Short-Term Human Prostate Primary Xenografts: An in Vivo Model of Human Prostate Cancer Vasculature and Angiogenesis


ABSTRACT

Transgenic spontaneously occurring and transplantable xenograft models of adenocarcinoma of the prostate (CaP) are established tools for the study of CaP progression and metastasis. However, no animal model of CaP has been characterized that recapitulates the response of the human prostate vascular compartment to the evolving tumor microenvironment during CaP progression. We report that primary xenografts of human CaP and of noninvolved areas of the human prostate peripheral zone transplanted to athymic nude mice provide a unique model of human angiogenesis occurring in an intact human prostate tissue microenvironment. Angiogenesis in human kidney primary xenografts established from human renal cell carcinoma and noninvolved kidney tissue, a highly vascular organ and cancer, was compared with angiogenesis in xenografts from the relatively less vascularized prostate. Immunohistochemical identification of the human versus mouse host origin of the endothelial cells and of human endothelial cell proliferation in the human prostate and human kidney xenografts demonstrated that: (a) the majority of the vessels in primary xenografts of benign and malignant tissue of both organs were lined with human endothelial cells through the 30-day study period; (b) the mean vessel density was increased in both the CaP and benign prostate xenografts relative to the initial tissue, whereas there was no significant difference in mean vessel density in the renal cell carcinoma and benign kidney xenografts compared with the initial tissue; and (c) the number of vessels with proliferating endothelial cells in primary xenografts of CaP and benign prostate increased compared with their respective initial tissue specimens, whereas the number of vessels with proliferating endothelial cells decreased in the benign kidney xenografts. Short-term primary human prostate xenografts, therefore, represent a valuable in vivo model for the study of human angiogenesis within a human tissue microenvironment and for comparison of angiogenesis in CaP versus benign prostate.

INTRODUCTION

The endothelium of the human vasculature forms a contiguous but not homogeneous system that responds differentially to tissue-specific angiogenic promoters/inhibitors, pharmaceutical agents, and diet (1–3). The vasculature of individual organs has been proposed to be sufficiently organ specific to allow targeting of the vasculature in a specific organ through “vascular addressing” (4–6). The endothelial cells of the vasculature of the human prostate express unique markers and proliferate at more than twice the rate of the endothelial compartment of other organs (0.01%), and survival of prostatic endothelial cells is dependent on the prostatic microenvironment, particularly androgen levels (6, 7). Consequently, the prostate microenvironment comprising the glandular epithelium, stromal cells, and neuroendocrine cells is likely involved integrally in the stability of prostatic vascular endothelial cells and their associated mural cells and the regulation of angiogenesis in the prostate (8–10). Signaling molecules that can originate in the tissue microenvironment such as HIF-1α, the vascular endothelial growth factors, transforming growth factors, epidermal growth factors, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factor, platelet-derived growth factor, and proteolytic enzymes regulate angiogenesis (3, 7, 11–17). In the prostate, vascular maturation, integrity, and angiogenesis are regulated predominantly by vascular endothelial growth factor-A expression, which is regulated by androgen (18). In the rat ventral prostate, androgen ablation by castration or antiandrogens causes rapid down-regulation of vascular endothelial growth factor-A expression followed by endothelial cell death, vascular involution, and local hypoxia (peaks at 48 h post-castration), which precedes and may contribute to the apoptotic death of adenocarcinoma of the prostate (CaP) cells (19–21). CaP can induce angiogenesis as a response to areas of localized hypoxia or production of mechanical stress by the growing tumor, or by the release of growth factors by the cancer cells or reactive stroma. The production of angiogenic factors in the local microenvironment of tumors contributes to the development of a complex vascular network of unstable, immature microvessels that are convoluted, leaky, and lack supporting smooth muscle cells and pericytes (22–27). In CaP, the prostatic microvasculature loses the periglandular localization observed in benign prostate; Benjamin et al. estimated that up to 40% of the small caliber vessels in CaP lack stabilizing interactions with mural cells (28). The unique biological characteristics of prostate vasculature and the regulation of vascular integrity by androgen suggest that the interaction of the prostate tissue microenvironment with the vascular compartment in CaP may represent an important determinant of the success/failure of androgen deprivation therapy.

