Magnetic Resonance Imaging Defines Cervicovaginal Anatomy, Cancer, and VEGF Trap Antiangiogenic Efficacy in Estrogen-Treated K14-HPV16 Transgenic Mice

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Abstract

Noninvasive detection of dysplasia provides a potential platform for monitoring the efficacy of chemopreventive therapy of premalignancy, imaging the tissue compartments comprising dysplasia: epithelium, microvasculature, and stromal inflammatory cells. Here, using respiratory-gated magnetic resonance imaging (MRI), the anatomy of premalignant and malignant stages of cervical carcinogenesis in estrogen-treated K14-HPV16 transgenic mice was noninvasively defined. Dynamic contrast enhanced (DCE)-MRI was used to quantify leakage across premalignant dysplastic microvasculature. Vascular permeability as measured by DCE-MRI, $K^{\text{trans}}$, was similar in transgenic (0.053 ± 0.020 min⁻¹; $n = 32$ mice) and nontransgenic (0.056 ± 0.029 min⁻¹; $n = 17$ mice) animals despite a 2-fold increase in microvascular area in the former compared with the latter. DCE-MRI did detect a significant decrease in vascular permeability accompanying diminution of dysplastic microvasculature by the antiangiogenic agent, vascular endothelial growth factor Trap ($K^{\text{trans}} = 0.052 ± 0.013$ min⁻¹ pretreatment; $n = 6$ mice versus $K^{\text{trans}} = 0.019 ± 0.008$ min⁻¹ post-treatment; $n = 5$ mice). Thus, we determined that the threshold of microvessel leakage associated with cervical dysplasia was <17 kDa and highlighted the potential of DCE-MRI to noninvasively monitor the efficacy of antiangiogenic drugs or chemoprevention regimens targeting the vasculature in premalignant cervical dysplasia. [Cancer Res 2009;69(20):7945–52]

Introduction

Cervical cancer is the second most common malignancy affecting women worldwide (1). Although effective screening has markedly diminished cervical cancer incidence in the United States, dysplasia remains a common clinical challenge requiring surgical extirpation for high-grade lesions (1, 2). As such, noninvasive therapy for premalignant, but high-risk, dysplasias would be a tremendous boon to gynecologic care.

Dysplastic lesions are characterized by disorganized epithelial differentiation. The uterine cervical squamous epithelium can harbor dysplasia initiated by oncogenic human papillomaviruses (HPV), most frequently types 16 and 18 (1). Normal cervical epithelium is composed of a basal layer that is immature and proliferative and multiple suprabasal layers of differentiating squamous cells that are ultimately shed from the top of the epithelium into the cervical canal or vaginal lumen. Increasing grades of dysplasia are characterized by progressive occupation of the suprabasal layer by basaloid squamous cells (1). High-grade dysplasia, if untreated, has an extremely high incidence of conversion to malignancy (2). Cervical dysplasia and dysplastic lesions, in general, activate an angiogenic switch that both increases the subepithelial microvasculature and produces stromal inflammation (3, 4). Angiogenesis and inflammation are features that could be exploited for imaging by a technique measuring tissue vascularity and leakage, such as dynamic contrast enhanced (DCE)-magnetic resonance imaging (MRI). DCE-MRI, studied extensively in solid malignancies (5–7), uses bolus contrast agent administration to noninvasively visualize tumor microvasculature and quantify leakage across microvessels (8, 9). Time-resolved images, collected as contrast agent enters and exits tissue (10), are pharmacokinetically modeled (11, 12), providing quantitative measures of important physiologic parameters, such as tissue microvascular integrity (vascular permeability).

