Multiple Acquired Renal Carcinoma Tumor Capabilities Abolished upon Silencing of ADAM17

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Abstract

Malignancy is a manifestation of acquired defects in regulatory circuits that direct normal cell proliferation and homeostasis. Most of these circuits operate through cell autonomous pathways, whereas others potentially involve the neighboring microenvironment. We report that the metalloprotease ADAM17 plays a pivotal role in several acquired tumor cell capabilities by mediating the availability of soluble transforming growth factor-α, an epidermal growth factor receptor (EGFR) ligand, and thus the establishment of a key autocrine signaling pathway. Silencing of ADAM17 in human renal carcinoma cell lines corrects critical features associated with cancer cells, including growth autonomy, tumor inflammation, and tissue invasion. Highly malignant renal carcinoma cells fail to form in vivo tumors in the absence of ADAM17, confirming the essential function of this molecule in tumorigenesis. These data show that ligand shedding is a crucial step in endogenous EGFR activation and endorse prospective therapeutic strategies targeting ADAM17 in human cancer. (Cancer Res 2006; 66(16): 8083-90)

Introduction

Cancer cells share numerous defects in distinct regulatory circuits that govern cell proliferation and homeostasis; these defects are often called hallmarks of cancer (1). Through successive genomic alterations, transformed cells develop intricate programs that drive tumor-associated phenotypes, including growth autonomy, resistance to apoptosis, and tissue invasion. These common, yet complex, acquired tumor cell capabilities are thought to define malignancy of the majority of neoplasia and have thus been exploited in anticancer therapeutic strategies. It is thought that these acquired capabilities arise from the alterations of functionally distinct genes and, as a consequence, unrelated regulatory circuits (2, 3). Whether these alterations occur during the earliest steps of transformation or follow a linear model of chronological events remains debatable. Certainly, an overwhelming number of independent pathways have been implicated in the process of tumorigenesis. Nonetheless, it is reasonable to speculate that self-sufficiency in growth signaling is the initial acquired capability during the process of cellular transformation and perhaps the most critical aspect of neoplasia. Normal cells require exogenous growth stimulatory cues to engage in cellular division. This is reflected by their ability to withdraw from the cell cycle and enter quiescence in the absence of exogenous growth factors (4). This tightly regulated growth control is lost in essentially all cancer cells, often as a consequence of the constitutive production of endogenous growth factors (1, 5, 6). Self-sufficiency in growth signaling can be triggered by the aberrant and constitutive production of growth factors in a process called an autocrine loop. A suitable example of acquired autocrine loop capacity is observed in clear cell renal carcinoma cells. Renal epithelia require exogenous growth factors to exit quiescence and enter the cell cycle (7, 8). In stark contrast, renal carcinoma cells are able to constitutively produce the soluble transforming growth factor-α (TGF-α), a mitogen of renal epithelial cells and a ligand of the epidermal growth factor receptor (EGFR; refs. 9–13). Inhibition of the TGF-α/EGFR growth stimulatory pathway is sufficient to prevent self-sufficiency in growth of renal carcinoma cells in culture and, as a result, tumor formation in vivo (9, 14, 15). In several systems, TGF-α undergoes ectodomain shedding by ADAM17, a metalloprotease also called TACE [tumor necrosis factor-α (TNF-α) converting enzyme; refs. 16, 17]. However, it remains unclear whether this process is a universal requirement for EGFR signaling and tumorigenesis (18, 19). ADAM17 is involved in the ectodomain shedding of a wide variety of membrane-bound ligands and cytokines that are implicated in diverse biological processes, including growth and inflammation (20). Recent preclinical studies have yielded encouraging results as to the potency of specific ADAM17 inhibitors in the treatment of inflammatory diseases, such as rheumatoid arthritis and ischemic stroke (21, 22). Given the number of anti-ADAM17 drugs that are currently in development and its prospective role in TGF-α processing and renal carcinoma tumorigenesis, we decided to examine the function of this specific sheddase in a model of human cancer for therapeutic purposes.