In benign prostate and prostatic intraepithelial neoplasia (PIN), capillaries are in close contact with the basement membrane of glands, with one or more capillaries forming a continuous ring around each gland (29, 30). Progression from PIN to CaP is associated with an increased proportion of shorter capillaries that display wider lumens and undulating external contours, increased levels of endothelial cell proliferation, and increased numbers of endothelial cells (26). Additionally, the neovascularization becomes interspersed within the tumor stroma (29, 30). The prognostic significance of microvessel density (MVD) in CaP is controversial. Both Microvessel Density and Mean Vessel Density has been reported to be, and not to be, correlated with stage/grade, recurrence, metastasis, or disease-specific survival (7, 31–42). In contrast to the prostate, the benign kidney is a highly vascular organ that exhibits a high number of blood vessels and a high ratio of endothelial cells to stromal cells per unit area, especially in the cortical region. Similarly, renal tumors, especially tumors arising from proximal tubule epithelial cells of the cortex [renal cell carcinoma (RCC)], are highly vascularized compared with CaP, however, Mean Vessel Density appears to have little prognostic significance in RCC (3, 43, 44). Therefore, there may be important differences in the regulation of angiogenesis by the tissue microenvironments of CaP and RCC.

This study demonstrates a new in vivo model for the study of human angiogenesis and human tumor neovascularization within an
intact human tissue microenvironment in primary xenografts of malignant and benign human prostate. Increases in the MVD of small caliber human vessels and the number of proliferatively active human endothelial cells were observed in primary xenografts established from CaP and benign human prostate tissues relative to the initial pretransplantation specimen. In contrast, neovascularization/angiogenesis was not apparent in primary xenografts established from RCC or benign renal tissue. Consequently, the unique interaction of the prostate vasculature with the prostate tissue microenvironment is maintained in the primary xenografts and provides a valuable model for investigation of human angiogenesis and neovascularization in CaP.

MATERIALS AND METHODS

Hosts for Human Xenografts. All experimentation involving laboratory animals was performed in accordance with the NIH guidelines and approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill. Three-month-old male athymic nude mice (Hsd: athymic Nude/Nude; Harlan Sprague Dawley, Indianapolis, IN) that were to be hosts for prostate xenografts received s.c. implants of 12.5 mg of sustained-release testosterone pellets (Innovative Research of America, Sarasota, FL) before transplantation of the prostate tissue to maintain serum testosterone levels in the host at ~4 µg/ml throughout the study, mimicking human serum levels. Mouse hosts for kidney xenografts did not receive testosterone pellet implants.

Tissue Collection, Cryopreservation, Implantation, and Harvest. All surgically resected tissues were collected in accordance with NIH guidelines on the use of human subjects, with approval by the Institutional Review Board at University of North Carolina Hospitals. Human tissue designated as excess prostate and kidney tissue was obtained from 42 and 10 patients, respectively, at the time of prostatectomy or nephrectomy. Gross morphological assessment of the resected organ/tumor by the surgeons (Dr. James L. Mohler, David K. Ornstein, and Raj S. Pruthi) was the basis for identification of the specimens as originating in noninvolved (benign) or tumor areas. Resected tissue specimens were submerged immediately in iced ViaSpan solution (Barr Laboratories Inc., Pomona, NY) and transported on ice for transplantation or processing. An initial tissue specimen, at least 3 mm², was cut from each tissue sample, placed in 10% formalin for fixation and paraffin embedded. Xenografts were established from tissue as described previously (45). In brief, the remainder of each tissue specimen was cut into wedge-shaped pieces 2–3 mm in length and 1–2 mm in width at the broadest end, and the wedges were transplanted immediately or cryopreserved in prostate growth medium (Rich- ter’s MDM, 2% fetal bovine serum, 1% antibiotic-antimycotic solution, 10 µg/ml Insulin, Transferin, Selenacide acid supplement (ITS), 0.1% epidermal growth factor, and 0.12% nicotinamide by volume/weight) with 10% DMSO at −140°C (46). Frozen tissue wedges were prepared for transplantation by thawing on ice and rinsing three times in sterile ViaSpan. For implantation, small (~3 mm) incisions were made in the skin on the right and left flanks of an athymic nu/nu mouse anesthetized with Domitor (Pfizer, Inc., New York, NY), tissue wedges to be implanted were dipped in Matrigel (BD Biosciences, Bedford, MA), and the tissue was inserted into the s.c. space through a 10-gauge trocar device (Popper & Sons, Inc., Lincoln, RI). Between 3 and 10 wedges from a single patient were implanted through a single incision along each flank. Incision sites were closed with Nexband tissue glue (Veterinary Products Laboratories, Phoenix, AZ). Mice were observed weekly after implantation. One month postimplantation, the host mice were euthanized, and the xenografts were harvested, placed in 10% formalin for fixation, and paraffin embedded. Paraffin blocks were sectioned (5 µm) onto ProbeOn Plus slides (Fisher Scientific International, Suwanee, GA).

