at the well-differentiated carcinoma state, might affect the progression of prostate cancer to poorly differentiated carcinoma, we did a double-blinded analysis of the tumor histology of representative sections of tumors from 50-week-old \(W^{10}/Adam9^-/-\), \(W^{10}/Adam9^+/+,\) and \(W^{10}/Adam9^+/-\) mice. The lesions were classified as well-differentiated carcinoma when gland formations lined with epithelial cells were present throughout the tumor sections, as moderately differentiated carcinoma when focal to extensive areas of solid cell nests and fused glands were mixed with well formed glands, and as poorly differentiated carcinoma when large areas of the tumor were composed of more solid sheets or nests, with rare gland formation (Fig. 2B, top, shows examples of each tumor state). This analysis revealed that 58% of tumors from \(W^{10}/Adam9^-/-\) mice contained only well-differentiated carcinoma, 42% of tumors contained some moderately differentiated carcinoma, whereas none had areas of poorly differentiated carcinoma (\(n = 12\); Fig. 2B, bottom). On the other hand, 50% of tumors in littermate controls (\(W^{10}/Adam9^+/+\) or \(W^{10}/Adam9^+/-\) mice) contained poorly differentiated carcinoma, 33% contained moderately differentiated carcinoma whereas 17% contained only well-differentiated carcinoma (\(n = 12\); Fig. 2B, bottom). Thus, there was significantly more well-differentiated carcinoma and less poorly differentiated carcinoma in \(W^{10}\) mice in the absence of ADAM9, compared with controls with one or both copies of WT ADAM9.

**Transgenic overexpression of ADAM9 in mouse prostate results in abnormal acinar epithelium and high-grade prostatic intraepithelial neoplasia.** The high expression of ADAM9 in epithelial cells of three mouse carcinoma models coupled with the results of the loss-of-function studies prompted us to assess how transgenic overexpression of ADAM9 affects prostate epithelial cells. For this purpose, we generated three
independent mouse lines that overexpress ADAM9 under control of the minimal probasin promoter (PB-ADAM9). PCR and Western blot analysis confirmed that overexpression of ADAM9 was restricted to the dorsolateral prostate lobes in all three lines (data not shown). Urogenital tracts from 4-, 6-, and 12-month-old male PB-ADAM9 mice and nontransgenic littermates were collected for further analysis. In 4- and 6-month-old PB-ADAM9 mice, histopathologic analysis of dorsolateral prostate lobes uncovered disorganized prostate ducts with areas that seemed to lack a proper epithelial lining and lumens filled with rounded cells (Fig. 3A). These abnormalities were not seen in any sections of prostate glands of WT littermates (Fig. 3D). Immunofluorescent analysis of adjacent cryosections stained with anti-ADAM9 antibodies confirmed high ADAM9 expression in transgenic glands and in cells filling the lumen of these glands (Fig. 3B).

Prostate ducts with a similar appearance as those seen in 4- and 6-month-old PB-ADAM9 mice (disorganized epithelia, lumen filled with cells) have also been described in transgenic mice overexpressing a dominant-negative form of the FGFR2iiib under the probasin promoter (19). In prostates from these animals, increased expression of the neuroendocrine marker synaptophysin was observed in the smooth muscle area adjacent to the basement membrane. When prostates from 4-month-old PB-ADAM9 mice and WT littermate controls were stained with antibodies against synaptophysin, we observed a clear increase of stained cells within the smooth muscle areas adjacent to the

Figure 1. ADAM9 is overexpressed in mouse models for prostate, breast, and intestinal carcinoma. In situ mRNA hybridization to detect expression of ADAM9 in prostate tumors from W10 mice (8), mammary tumors from MMTV-PyMT mice (11), and intestinal tumors from ApcMin/+ mice (12). A to /, prostate sections. Whereas ADAM9 is weakly expressed in normal WT prostate (A-C), high expression is observed in epithelial cells of a well-differentiated prostate tumor (WDCA, arrows in D-F), relative to the adjacent negative stroma. Little ADAM9 is detected in a poorly differentiated carcinoma (PDCA, G-I) and a small lung metastasis (MET, J-L). M to O, MMTV-PyMT mammary tumor. High levels of ADAM9 mRNA are seen in tumor cells filling an enlarged duct (lobular carcinoma in situ, arrow). P to R, ApcMin/+ model. High expression of ADAM9 is visible throughout the adenoma (arrow), whereas ADAM9 expression is low in the apparently normal adjacent villi (indicated by an asterisk in O). Each panel shows result that is representative for at least five different tumors. Magnifications were ×100 in the left and middle panel of each row and ×600 in the right panel of each row.
basement membrane in PB-ADAM9 mice compared with non-transgenic littermates (compare Fig. 3C and F).