Here, we used respiratory-gated MRI to obtain high-resolution images of the entire mouse reproductive tract and delineated an invasive squamous carcinoma in a late-stage (13–15), estrogen-treated K14-HPV16 transgenic mouse. We also interrogated the microvascular biology associated with premalignant dysplasia using DCE-MRI with a 17-kDa contrast agent, Gadomer. No differential dysplastic microvascular leak was detectable, consistent with experiments showing undetectable physical leakage of an i.v. injected marker protein, suggesting that dysplastic microvessels have a maximal permeability pore size <17 kDa. However, DCE-MRI detected a significant decrease in dysplastic vascular leak following treatment with the antiangiogenesis agent, vascular endothelial growth factor (VEGF) Trap (16, 17). Thus, DCE-MRI can serve as a platform for noninvasively monitoring antiangiogenic drug efficacy, potentially deployed with chemoprevention programs, for high-risk premalignant disease.

Materials and Methods

Transgenic mice. One-month-old female K14-HPV16:FVB/n congenic transgenic mice and nontransgenic littermate controls were treated for 3 or 6 months with s.c. insertion of 17β-estradiol pellets, 0.05 mg/60 day release (Innovative Research of America; “estrogen”; refs. 13, 15). The Animal Studies Committee of Washington University in St. Louis approved all procedures in this study.

MRI. MR images were collected on a Varian NMR Systems 4.7T INOVA scanner described previously (18) using Stark Contrast 2.0 or 2.5 cm birdcage RF coils. Mice were anesthetized and maintained on isoflurane/O₂ (1–1.25% v/v), and a 3-inch length of PE-10 tubing (Becton Dickinson) was...
inserted via the urethra into the bladder for drainage throughout data collection. Core body temperature was maintained at 37 ± 1°C by warm air circulation through the magnet bore.

For anatomic imaging, 500 µL Omniscan (gadodiamide; GE Healthcare) contrast agent, diluted 1:10 in saline to yield a 50 mmol/L solution, was administered i.p. immediately before placing the animal into the magnet. High-resolution images were collected using a respiratory-gated, two-dimensional, multi-slice spin-echo sequence (18), and all images were collected during post-expiratory periods. Imaging parameters were repetition time ~3 s, echo time 20 ms, field of view 2.5 × 2.5 cm² (transaxial)/4.0 × 4.0 cm² (coronal), 128 × 128 data points, and slice thickness 0.5 mm.

DCE-MRI data were collected using a T1-weighted, gradient spoiled, multi-slice gradient-echo sequence. Imaging parameters were flip angle 30°, repetition time 0.06 s, echo time 0.002 s, field of view 2.5 × 2.5 cm², number of slices 15, in-plane resolution 195 µm, and slice thickness 0.50 mm. The temporal resolution was ~15 s. Early DCE-MRI data were collected following i.v. injection of Omniscan. However, values of vascular permeability (Ktrans) were all derived from experiments using Gadomer (Bayer Schering Pharma). Pre-contrast, T1 maps were produced using a variable flip-angle, three-dimensional, gradient-echo sequence (19, 20) with flip angles of 2.5°, 5.0°, 7.5°, 10.0°, and 15.0°. After 1 min of scanning (four images at 15 s/image), 60 µL of a solution of Gadomer that is 50 mmol/L Gd (a dose of 0.12 mmol Gd/kg body weight for a 25 g mouse) were injected over 10 s using a Harvard 2 dual syringe pump (Harvard Clinical Technology) via either a tail or a jugular venous catheter.

Modeling DCE data. The starting point for our analysis was the Patlak model containing independent parameters $v_p$ (fractional blood-plasma volume) and $K_{trans}$ (volume transfer constant between blood plasma and extravascular extracellular space; refs. 21, 22). Significant deviation from linearity in the Patlak plot led us to include a third independent parameter, $v_e$ (fractional volume of extravascular extracellular space), to account for contrast agent efflux from extravascular extracellular space to blood plasma (21, 23). Conversion of signal intensity to concentration of contrast agent was achieved by standard methods (10). Negligible $T_2^*$ weighting of the images and the fast-exchange limit were both assumed (10, 12). Values of $T_{10}$ were 1.6 s for cervix (24) and 1.4 s for leg muscle (see below; ref. 25); $r_1$ of Gadomer was taken to be 9.1 mmol/L⁻¹ s⁻¹ (26).