Tissue Evaluation. A histological section from each initial tissue specimen and each xenograft specimen was stained with H&E for definitive identification and grading of CaP and RCC or confirmation of the specimen as benign (noninvolved) by the surgical pathologist (W. K. F.). Twenty-two benign prostate, 22 prostate tumor, 4 benign kidney, and 5 kidney tumor specimens for which both an initial tissue specimen and a xenograft specimen were available were selected for characterization by immunohistochemical (IHC) analysis.

RESULTS

Establishment of Primary Human Xenografts. Xenografts were established from primary human tissue specimens (44 prostatectomy and 10 nephrectomy) by s.c. implantation into athymic nude mice. Xenografts were harvested after 1 month in the host animal and were evaluated for viability at the time of harvest. Viable xenografts were pink-to-red in color, indicating anastomosis to the host vasculature and adequate perfusion. Nonviable xenografts were pale or chalky white with no visible sign of attachment to the host vasculature and were determined to be necrotic by histological analysis. The implantation protocol was efficient with the proportion of surgical specimens that yielded at least one viable xenograft being 92.7% (n = 44) for CaP and 97.3% (n = 42) for benign prostate and 100% for malignant and benign kidney surgical specimens (data not shown). Xenografts were established successfully from both freshly harvested and cryopreserved tissue fragments from nearly 100 successive patients, with per fragment take rates of approximately 60% for freshly harvested tissue and 30% for previously cryopreserved tissue. The histology of the xenografts of CaP and benign prostate generally were consistent with their respective initial tissue specimens, although squamous metaplasia, basal cell hyperplasia, and atrophic glands were observed in the benign prostate xenografts. Xenografts from both RCC and benign kidney specimens contained areas of necrosis, fibrosis, and/or fat deposition that were not observed in their corresponding initial tissue specimens.

Vasculature in the Xenografts. The central end point of this study was the determination of the efficiency and distribution of neovascu-
larization or angiogenesis during xenograft establishment in the benign and malignant xenografts compared with the corresponding initial tissue specimens. Vasculature in all initial prostate and renal tissue specimens and the corresponding xenografts was identified by IHC analysis with a polyclonal antibody specific for both human and mouse endothelial cell marker CD34. In CaP initial tissue specimens and in CaP xenografts, the vasculature was found both adjacent to glands and distributed through the stroma (Fig. 1, A and B). In contrast, whereas the vasculature in initial tissue specimens of benign prostate was localized adjacent to glandular structures (Fig. 1C), the vessels in the benign prostate xenografts were disseminated throughout the stroma (Fig. 1D). Areas of necrosis were not observed in the xenografts of prostate tissues, indicating that the efficiency of angiogenesis and the rapidity of reperfusion of the prostate tissue were sufficient to ensure viability of the entire implant.

The vasculature in the initial tissue specimens and xenografts of RCC and benign kidney tissue was distributed around the glomeruli and was interspersed among the tubules and ducts. (Fig. 1, E–H). Compared with glomeruli found in their corresponding initial tissue specimens, glomeruli in the xenografts often were atrophic, circumscribed by scarring, and/or were necrotic (Fig. 1, F and H). The consistent observation of areas of necrosis in the xenografts of renal tissues indicated that the efficiency of angiogenesis and the kinetics of reperfusion of renal tissues were sufficient to support establishment of the xenografts but inadequate to ensure viability of the entire implant.