We report that blocking TGF-α shedding by stably silencing ADAM17 in two independent human renal carcinoma cell lines, 786-O and KTCL, is sufficient to suppress EGFR activation and multiple unrelated acquired tumor cell capabilities. Silencing of ADAM17 restored dependence on exogenous growth factor signaling, suppressed acquired pathways involved in tissue invasion, and prevented in vivo tumor formation of highly malignant renal cancer cells. These data show that the frequently altered regulatory circuits implicated in the process of tumorigenesis intersect at ADAM17, providing compelling evidence to target this particular metalloprotease in anticancer therapy.

Materials and Methods

Cell culture and reagents. The human sporadic von Hippel-Lindau(−/−) [VHL(−/−)] renal carcinoma cell line 786-O was obtained from the American Type Culture Collection (Rockville, MD). The 786-O cells stably transfected with hemagglutinin-tagged VHL (786-O + VHL) were a kind gift from Dr. W.G.
Kaelin (Harvard University, Boston, MA). The KTCL cell line was a kind gift from Dr. Peter Ratcliffe (University of Oxford, Oxford, United Kingdom). Cell lines were incubated at 37°C under a 5% CO2 environment. Cell lines were maintained in serum-containing medium consisting of DMEM with 5% fetal bovine serum (FBS). Serum-free medium consisted of DMEM supplemented with 1% insulin-transferrin-selenium (ITS; Invitrogen, Burlington, Ontario, Canada).

ADAM17 RNA interference. For transient silencing of ADAM17, the VHL(-/-) renal carcinoma cell lines 786-O and KTCL were transfected with commercially available double-stranded 21-nucleotide-long small interfering RNA (siRNA) targeting ADAM17 or a control siRNA (Ambion, Austin, TX). The cell lines were also stably transfected to express one of two independent short-hairpin RNA (shRNA) sequences targeting ADAM17 with Effectene reagent (Qiagen, Valencia, CA). For each sequence, two complimentary ssDNA oligonucleotides designed with overhangs encoding BamHI/HindIII restriction enzyme sites were synthesized and subsequently annealed with 1X DNA Anneling Solution according to the protocol of the manufacturer (Ambion). The annealed inserts were subsequently ligated into a pSilencer 3.1-H1 neo vector (Ambion). Sequence 1 (5’-3’): shRNA ADAM17-1 forward GAAGUGAUAUGAGCAGAUU and ADAM17-1 reverse AUAUCGUCAUAUACUC (Ambion, siRNA ID: 12917). Sequence 2 (5’-3’): shRNA ADAM17-2 forward AACGCTGTATCTGTCTCCTCA and shRNA ADAM17-2 reverse AATAGAGAGCAAAGAATCAAGC (23). All constructs were verified by standard DNA sequencing. A pSilencer 3.1-H1 neo vector encoding scramble shRNA was purchased and served as a negative control. Positive clones were selected and maintained in neomycin-containing medium. The 786-O cell lines stably transfected with shRNA-targeting EGFR were generated by our group as previously described (15).

Measurement of TGF-α levels. An equal number of cells were plated and incubated for indicated times in DMEM supplemented with 5% FBS. In experiments involving matrix metalloprotease inhibitors, cells were treated with 10 to 100 μmol/L GM6001 (Calbiochem, San Diego, CA) or corresponding volumes of DMSO as a vehicle control. The cell lysates and conditioned medium were collected and TGF-α levels were analyzed according to ELISA kit instructions (Oncogene, Boston, MA).

Western blot analysis. Cells were washed with PBS and harvested in 4% SDS in PBS. Protein concentrations were quantified using a BCA protein assay kit and samples (50 μg protein) were separated on a denaturing polyacrylamide gel containing SDS and transferred to a methanol-activated polyvinylidene difluoride membrane (NEN, Boston, MA). Before immunodetection, membranes were blocked with 5% (w/v) skim milk powder in a 0.2% Tween-PBS solution. Membranes were then incubated with anti-actin (Sigma, St. Louis, MO), anti-HIF2α (Novus, Littleton, CO), anti-ADAM17 precursor (Santa Cruz Biotechnology, Santa Cruz, CA), anti-EGFR (LabVision, Freemont, CA), or anti-py-EGFR (Santa Cruz Biotechnology) primary antibody overnight at 4°C followed by incubation with horseradish peroxidase (HRP)-conjugated anti-mouse (Amersham Biosciences, Piscataway, NJ) or anti-rabbit (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) secondary antibody for 1 hour at room temperature. The bands were detected by use of a chemiluminescent HRP substrate (Pierce, Rockford, IL).