When we inspected prostates of 12-month-old PB-ADAM9 mice, we observed considerable epithelial cell hyperplasia with stratification of the nuclei (Fig. 3G and H), whereas prostates from nontransgenic littermates did not have any evident abnormalities (Fig. 3I and J). In some sections of PB-ADAM9 prostates, more severe abnormalities could be seen, including extensive layering of the acinar epithelial cells, cells with increased nuclear to cytoplasmic ratio, variability in nuclear shape, condensed chromatin, prominent nucleoli, and appearance of mitotic figures. These abnormalities are consistent with the changes associated with prostatic intraepithelial neoplasia, considered to be a precursor of invasive prostate carcinoma in humans.

ADAM9 cleaves FGFR2iiib and epidermal growth factor in cell-based assays. The similarities between the histopathologic changes seen in 4-month-old PB-ADAM9 animals (Fig. 3A-C) and in mice expressing a dominant-negative FGFR2iiib under control of the probasin promotor (19) raised the question whether overexpressed ADAM9 could cleave and potentially down-regulate FGFR2iiib. If this were the case, ADAM9 might affect signaling via FGFR2iiib in a similar manner as overexpression of a dominant-negative receptor. To address this possibility, we cotransfected WT ADAM9 or a catalytically inactive E>A mutant together with FGFR2iiib in Cos-7 cells. Overexpression of ADAM9 resulted in a strong increase in ectodomain shedding of FGFR2iiib into the supernatant, whereas overexpression of the inactive ADAM9E>A control did not (Fig. 4B). ADAM9-dependent shedding of FGFR2iiib was only weakly enhanced by phorbol 12-myristate 13-acetate (PMA), a phorbol ester that is commonly used to stimulate ectodomain shedding. No shedding was observed when a mutant FGFR2iiib lacking 14 membrane-proximal amino acid residues (del 363-376aa) was cotransfected with ADAM9 WT, strongly suggesting that FGFR2iiib is cleaved in its juxtamembrane region (Fig. 4B).

The EGFR signaling pathway has also been implicated in the pathogenesis of prostate cancer (20–22), and proteolytic processing...
of EGFR ligands is important for regulating the function of these growth factors (1, 23–25). Because ADAMs participate in the shedding of six of seven EGFR ligands (15), we tested whether overexpression of ADAM9 triggers the release of one or more of these proteins. When ADAM9 WT was overexpressed in Cos-7 cells together with different EGFR ligands, we observed a significant increase in the release of EGF, but not of HB-EGF, transforming growth factor-α (TGF-α), betacellulin, amphiregulin, or epiregulin (Fig. 4C). ADAM9-dependent shedding of EGF was only weakly enhanced by treatment with PMA, and was not seen in the presence of the overexpressed catalytically inactive ADAM9E>A mutant (Fig. 4C). The apparent mass of soluble EGF released by ADAM9 differed from that of endogenously released EGF, indicating that ADAM9 has a different cleavage site than the endogenous EGF sheddase (most likely ADAM10; ref. 15).

Discussion

Membrane-anchored peptidases can function as critical regulators of cell-to-cell signaling. This study was initiated because the membrane anchored metalloprotease-disintegrin ADAM9 is highly expressed in several human carcinomas (3–6). We found that ADAM9 is also prominently expressed in epithelial cells undergoing neoplastic changes in mouse models for prostate, breast, and intestinal cancer. In addition, the more detailed analysis of different tumor states in the W10 mouse model for prostate cancer uncovered highest expression of ADAM9 in well-differentiated tumors of the prostate. The observed up-regulation of ADAM9, a cell surface peptidase with a potential role in cell-to-cell signaling, raised questions about whether the enhanced expression is merely correlative, or whether it might also have a causative role in tumorigenesis. To address this, we did loss-of-function experiments with W10 mice crossed with mice lacking ADAM9, and gain-of-function experiments by transgenic overexpression of ADAM9 under the prostate specific probasin promoter (PB-ADAM9 mice).