The determination of $v_p$, $v_e$, and $K_{trans}$ requires knowledge of contrast agent concentration in the blood plasma [artificial input function (AIF)] throughout the experimental time course. Traditionally, the AIF is measured from a large vessel within the image, carefully avoiding inflow and partial volume artifacts (10, 27, 28). However, direct AIF determinations in mice are often difficult due to small spatial dimensions and motion effects. Instead, we derived the AIF from leg muscle reference tissue (29, 30) using the following parameter values for muscle tissue: $K_{trans}$ (Gadomer) = 0.002 min⁻¹, $v_p$ = 0.021, and $v_e$ = 0.085 (31). To convert contrast agent concentration data into physiologic parameters, a region of interest (ROI) was drawn over the transformation zone of the cervix and the data were modeled in Matlab (Mathworks) using a variable projection (VARPRO) nonlinear least-squares approach (32).

Detection of antiangiogenesis mediated by VEGF Trap. K14-HPV16 transgenic mice were scanned by DCE-MRI before treatment, treated biweekly for 2 weeks with 500 µg VEGF Trap (Regeneron Pharmaceuticals) or vehicle (5 mmol/L phosphate, 5 mmol/L citrate, 100 mmol/L NaCl, 0.005% Tween 20) via i.p. injection, and then rescanned to test therapy-associated microvascular permeability alterations.

Determination of microvessel density. Isoturane anesthetized mice were i.v. injected with 50 µg FITC-conjugated Lycopersicon esculentum lectin (Vector Laboratories); after 3 min, the left ventricle was perfused with 10% formalin (Fisher Scientific International) for 3 min followed by 10% sucrose for 1 min (Perfusion One Rodent System; McCormick Scientific). The entire reproductive tract was removed, and the vaginal cavity was filled with OCT freezing medium, embedded in OCT (posterior-side down), flash-frozen using liquid nitrogen, and stored at −80°C. Cryosections (60 µm) were mounted using SlowFade Gold with 4′,6-diamino-2-phenylindole (Invitrogen) and viewed under appropriate filter sets using an Olympus BX61 microscope equipped with a Fire Wire Colorview II camera (Olympus). Images of lectin-perfused vessels in the cervical transformation zone taken at ×40 magnification were analyzed using Olympus MicroSuite Biological Suite software. For each image, four equally sized rectangular ROIs were identified along the epithelial-stromal border of the transformation zone. Subepithelial microvasculature was delineated by creating RGB color detection profiles to increase signal-to-noise and identify as many vessels as possible. These profiles were used for all images.

Detection of physical molecular leakage. ChromPure sheep IgG, Fc fragment, 50 µg in 50 µL PBS (Jackson ImmunoResearch Laboratories), was injected i.v. and allowed to circulate for 2 h followed by FITC-lectin injection, formalin perfusion, OCT whole-organ embedding, and 60 µm cryosectioning as described above. Air-dried sections were rinsed in PBS three times, blocked for 3 h with DAKO protein block, incubated overnight.
at 4°C with anti-sheep Cy3-conjugated AffiniPure Donkey IgG (Jackson Immuno Research Laboratories), diluted 1:100 in DAKO Antibody Diluent (DAKO), and mounted using SlowFade Gold with 4′,6-diamino-2-phenylindole. For visualizing Fc Fragment leakage, images were captured using the Cy3 filter from the sample with the highest signal, which was used to determine the optimal camera gain settings. A control image from a noninjected mouse was used to correct for the Cy3 background signal. A ROI obtained from the control image was used for background subtraction for analysis of signal intensity of Fc-injected experimental tissue sections using the MicroSuite software.

**Statistical analysis.** Data are mean ± SD. Mann-Whitney U, paired or unpaired Student’s t tests were used to determine statistical significance (GraphPad Prism).