Measurement of cell proliferation. Cells were plated at low density on glass coverslips and incubated overnight in DMEM supplemented with 5% FBS. At the start of experiments, cells were washed and supplemented with fresh serum-containing or serum-free medium. Following indicated time periods/treatments, cells were labeled with 5-bromo-2’-deoxyuridine (BrdUrd), fixed, and immunostained using a Zeiss Axiovert S100TV microscope (Thornwood, NY).

RNA isolation and reverse transcription-PCR analysis. Total RNA was collected using TriPure Isolation Reagent (Roche Molecular Biochemicals) according to the protocol of the manufacturer. Reverse transcription-PCR (RT-PCR) was done on 1 μg RNA using the One-Step Superscript RT Platinum TaqRT-PCR kit (Invitrogen) and 0.6 μl/mL of each primer. All primers and cycle details for RT-PCR analysis of vascular endothelial growth factor (VEGF), Glut-1, TGF-α, and actin mRNA levels were described elsewhere (14). Products were analyzed with gel electrophoresis and ethidium bromide staining, and visualized using a Kodak Digital Science IC440 system.

Immunofluorescence. Cells were plated at low density on glass coverslips and incubated for 6 days in DMEM supplemented with 5% FBS. Cells were fixed in 95% ethanol for 30 minutes at –20°C. The ethanol was aspirated and coverslips were allowed to air dry at 4°C. Cells were immunostained with antifibronectin antibody (Abcam, Inc., Cambridge, MA) and fibronectin deposition was assessed as previously described (24).

In vitro tumor spheroids. Multicellular spheroids were prepared as previously described (15, 25). Briefly, 24-well plates were coated with preheated 1% Seaplaque agarose (Cambrex, Rockland, ME) in serum-free medium. One hundred thousand of the indicated cells were plated per 1 mL.

Figure 1. TGF-α ectodomain cleavage is required for efficient EGFR activation. A, the effect of inhibiting TGF-α shedding with general matrix metalloprotease inhibitor, GM6001, on EGFR activation in renal carcinoma cell lines was examined. ELISA analysis of soluble TGF-α levels in serum-starved VHL(-/-) renal carcinoma 786-O and KTCL cell line conditioned medium following a 48-hour treatment with GM6001 (10-100 μmol/L) or an equivalent volume of DMSO as a vehicle control. B, Western blot analysis of total (EGFR) and phosphorylated EGFR (pEGFR) levels in cell lysates from (A). Cell lines expressing shRNA directed against EGFR (shRNA EGFR) were used as negative controls. Actin Westerns were done as loading controls. C, the effect of transiently silencing ADAM17, the major TGF-α sheddase, on TGF-α shedding and EGFR activation in renal carcinoma cell lines was examined. Western blot analysis of ADAM17, pEGFR, and total EGFR levels in 786-O and KTCL cell lines transiently transfected with 50 nmol/L siRNA against ADAM17, 50 nmol/L scramble siRNA (Control), or an equivalent volume of Effectene transfection reagent for 96 hours. D, ELISA analysis of TGF-α levels in conditioned medium of cell lines described in (C). Bars (A and D), SD of at least three independent experiments done in triplicate.
of medium per well. To promote cell-to-cell adhesion, the plates were gently swirled 30 minutes after plating. Spheroids were grown for 6 days at 37°C under 5% CO2 in serum-containing medium. Spheroids were then collected and fixed in 10% formaldehyde, embedded in paraffin, sectioned, mounted on slides, and stained with H&E.