Comparison of Vessel Density in Xenografts. CD31 (PECAM-1), CD34 (myeloid progenitor cell antigen), and vWF (factor VIII-related antigen) are markers of endothelial cells that have been used to quantitate microvessel density in CaP (40, 47). As shown in Fig. 2, serial sections of benign prostate tissue and corresponding xenograft specimens were stained for CD31 (Fig. 2, A and B), CD34 (Fig. 2, C and D), and vWF (Fig. 2, E and F) to establish which marker detected the largest number of vessels in the xenografts. Each of the three markers failed to stain all of the vessels in the tissue specimens or the xenografts; CD34 and CD31 generally stained approximately equal number of vessels, and both stained significantly more vessels than vWF. The vessels positive for each marker in the prostate initial tissue and xenografts were counted in three highly vascular fields (0.5 mm²) per section, and the results were quantitated as the MVD (Fig. 3). The MVD in the xenografts from CaP tissue was increased significantly compared with the MVD in the corresponding CaP initial tissue specimens: by CD31, MVD increased 1.8-fold in xenografts; CD34-positive vessels increased 2.1-fold; and vWF-positive vessels increased 4.1-fold (Fig. 3A). Similarly, the MVD measured by these three vessel markers was increased significantly in the benign prostate xenografts compared with the benign prostate initial tissue specimens: CD31 increased 4.8-fold; CD34 staining increased 2.6-fold; and vWF staining increased 6.9-fold (Fig. 3A). In contrast, there was no increase in MVD in xenografts established from the more highly vascularized RCC or from benign renal tissues compared with their initial tissue specimens (Fig. 3B). CD31 or CD34 stained fewer vessels in the prostate and renal initial tissue specimens, however they stained approximately equal numbers in the prostate and renal xenografts. Because CD31 is proposed to be more specific for angiogenic endothelial cells than CD34 (40, 48, 49), CD31 was used for quantification of vasculature in subsequent studies.

Species-Specific Vascular Labeling. Xenograft establishment is presumed to depend on vascularization via angiogenesis by host vessels. The studies presented in Fig. 3 that used antihuman CD31 to identify the vasculature in the CaP and benign prostate xenografts suggested that the angiogenesis apparent in the prostate xenografts was by human vessels in the human xenograft, not by host vessels. Consequently, the origin of the vasculature in the xenografts was analyzed using species-specific anti-CD31 antibodies. The species specificities of a rat monoclonal antibody specific for mouse CD31 (msCD31) and a mouse monoclonal antibody specific for human CD31 (huCD31) were verified by IHC analysis of human and mouse control tissues (Fig. 4). The huCD31 antibody labeled endothelial cells in human benign prostatic hyperplasia specimens (Fig. 4A), but did not label endothelial cells in mouse prostate tissue (Fig. 4C). In contrast, the msCD31 antibody labeled mouse endothelial cells in mouse prostate tissue (Fig. 4D) but showed no vascular reactivity in human benign prostatic hyperplasia tissue (Fig. 4B).

The species of origin of the vasculature in the prostate and kidney xenografts was determined by IHC analysis of serial sections with huCD31 and msCD31 (Fig. 5, C–F). The number of vessels positive for each of the species-specific CD31 antibodies were counted in three highly vascular fields (0.5 mm²) per section and averaged. The percentage of human vessels in the xenografts at 1 month after implantation was calculated as the number of huCD31-positive vessels divided by the sum of the huCD31-positive and msCD31-positive vessels per field. The percentage of human vessels in the prostate xenografts, despite the significant increase in the number of vessels per field relative to the initial tissue, was 79.3 ± 4.8% (n = 30). The percentage of human vessels in kidney xenografts was 90.4 ± 1.8% (n = 10), although there was no significant increase in MVD. In xenografts of either prostate or renal tissue, the vessels that stained with the mouse-specific CD31 (msCD31) were localized mainly in a compressed connective tissue layer at the periphery of the xenografts that presumably was host tissue (not shown), however, vessels of mouse origin occasionally penetrated into the xenografts, suggesting angiogenesis by host vasculature.