**Results**

**Cervical transformation zone MRI and histopathologic correlation.** First, we developed MRI techniques to visualize the entire mouse female reproductive tract, including the vagina, cervix, and lower uterus (Fig. 1B). High-resolution, *in vivo* respiratory-gated spin-echo coronal and transaxial MR images were obtained of a 3-month-old, estrogen-treated, nontransgenic mouse with an in-plane resolution of 150 μm (Fig. 1I and C). The coronal MR image (Fig. 1A) was a striking reproduction of the actual organ anatomy delineating the cervical isthmus, canal, outer cervix, and upper vagina (Fig. 1B). Transaxial images also delineated all three zones of the cervix: the upper cervical-uterine junction (data not shown), the mid-cervix with the transformation zone and isthmus division septum leading to the two uterine horns (Fig. 1C, top), and the lower cervix, here containing a single central canal and laterally bounded by the adjacent vaginal walls (Fig. 1C, middle), and the vagina (Fig. 1C, bottom).

Anatomic MRI also detected cervical cancer that occurs in 80% of K14-HPV16 transgenic mice treated with estrogen for 6 months (refs. 13, 15; Fig. 2A and B, multiple delineating black and red arrowheads, respectively). The malignancy was first evident in the mid-cervix both histologically and in the MRI (data not shown). Histologic analysis further showed spread of the cancer to the lower cervix, as determined by the single “X”-shaped lumen (Fig. 2A, green arrows indicate upper vagina), which invaded almost through the anterior cervical wall adjacent to the bladder (Fig. 2A, six o’clock position), and effaced the right portion of the cervical canal. MRI delineated the same extent of tumor invasiveness (Fig. 2B, red arrowheads), but with the additional feature of obliteration of the right cervical lumen, not evident on the histopathology (Fig. 2A). This slight discordance of the MRI vis-à-vis histology is due either to volume averaging in the former or, more likely, tissue shrinkage during processing in the latter.

**Differential histology and microvascular area in transgenic premalignant high-grade dysplasia.** As high-grade dysplasia, usually arising from the cervical transformation zone, is the source of subsequent invasive cervical cancer in both humans (1) and this transgenic model (13) and is a potential target for chemoprevention, we focused our histopathologic (Fig. 3A-D), microvascular (Fig. 4A-D), and MRI analyses on this anatomic region and this stage of progression in the transgenic mice treated with estrogen for 3 months (13). Moderate to high-grade cervical dysplasia (Fig. 3B and D), but no cervical cancer, was evident, similar to our previous experience (13). Nontransgenic littermates treated with the same dosage and duration of estrogen evidenced hyperplasia without dysplasia (Fig. 3A and C).

Induction of angiogenesis and increased microvascularity has been documented previously in patients with cervical dysplasia and also in dysplastic skin lesions in K14-HPV16 transgenic mice (33). Thus, we first determined differences in microvascular morphology in transgenic cervical dysplasia (Fig. 4A, top and bottom right) compared with nontransgenic estrogen-induced hyperplasia (Fig. 4A, top and bottom left). There were two distinct zones of microvasculature in the mouse cervix: the subepithelial region and the deep stromal area (Fig. 4A, top left, white arrowheads and arrow). The subepithelial region was most affected by mouse genotype, with the transgenic microvasculature forming tufts and projections into the overlying dysplastic epithelium (Fig. 4A, top right, arrowhead, and higher-magnification image in bottom right). In contrast, nontransgenic microvasculature was flattened and linearly arrayed in the stroma immediately beneath the hyperplastic epithelium (Fig. 4A, top and bottom left). Image analysis revealed that the area of the subepithelial microvasculature in K14-HPV16 mice was 42.6 ± 8.8% compared with 24.3 ± 8.5% in nontransgenic cervixes (Fig. 4B). These data were similar to immunohistochemical analysis of dysplastic microvessel density in this model (14).

**Using DCE-MRI to detect vascular permeability changes in premalignant cervical dysplasia.** The increased subepithelial microvascularity in 3-month-old, estrogen-treated transgenic cervices led us to investigate microvessel biology using DCE-MRI, particularly because K14-HPV16 transgenic mice were known to accumulate activated stromal inflammatory cells that could, via chemokine/cytokine release, induce leakage even in premalignant dysplasia (14). Initially, we conducted these DCE-MRI experiments using Omniscan, a low-molecular weight, Gd-based contrast agent.