Migration and invasion assays. Colorimetric cell migration assays (Chemicon, Temecula, CA) were done according to the protocol of the manufacturer. Briefly, 5.0 × 10^4 cells were plated in Boyden chambers. Cells were allowed to migrate for 16 hours toward serum-containing (10% FBS) or serum-free (5% bovine serum albumin) medium. The chambers were then removed and placed in staining reagent provided in the kit. The dye was solubilized and the absorbance was measured on a 96-well microplate reader at 574 nm. Colorimetric cell invasion assays were done on BD BioCoat Matrigel invasion chambers (BD Biosciences, San Jose, CA). Cells (1.5 × 10^5) were plated in the chambers in the presence (10% FBS) or absence (1% ITS) of serum and incubated for 48 hours. The chambers were removed and stained with crystal violet. The dye was solubilized with 10% acetic acid and its absorbance was read at 574 nm.

Nude mouse xenograft assays. Nude mouse xenograft assays were done as previously described (15, 26). Briefly, female nude mice (Charles River,
Wilkinson, MA) were injected in the flanks with \(10^7\) control (parental or scramble shRNA) and ADAM17 shRNA-expressing cells. Mice were sacrificed 7 to 9 weeks postinjection according to facility protocol (University of Ottawa). Tumor size was measured weekly and the tumors sacrificed 7 to 9 weeks postinjection according to facility protocol.

**Results**

**TGF-\(\alpha\) ectodomain cleavage is required for efficient EGFR activation in renal carcinoma cell lines.** Self-sufficiency in growth signaling requires activation of an autonomous program that constitutively drives cell proliferation. A model that precisely exhibits characteristics of acquired growth autonomy by production of soluble growth factors is renal carcinoma. Human renal carcinoma often harbor defective alleles of the \(VHL\) tumor suppressor and, as a consequence, overproduce hypoxia-inducible factor (HIF), a transcription factor that activates an array of genes involved in oxygen homeostasis (27, 28). One HIF target gene of particular interest with respect to tumor formation is \(TGF-\alpha\). We recently showed that renal carcinoma engage in a classic TGF-\(\alpha\)/EGFR autocrine circuit required for autonomous proliferation and \textit{in vivo} tumorigenesis (9, 14, 15). TGF-\(\alpha\) is synthesized as a transmembrane precursor protein (pro-TGF-\(\alpha\)) that undergoes ADAM17-mediated proteolytic cleavage to release the mature ligand (16). There have been conflicting accounts in the literature regarding the requirement for TGF-\(\alpha\) shedding in EGFR activation. Although recent reports suggest that ADAM17-dependent shedding of EGFR ligands is a requirement for EGFR activation, it has also been reported that the proteolytic cleavage of TGF-\(\alpha\) is defective in many cancer cells leading to the accumulation of membrane-bound forms and enhanced EGFR activation (18, 19). We therefore decided to examine the role of ADAM17 in a model system of human renal cancer where participant molecules in this pathway, including TGF-\(\alpha\), EGFR, and ADAM17, are produced endogenously.

To determine whether TGF-\(\alpha\) cleavage is necessary for EGFR activation, two independent sporadic renal carcinoma cell lines derived from human primary renal tumors with clear cell type histology and harboring loss of function mutations in the \(VHL\) tumor suppressor gene, 786-0 and KTCL, were treated for 48 hours with a general sheddase/matrix metalloprotease inhibitor, GM6001. The amount of TGF-\(\alpha\) secreted into the medium by both renal carcinoma cell lines (Fig. 1A), and corresponding phosphorylation of the EGFR, decreased in a dose-dependent manner (Fig. 1B) without affecting total levels of cellular TGF-\(\alpha\) or EGFR (Fig. 1B and data not shown). To confirm that the observed effect of the drug on TGF-\(\alpha\) shedding and EGFR phosphorylation was not a result of the inhibition of other metalloproteases or alternative pathways, ADAM17 was transiently silenced using small-interfering RNA (siRNA) technology. ADAM17 knockdown was confirmed by Western blot analysis of cellular levels of the full-length ADAM17 precursor protein (Fig. 1C). Predictably, the transient silencing of ADAM17 inhibited TGF-\(\alpha\)-ectodomain cleavage (Fig. 1D) and resulted in reduced EGFR phosphorylation (Fig. 1C). The decrease in EGFR activation following transient silencing of ADAM17 only became evident after 96 hours, as it required additional time following the efficient silencing of ADAM17 to observe these downstream effects. Nonetheless, the data indicate that TGF-\(\alpha\) shedding is required for maximal EGFR activation and that this process is most likely mediated by ADAM17.