Quantitation of Human Vessel Density and Endothelial Cell Proliferation. The magnitude of the angiogenic response associated with establishment of the xenografts was compared with the vascularization of the initial tissue by IHC analysis of serial sections of initial tissue specimens and xenografts of benign and carcinomatous prostate and kidney specimens with huCD31, msCD31, and Ki-67 (proliferation marker) plus high-molecular weight cytokeratin (34BE12, basal cell marker; Fig. 5). Normal prostate glandular architecture, characterized by epithelial cell lined glands with adjacent stroma, was observed in the initial benign tissue specimens (Fig. 5A) and in benign prostate xenografts (Fig. 5B). The presence of a basal cell layer that stained positive for 34BE12 surrounding the glandular structures confirmed that the specimens were benign prostate. A low number of huCD31-positive vessels (dark brown) were observed in the initial tissue specimen (Fig. 5C). In contrast, a large number of small-to-medium caliber huCD31-positive vessels were dispersed throughout the xenografts (Fig. 5D) and represented a 5-fold increase in MVD compared with the initial prostate tissue specimen (Fig. 6B). MsCD31-positive vessels generally were absent from the interior of xenograft specimens (Fig. 5, E and F), consistent with the peripheral location of mouse vessels described previously. In initial benign tissue specimens, essentially no vessels were observed with Ki-67-positive endothelial cells (Fig. 5G). However, the PMVD of the benign prostate xenografts increased 8-fold compared with their initial tissue specimens, with endothelial cells with Ki-67-positive nuclei observed in vessels near glandular structures as well as in vessels dispersed within the stroma (Fig. 5H). CaP xenografts also demonstrated significant increases in MVD (2-fold) and PMVD (5-fold) compared with the initial CaP specimens (Fig. 6A). In contrast, there was a decrease, although not statistically significant, in MVD in the RCC xenografts compared with the initial RCC tissue specimens, whereas the PMVD remained unchanged (Fig. 6A). The MVD and PMVD were unchanged in the benign kidney xenografts compared with the initial tissue specimens (Fig. 6B).
Fig. 1. Spatial analysis of prostate vasculature using immunohistochemistry. Endothelial cells were stained with anti-CD34 antibody to analyze the architectural relationship to histological structures (e.g., prostate glands and kidney glomeruli) and images captured at ×200. A and B, CaP (arrows indicate stained vessels). C and D, benign prostate (black arrows indicate vessels near glands, and red arrows indicate vessels in the stroma). E and F, RCC (arrows indicate stained vessels). G and H, benign kidney (black arrow indicates a normal glomerulus, and red arrow indicates an atrophic glomerulus).
Quantitation of Vessel Dimensions. CD34 staining of the vasculature in xenografts presented in Fig. 1 suggested that the size of the angiogenic vessels in the xenografts of CaP and benign prostate were substantially smaller than the vessels in initial prostate tissue specimens (Fig. 1, A–D). In contrast, there appeared to be little difference in vessel size between the initial renal tissue specimens and the corresponding benign and RCC xenografts (Fig. 1, E–H). Consequently, vascular dimensions in huCD31-stained serial sections of benign prostate, CaP, benign kidney, and RCC initial tissue specimens and xenografts were characterized by measurement of the area and perimeter of all huCD31-stained vessels in three microscopic fields (0.5 mm²). The MVA and MVP were reduced significantly (1.7-fold) in CaP xenografts compared with the corresponding initial tissue specimens (Fig. 7A). Similarly, the MVA was reduced 7-fold in benign prostate xenografts compared with the initial tissue specimens, and the MVP showed a 2.5-fold decrease (Fig. 7B). In contrast, the RCC and benign kidney xenografts showed no significant difference in MVA and MVP compared with the corresponding initial tissue specimens (Fig. 7, A and B).