![Figure 2. MRI detection of an invasive squamous cervical cancer. Paired transaxial MRI (B) and histologic cross-section of a cervix (A) obtained from a K14-HPV16 transgenic mouse treated with estrogen for 6 mo. A hyperintense lesion was detected extending from the upper to lower cervix on contrast agent–enhanced, T1-weighted images at the 6-mo time point (B, red arrowheads; green arrows, upper vagina). Matched cervical cross-sectional histology verified the cancer and distortion of the cervical canal (A, black arrowheads, cancer; green arrows, upper vagina). Magnification, ×40 (A).](image-url)
Visual analysis of sequential temporal images from a DCE-MRI experiment using a nontransgenic mouse revealed a distinct pattern and distribution of Omniscan contrast agent over time (Fig. 5A; Supplementary Movie). Initial pre-injection images were dark (Fig. 5A, left). Immediately following injection, the pelvic branches of the internal iliac artery were visualized; coincidently, the luminal lining of the isthmus and cervical canal was brightly enhanced (Fig. 5A, middle). This compartment was presumably the subepithelial microvasculature. Finally, contrast was evenly distributed in the cervix, consistent with permeation throughout the deep cervical stroma (Fig. 5A, right). We observed a swift, large signal enhancement following Omniscan injection in both transgenic and nontransgenic animals, consistent with rapid extravasation, making it very difficult to collect high-resolution DCE-MRI data rapidly enough for determination of contrast agent kinetics.

As such, we switched to Gadomer, a 17-kDa, dendrimer-based contrast agent with permeability that was significantly lower than that of Omniscan, for DCE-MRI. Following data collection, ROIs were drawn in the cervical transformation zone (Fig. 5B, left) and image intensity versus time curves were derived from within these ROIs (Fig. 5C, left). Intensity versus time data were converted to concentration versus time curves, and physiologic parameters were derived, as described above. The red circles in Fig. 5C are experimental data points, whereas the red curve represents the modeling of this concentration versus time data using an AIF derived from a reference tissue, muscle (refs. 29, 30; Fig. 5B, right). A representative contrast agent concentration versus time curve for muscle is plotted as the blue circles, whereas the derived AIF is shown as the dashed blue curve (Fig. 5C, left). Based on our analysis of the DCE data, we determined the transfer constant ($K_{\text{trans}}$) for a ROI within the cervical transformation zone in 32 transgenic and 17 nontransgenic mice (Fig. 5C, right). Average $K_{\text{trans}}$ values for the nontransgenic (0.056 ± 0.029 min$^{-1}$) and transgenic mice (0.053 ± 0.020 min$^{-1}$) were indistinguishable.

To investigate the possibility of low-level microvascular leak to which DCE-MRI was insensitive, we determined the physical leakage (Fig. 5D). Injection of Fc fragments of 50-kDa molecular weight showed a robust microvascular leakage both within an invasive cancer (Fig. 5D, left) and within a dysplasia adjacent to the malignancy (Fig. 5D, middle). In contrast, we did not detect microvascular leak in dysplastic microvessels in 3-month-old, estrogen-treated transgenic mice (Fig. 5D, right). Thus, despite a 2-fold increase in microvascular density (Fig. 4B), the switch to leaky vessels occurred later in this model, possibly at the 4.5-month point wherein carcinoma in situ and microinvasive cancer first appear (15), or was restricted to frank invasive malignant lesions.
Anatomic response of microvasculature associated with premalignant cervical dysplasia to antiangiogenic therapy. Noninvasive detection of the response of dysplastic lesions to antiangiogenic or antineoplastic therapies would be a tremendous boon to assess efficacy of cancer prevention. We determined the morphologic response of the transgenic cervical microvasculature to VEGF Trap and then the sensitivity of DCE-MRI to detect alterations of microvascular leakage. We used VEGF Trap (16, 17), because VEGF has also been shown to be incrementally up-regulated in both human dysplastic and malignant cervix (34) and the cervical dysplasias and malignancies of estrogen-treated, K14-HPV16 transgenic mouse (14).