**Stable inhibition of ADAM17 inhibits TGF-\(\alpha\) shedding and EGFR activation in renal carcinoma cell lines.** To further examine the specific role of ADAM17 in the establishment of the TGF-\(\alpha\)/EGFR autocrine signaling loop, we generated renal carcinoma cell lines with stably inactivated ADAM17 using a shRNA strategy. Two independent shRNA sequences targeting ADAM17, called shRNA ADAM17-1 and ADAM17-2, were used to silence ADAM17 protein expression in the 786-0 and KTCL cell lines. Renal carcinoma stably expressing either of the shRNA sequences against ADAM17 exhibited a significant decrease in ADAM17 protein levels compared with parental cells and cells stably expressing control scrambled shRNA (Fig. 2A). Stable expression of shRNA against ADAM17 inhibited the secretion of TGF-\(\alpha\) by 786-0 and KTCL cells compared with parental and control cell lines suggesting that ADAM17 is the major TGF-\(\alpha\) ectodomain sheddase in renal carcinoma (Fig. 2B). The observed decrease in secreted TGF-\(\alpha\) was not accompanied by a measurable increase in cellular TGF-\(\alpha\) levels (Figs. 2C and 4B). The parental renal carcinoma cell lines shed low levels of TGF-\(\alpha\) over time, such that any resulting increases in cell-associated TGF-\(\alpha\) were not
detectable at steady state in the cell lines where ADAM17 was silenced. Importantly, there was a concomitant reduction in phosphorylated EGFR levels, but not total EGFR levels, in shRNA-expressing cells (Fig. 2A). Addition of exogenous TGF-α rescued EGFR phosphorylation, indicating that silencing ADAM17 did not affect the ability of the receptor to become activated in response to ligand stimulation (Fig. 2D). These data show that ADAM17-mediated TGF-α ectodomain shedding is a prerequisite for efficient EGFR activation in cells that produce endogenous ligand and receptor.

Inhibition of ADAM17-mediated TGF-α shedding abolishes the ability of renal carcinoma to engage in autonomous growth. Based on the data shown in Fig. 2, we decided to examine the effect of preventing TGF-α cleavage on the ability of renal carcinoma to engage in autonomous proliferation upon serum withdrawal (29). Stable silencing of ADAM17 was sufficient to abrogate the ability of both 786-0 and KTCL cells to incorporate BrdUrd in serum-free medium (Fig. 3A) to levels similar to those observed in renal carcinoma cells upon stable silencing of EGFR (15). After the cells had been cultured for 72 hours in the absence of serum, addition of exogenous TGF-α activated the EGFR (Fig. 2D) and reinitiated cell proliferation demonstrating that loss of growth autonomy observed in shRNA-expressing cells was not due to a dominant effect on the cell cycle (Fig. 3A). Similar data were also obtained with transient silencing of ADAM17 (Fig. 3B) and the treatment of cells with GM6001 (Fig. 3C), indicating that the results obtained with stable silencing of ADAM17 are not a consequence of fortuitous effects of shRNA expression or single cell clonal effects. These data suggest that ADAM17-mediated processing of pro-TGF-α into its soluble form is essential for EGFR phosphorylation and critical for the establishment of the TGF-α/EGFR autonomous growth circuit observed in renal carcinoma cells.