The loss-of-function experiments revealed a critical function of ADAM9 in prostate cancer growth and progression in W10 mice. Remarkably, most of the tumors in W10/Adam9−/− mice were well-differentiated carcinomas, whereas most of the tumors in littermates with one or both copies of WT ADAM9 contained significant areas of poorly differentiated carcinoma. Moreover, 5 of 22 W10 mice with WT ADAM9 had large or very large tumors. No poorly differentiated or very large tumors were seen in W10 mice lacking ADAM9. These results suggest that prostate tumor initiation or progression is delayed in W10/Adam9−/− mice, or that tumor progression is possibly even arrested at the
Overexpression of ADAM9 results in ectodomain shedding of tumor model. These mice accelerates tumor growth and progression. Nevertheless, it is tempting to speculate that less FGFR2iiib might mimic the dominant-negative FGFR2iiib in the prostate to disrupt FGF-7 signaling (19). Because changes in the FGF signaling pathway are believed to be critical for the development of prostate cancer (26), we tested whether ADAM9 can cleave FGFR2iiib in cell-based assays. We found that overexpressed ADAM9 enhances shedding of FGFR2iiib from cells, which is consistent with a model in which ADAM9 overexpressed in prostate epithelium could release and down-regulate FGFR2iiib. In addition, the soluble ectodomain of FGFR2iiib has been reported to function in a dominant-negative fashion and disrupt FGF signaling in vivo (27). In PB-ADAM9 transgenics, cleavage of FGFR2iiib by ADAM9 could, therefore, conceivably contribute to the early changes seen at 4 and 6 months by disrupting FGFR2iiib signaling. This notion is also supported by the observed up-regulation of the neuroendocrine marker synaptophysin in PB-ADAM9 mice, which was also seen in transgenic mice expressing the dominant-negative FGFR2iiib.

With respect to the possible role of the FGFR2iiib at later stages of tumorigenesis, it is noteworthy that this receptor is frequently down-regulated during progression of human prostate cancer and animal tumor models to malignancy, such as in the TRAMP model (28). This down-regulation of FGFR2iiib is considered a feature of malignant stroma-independent epithelial cells (29, 30). On the other hand, transfection of WT FGFR2iiib into malignant prostate cancer cells is sufficient to inhibit growth and promote differentiation (31, 32). In the context of the loss-of-function experiments in W10 mice, it is tempting to speculate that less FGFR2iiib might shed from Adam9+/− epithelial cells than from Adam9+/+ cells. Less FGFR2iiib shedding in Adam9+/− cells could conceivably contribute to the early changes seen at 4 and 6 months by disrupting FGFR2iiib signaling. This notion is also supported by the observed up-regulation of the neuroendocrine marker synaptophysin in PB-ADAM9 mice, which was also seen in transgenic mice expressing the dominant-negative FGFR2iiib.

Gain-of-function experiments provided additional evidence for a causal role of ADAM9 in tumorigenesis. We found that PB-ADAM9 mice at different ages had significant abnormalities compared with age-matched littermate controls. Specifically, at 4 and 6 months of age, transgenic animals of three different lines invariably displayed areas of structurally abnormal and disorganized acinar ducts that were filled with round epithelial cells, with a concomitant increase of synaptophysin-positive cells in the adjacent smooth muscle surrounding the ducts. After 1 year, the dorsolateral prostate lobes of mice from all three lines displayed high-grade prostatic intraepithelial neoplasia lesions, with crowded epithelial cells showing nuclear and nucleolar enlargement.