Following a 2-week course of VEGF Trap, there was an obvious pruning of the epithelial tufting and a marked overall reduction in microvascular density in the treated mice (Fig. 6A, vehicle, left, and VEGF Trap–treated, right). Subepithelial microvascular area decreased 50% in VEGF Trap–treated mice compared with vehicle-treated transgenic mice (Fig. 6B), to a level below that of estrogen-treated nontransgenic controls (Fig. 4B). Despite the marked subepithelial microvascular pruning, there was no difference in the extent or grade of epithelial dysplastic histopathology in VEGF Trap–treated versus vehicle-treated transgenic mice (data not shown).

DCE-MRI detects antiangiogenic efficacy in premalignant dysplasia. Next, we tested the ability of DCE-MRI to detect a permeability response that potentially accompanied the VEGF Trap–mediated decrease in microvascular area in cervical dysplasia. Pre- and post-VEGF Trap DCE-MRI showed a significant 63% decrease in $K^{\text{trans}}$ in all of the treated transgenic mice (0.052 ± 0.013 min$^{-1}$ pretreatment versus 0.019 ± 0.008 min$^{-1}$ post-treatment; $n = 6$ mice; Fig. 6D). DCE-MRI data in vehicle control transgenic mice were heterogeneous and bivariant (Fig. 6C), but all values fell within the range that we previously determined for this group (Fig. 5C, right). Overall, there was no statistical difference in the pretreatment/post-treatment values either for each vehicle-treated mouse in a paired Student’s $t$ test (Fig. 6D) or within the entire vehicle-treated group (0.048 ± 0.015 min$^{-1}$ baseline and 0.045 ± 0.021 min$^{-1}$ after 2 weeks; $n = 5$ mice). The uniformity of $K^{\text{trans}}$ reduction in VEGF Trap–treated mice compared with the variable response of vehicle-treated transgenic mice suggests that, at a 17-kDa cutoff, DCE-MRI was detecting the marked reduction in microvascular area in VEGF Trap–treated mice rather than an inherent effect on the microvessel stability of neoplastic vessels.

Discussion

The estrogen-treated K14-HPV16 transgenic mouse model of cervical carcinogenesis has been extensively studied since inception (14, 35–37), and its relevance for human disease has been validated by both detailed histopathologic and genome-wide expression analysis (15, 36, 38). Other work has shown angiogenesis induction coincident with high-grade dysplasia, similar to our findings (14). Moreover, dysplastic and malignant angiogenesis in this model has been linked to both macrophage and neutrophil expression of proteases and angiogenic factors (3, 14). Thus, the emerging importance of the K14-HPV16 transgenic mouse as a preclinical platform for testing drugs that target both malignant and dysplastic angiogenesis (3, 14) motivated us to undertake a detailed MRI-based analysis to both noninvasively determine cervicovaginal anatomy and interrogate microvascular biology associated with premalignant dysplasia.

Here, in vivo MRI at 4.7 T clearly distinguished epithelium and subepithelial microvasculature from the relatively avascular deep