Silencing ADAM17 does not affect proangiogenic phenotypes characteristic of VHL-loss renal carcinoma. Secretion of proangiogenic factors, such as the VEGF, in conjunction with disruption of extracellular matrix deposition has been shown to promote and maintain renal carcinoma tumor angiogenesis (30). To assess whether ADAM17 has functions unrelated to TGF-α processing that may affect the well-established pathways involved in renal carcinoma tumorigenesis, we examined the effect of ADAM17 knockdown on other defects associated with VHL-loss. Silencing ADAM17 did not alter the expression of HIF2α protein (Fig. 4A) nor the expression of classic hypoxia-inducible mRNAs, such as glucose transporter-1 (GLUT1) or VEGF (Fig. 4B). Additionally, silencing ADAM17 did not restore the ability of renal carcinoma cells to deposit extracellular fibronectin matrix (Fig. 4C), a process that can be corrected by reintroduction of VHL (31). Thus, ADAM17 function is unrelated to the major proangiogenic characteristics associated with VHL-loss.

Figure 5. Inhibition of ADAM17 abolishes multiple acquired tumor capabilities in vitro. The ability of VHL(-/-) renal carcinoma 786-0 cells, VHL(+) renal carcinoma cells (VHL), and 786-0 cells stably expressing shRNA (Control, EGFR, and ADAM17) to grow in an anchorage-independent manner, migrate toward chemoattractant, and invade basement membrane was assessed in vitro. A, stable silencing of ADAM17 hinders the ability of renal carcinoma to grow as tumor spheroids. Cells were grown as three-dimensional tumor spheroids for 6 days in vitro. Histology from spheroids is visualized at a magnification of ×400. Spheroid density was measured at a magnification of ×100 in at least three independent spheroid sections and reported as nuclei/field. B, stable silencing of ADAM17 abolishes the ability of renal carcinoma to migrate. Cells were plated in Boyden chambers to assess their ability to migrate through a porous membrane in the presence or absence of chemoattractant (serum) over a 16-hour period. Photographs depict migration in a serum-free environment (<200). C, stable silencing of ADAM17 abolishes the ability of renal carcinoma to invade basement membrane. Invasion of Matrigel in the presence or absence of serum over a 48-hour incubation period. Photographs depict invasion in a serum-free environment (<200). Absorbance readings were taken at 574 nm. Bars, SD of at least three independent experiments done in triplicate.
Inhibition of ADAM17 abolishes multiple acquired tumor capabilities in vitro. Although the self-sufficiency in growth is likely to be a critical initial step in cellular transformation, cancer cells must also acquire several other characteristics for malignancy. Silencing of ADAM17 prevented the formation of highly dense spheroids (Fig. 5A) in an avascular in vitro tumor assay that measures the tumorigenic potential of cancer cells (25). No difference in the number of apoptotic cells, as determined by propidium iodide exclusion and nuclear morphology assays, was observed in renal carcinoma cells expressing shRNA against ADAM17 or EGFR compared with parental renal carcinoma (data not shown). However, the migratory ability of renal carcinoma cells expressing shRNA against ADAM17 or EGFR was severely compromised compared with parental and control cells (Fig. 5B), and hence they failed to invade basement membrane in vitro (Fig. 5C). Taken together, these results show that ADAM17 mediates multiple acquired tumor capabilities required for overt malignancy.

Silencing of ADAM17 is sufficient to suppress renal carcinoma tumor formation in vivo. Finally, we examined the consequence of silencing ADAM17 on renal carcinoma tumor formation in vivo. Parental and control VHL-defective 786-0 renal carcinoma cells formed large tumors detectable 4 to 5 weeks after injection. Reintroduction of a functional copy of VHL was sufficient to prevent tumor formation of VHL-defective renal carcinoma cells, as expected (26). 786-0 cells expressing shRNA ADAM17-1 or ADAM17-2 failed to form tumors in the xenograft mouse assay (Fig. 6A and B). Similar data were obtained with the VHL-defective KTCL cell line expressing shRNA directed against ADAM17 mRNA although the size of the control tumors was smaller compared with 786-0 renal carcinoma (Fig. 6B). Prolonged incubation time resulted in the formation of very small tumors in only two of the 786-0 renal carcinoma cell lines expressing shRNA ADAM17-2, indicating that abolishing ADAM17 function efficiently abrogated the tumorigenic potential of highly malignant renal carcinoma cells. Interestingly, analysis of H&E-stained sections of these small tumors revealed that the typical leukocyte infiltration, observed as darkly stained nuclei, in parental and control renal carcinoma tumors, was significantly less evident when ADAM17 was silenced (Fig. 6C). This effect is likely due to the involvement of ADAM17 in the cleavage of the proinflammatory cytokine TNF-α (32). Thus, in addition to its role in suppressing cell proliferation and tumorigenesis, silencing ADAM17 may reduce tumor inflammation, a negative prognostic factor in renal carcinoma (33). Therefore, silencing of ADAM17 is sufficient to abolish in vivo tumor formation of highly malignant cell lines derived from human primary renal tumors.