Taken together, the gain- and loss-of-function studies provide independent lines of evidence for a role of ADAM9 in carcinogenesis. Whereas the loss-of-function studies establish a critical role for ADAM9 in an in vivo tumor model, the gain-of-function experiments show that overexpression of ADAM9 alone is sufficient to trigger preneoplastic changes, including high-grade prostatic intraepithelial neoplasia. Further studies will be necessary to determine whether transgenic overexpression of ADAM9 at the well-differentiated carcinoma state in W10 mice accelerates tumor growth and progression. Nevertheless, it is tempting to speculate that ADAM9, which is highly expressed at the well-differentiated carcinoma state in W10 mice (see Fig. 1D-F), might contribute to cell proliferation and promote neoplastic changes in W10 mice in a similar manner as when it is overexpressed in normal prostate epithelia in PB-ADAM9 mice.

The early lesions observed in PB-ADAM9 mice share striking similarities with the disorganized prostatic ducts in mice expressing a dominant-negative FGFR2iiib in the prostate to disrupt FGF-7 signaling (19). Because changes in the FGF signaling pathway are believed to be critical for the development of prostate cancer (26), we tested whether ADAM9 can cleave FGFR2iiib in cell-based assays. We found that overexpressed ADAM9 enhances shedding of FGFR2iiib from cells, which is consistent with a model in which ADAM9 overexpressed in prostate epithelium could release and down-regulate FGFR2iiib providing one possible explanation for the higher percentage of well-differentiated carcinomas in 50-week-old W10/Adam9+/− compared with littermate W10/Adam9+/+ controls.

Whereas the changes observed in 4- and 6-month-old transgenic PB-ADAM9 mice resemble those seen in animals expressing a dominant-negative FGFR2iiib, the development of prostatic intraepithelial neoplasia in 1-year-old transgenic PB-ADAM9 mice suggests that additional mechanisms might be affected by overexpression of ADAM9 in prostate epithelium (33). Because ADAMs have also been implicated as regulators of EGFR ligands,
we asked whether ADAM9 can shed various EGFR ligands. We found that overexpression of ADAM9 leads to a clear enhancement of EGF shedding, but not of any of the other EGFR ligands tested here, including HB-EGF, which was previously reported as a substrate for ADAM9 (34). Whereas the reason for this discrepancy remains to be determined, the unequivocal ability of ADAM9 to cleave EGF has potential implications for its role in prostate cancer. EGF is present in large amounts in human prostatic fluid (35), is up-regulated in benign prostatic hyperplasia and tumors (36), has been implicated in the proliferation of epithelial and stromal prostate cells (37), and is expressed in epithelial cells in the TRAMP mouse model for prostate cancer (38), which is similar to the W10 model. Moreover, chronic systemic treatment with EGF induces prostate growth in young rats (39), and EGF has a role in androgen-independent transactivation of the androgen receptor, which in turn is thought to contribute to androgen-independent tumor growth (40, 41). Because ADAM9 can shed EGF in cell-based assays, we hypothesize that it might also increase EGF release and, thus, EGFR activation when it is overexpressed in epithelial prostate cells. In theory, the combination of EGFR activation and inactivation of FGR2riib could both promote epithelial cell proliferation, which in turn might raise the likelihood of acquiring additional deleterious mutations in the transgenic PB-ADAM9 mice. Likewise, ADAM9-dependent release of EGF at the well-differentiated state of prostate cancer in W10 mice could also contribute to progression of prostate cancer in this model, although ADAM9 might also have other mechanisms of action. Taken together, these results provide the first evidence for a causal role of ADAM9 in the pathogenesis of cancer. ADAM9 is up-regulated, directly or indirectly, in three different mouse tumor models, in several human carcinomas, and its expression is also known to be induced by the oncogene c-myc (42). ADAM9 deficiency delays or prevents prostate tumor progression to advanced states in a mouse prostate cancer model, and dysregulated expression of ADAM9 initially disrupts epithelial-stromal homeostasis in the prostate epithelium, and later leads to prostatic intraepithelial neoplasia lesions. Cell-based studies show that ADAM9 has the ability to process and shed FGR2riib and EGF, both of which have well-established roles in prostate cancer. Moreover, ADAM9 could also have other yet to be identified substrates or other functions that contribute to its role in tumorigenesis, such as roles in cell-to-cell interactions or signaling via its cytoplasmic domain. These findings suggest that ADAM9 might, therefore, be a novel target for the treatment of carcinomas in which this enzyme is highly expressed.

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