![Figure 4](image-url). Microvascular anatomy and density in estrogen-treated cervixes. Fluorescent images of FITC-conjugated L. esculentum lectin-perfused cervixes from nontransgenic (A, top and bottom left) and transgenic (A, top and bottom right) mice revealed subepithelial and interior stromal microvascular compartments (A, top left, arrowheads and arrow, respectively). Subepithelial microvessels were linearly arrayed in nontransgenic (NTG) mice, whereas they formed tufts projecting into the neoplastic epithelium in transgenic mice (A, top right, arrowhead, and bottom). Microvessel quantification (see Materials and Methods) revealed a statistically significant increase in subepithelial microvascular density in the transgenic cervix (B).
cervical stroma and detected cervical cancers in transgenic mice. The next challenge was determination of microvascular leak in premalignant cervical dysplasia using DCE-MRI. DCE-MRI of the lower female reproductive tract poses several unique data acquisition and analysis challenges, including the relatively small size of the target organs (only a few pixels in many images), bladder proximity, and respiratory motion effects. Nonetheless, we successfully determined the transfer constant ($K_{\text{trans}}$), describing vascular permeability/leak in the cervical transformation zone. Within each of these groups of animals, we observed a wide range of $K_{\text{trans}}$, although the average values for transgenic and nontransgenic mice were indistinguishable. The DCE-MRI data were supported, in part, by the lack of detection of immunofluorescent analysis of Fc fragment leakage, although the molecular mass of this protein (50 kDa) was larger than Gadomer (17 kDa). Thus, the DCE-MRI data suggest that microvessels associated with dysplastic lesions at the midpoint of carcinogenic progression do not elaborate fenestrations or other leakage-associated structures (39) despite their increased density, abnormal morphology, and associated stromal inflammation (3, 4, 14).

In contrast, we were clearly able to detect and monitor the decrease in cervical microvasculature due to VEGF Trap (16, 17) in transgenic dysplasias. The antiangiogenic potency of VEGF Trap was highlighted by the marked 50% reduction in $K_{\text{trans}}$ in VEGF Trap–treated compared with vehicle-treated transgenic mice. VEGF Trap also decreased dysplastic microvascular area to a level that was 25% lower than the estrogen-treated nontransgenic controls. These data suggested that VEGF was the predominant coordinator of angiogenesis of cervical dysplasia despite the

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**Figure 5.** T1-weighted, gradient-echo images of the mouse female reproductive tract (A), showing the time course for contrast illumination of the pelvic internal iliac artery branches, the presumed epithelial/subepithelial tissue, followed by stromal permeation following i.v. injection of Omniscan (60 μL of 50 mmol/L contrast agent over 10 s). A, collected pre-contrast, 1-min post-contrast injection, and ~15 min post-injection.

All other DCE-MRI data reported in this figure were collected following i.v. injection of Gadomer (60 μL of a solution that is 50 mmol/L Gd injected over 10 s).

ROIs drawn in the cervical transformation zone (B, left) and in a reference muscle region (B, right) were used to derive the AIF. For a typical mouse, the cervical transformation zone was defined by 30 to 50 pixels and the muscle region by 2,400 to 3,000 pixels. Contrast agent concentration versus time curves were constructed for a cervical transformation zone ROI: red circles, experimental data points; red curve, the model (C, left). The reference contrast agent concentration versus time curve for muscle, plotted with blue circles, and the derived AIF, shown as the dashed blue curve (C), $K_{\text{trans}}$ values (C, right) for a ROI within the cervical transformation zone were statistically indistinguishable. C, right, the horizontal line within each box is the median value of $K_{\text{trans}}$, the dot at the middle of each box is the mean, the edges of the box are ±1 SD, and the whiskers are the maximum and minimum values. Injection of sheep Fc fragment (50 kDa) and immunofluorescent analysis showed leakage in a large cervical cancer in a 6-month-old, estrogen-treated transgenic mouse (D, left), and adjacent dysplasia (D, middle), whereas leak was undetectable in the 3-month-old, estrogen-treated transgenic cervix (D, right).
Figure 6. Effect of VEGF Trap on the transgenic cervical microvasculature. VEGF Trap treatment of transgenic mice produced a marked pruning of the cervical microvasculature in VEGF Trap (A, right), compared with vehicle control transgenic mice (A, left), with a statistically significant decrease in subepithelial microvascular area (B). DCE-MRI detected a significant decrease in microvessel leakage within VEGF Trap–treated transgenic mice (D) compared with lack of significance within the highly variant vehicle-treated transgenic group (C). *, P < 0.05, Mann-Whitney U test (B) or paired Student’s t test (C and D).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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