Discussion

The primary event leading up to the development of cancer is the establishment of an actively proliferating population of cells. This inappropriate initiation of cell division hinges upon the ability of cancer cells to engage in autonomous proliferation through self-induced signaling cascades, such as the TGF-α/EGFR growth stimulatory circuit (6). Uncontrolled cellular proliferation alone is not sufficient, however, for the progression and persistence of a malignancy. Tumors would not survive without the oxygen and nutrients supplied by the circulatory system (34), nor would they metastasize to distal organs, the major cause of cancer-associated death, without the means to compromise and invade the extracellular matrix (35). The endurance of tumor cells is dependent on five additional acquired traits that bestow them with fundamental growth and survival advantages. In contrast to their normal counterparts, cancer cells are able to resist growth-inhibiting signals and programmed cell death, induce angiogenesis, migrate and invade surrounding tissues, and replicate limitless (1).

Together, the results of this study show that silencing one biologically relevant enzyme, ADAM17, is sufficient to abrogate cancer cell growth autonomy, migration and invasion, and likely tumor inflammation. ADAM17 function was analyzed in a model system with endogenous participants, eliminating the possibility of interference due to overproduction of molecules. In this setting, ADAM17-mediated shedding of membrane-bound TGF-α is required for EGFR activation and its ability to drive autonomous proliferation. The ability of TGF-α to promote cell migration has been shown in a multitude of cell types, ranging from mammary epithelial cells to ovarian cancer cell lines (36–38). Furthermore, inhibition of EGFR signaling with receptor tyrosine kinase inhibitors is sufficient to prevent migration of glioblastomas in
and resistance to apoptosis of the cells seemed to be unaffected by capabilities characteristic of cancer cells, the angiogenic potential in vivo has an emerging role as a modulator of integrin-mediated kinase–mediated cell migration (44). Although a full-scale study of ADAM17-associated integrins has not yet been conducted, ADAM17 has an emerging role as a modulator of integrin-mediated migration that warrants additional examination (42, 43).

Although silencing of ADAM17 was sufficient to block tumor formation in vivo by eradicating at least three of the acquired capabilities characteristic of cancer cells, the angiogenic potential and resistance to apoptosis of the cells seemed to be unaffected by the knockdown. Our results suggest that there is no correlation between ADAM17 expression and HIF2α activation and resistance to cell detachment–induced apoptosis. Based on our observations, inhibition of cell proliferation, rather than enhancement of cell death or blockade of angiogenesis, is sufficient to prevent in vivo renal carcinoma tumor formation.

The results shown here argue that ADAM17-mediated shedding of TGF-α is required for renal cancer growth autonomy and in vivo tumor formation. The data support the notion that ADAM17-mediated ectodomain cleavage of TGF-α is required for EGFR activation and tumor formation in a biologically relevant human cancer model system that expresses endogenous ADAM17, ligand, and receptor. The central role of EGFR signaling in the development of human cancer is well documented and therapeutic agents directed at inhibiting ligand-dependent receptor activation, such as monoclonal antibodies targeting the EGFR and receptor tyrosine kinase inhibitors, have been in clinical trials for a number of years. However, such therapeutic strategies have yielded poor response rates in patients and effective therapies remain elusive (45–48). In light of the fact that ADAM17 plays a central role in acquired tumor cell capabilities, such as tumor cell growth autonomy, inflammation, migration, and invasion, we suggest that ADAM17 is an ideal therapeutic target in the treatment of VHL-defective renal carcinoma and other EGFR autocrine signaling-dependent human cancers.

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