DISCUSSION

This study established that a florid angiogenic response was elicited during xenograft establishment of both CaP and benign (uninvolved) human prostate tissue, as evidenced by the significant increases in MVD with significant decreases in MVA and MVP measurements. Xenografts from RCC and benign kidney specimens were analyzed in parallel with prostate specimens, because kidney is a more vascularized organ than the prostate, with the expectation that the xenografts would demonstrate a more vibrant angiogenic response than transplanted prostate tissue. The MVD in initial tissue specimens of both the RCC and benign kidney was higher than in CaP and benign prostate initial tissue specimens; however, in the 1-month xenografts, the MVDs in prostate tissue had increased to about the same level as the MVDs in the kidney tissue. In marked contrast to the prostate, xenografts of both RCC and benign (uninvolved) kidney demonstrated a low rate of endothelial cell proliferation, showed no significant increase in vessel densities, and did not demonstrate a significant decrease in MVP or area relative to the initial renal tissues, even though the human vasculature of the kidney xenografts contained RBCs, indicating successful anastomosis with the host vasculature. Our findings of low levels of angiogenesis in the RCC and benign kidney xenografts, often associated with glomerular atrophy and areas of fibrosis/necrosis, are consistent with reports that the s.c. compartment is not an optimal location for RCC xenografts (50, 51). The areas of fibrosis and/or necrosis observed in the renal xenograft specimens imply that the number of connections and/or the low level of angio-
Genesis is not sufficient to maintain the entire cellular mass during xenograft establishment. Therefore, RCC xenografts do not appear to be an attractive model for the study of human tumor angiogenesis. In contrast, the increase in small vessels in the human prostate primary xenografts is consistent with a simple definition of angiogenic vessels that arise from existing vessels, being of small caliber and containing proliferatively active endothelial cells. Dissemination of the neovascularization throughout the stroma may be a hallmark of a human prostate angiogenic switch, whether it occurs in CaP or benign tissue, similar to the increase in intraductal microvessel density observed in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model (26, 52). The marked angiogenic response observed in the prostate xenografts implies that the model is well suited for additional investigation of hallmarks of vascular dynamics in human tumors, such as the formation of associations between the new endothelial cells and perivascular support cells, and of the response of the new vessels to tissue microenvironmental signals during tumor progression or treatment (22, 23, 28).

Although this study focused on human prostate and kidney angiogenesis, additional mechanisms reported to increase tumor perfusion could readily be investigated using this short-term human prostate primary xenograft model. The use of CD31 to quantify neovascularization does not exclude vasculogenesis from circulating endothelial precursors, which express CD31 along with CD34 and AC133, as the source of neovascularization in syngeneic xenograft/host models (53, 54). However, in our model, in which the majority of circulating endothelial precursors would be of mouse host origin, vasculogenic vessels would be counted as mouse host vessels (55). An additional consideration is to what extent could circulating endothelial precursors contribute to new vessel formation during our 30-day experimental period. In a cardiac ischemia model, Jackson et al. demonstrated that the mean percent of endothelial cell engraftment from hemopoietic stem cells, which were committed to a pre-endothelial lineage, was 3.3% over a 2- to 4-week period of reperfusion (56). Mosaic vessels and vascular mimicry in tumors could be evaluated as vessels that express human CD31 in our model (57, 58). Sharma et al. reported that CD31-
Fig. 5. Analysis of species of origin and proliferation of endothelial cells of benign prostate initial tissue and xenograft specimens. A and B, benign prostate tissue architecture (H&E). C and D, human vessel staining (arrows indicate human vessels; huCD31). E and F, mouse vessel staining (arrows indicate unstained vessels; msCD31). G and H, cellular proliferation marked by nuclear staining by Ki-67 (dark blue; arrows indicate proliferating vessels). Basal cells of prostate glands visualized by staining for high-molecular weight cytokeratin (HMWCK; 34BE12 antibody). Magnification, ×100.
positive tumor-lined channels were not found in Gleason grade 0–3 specimens, and only some were found in grade 4–5 specimens but were limited to a CD31-positive tumor epithelial cell compartment, suggesting the presence of atypical blood channels. In this study, only 43% of our tumor tissue histological sections contained areas of Gleason grade 4 or greater with only one containing an area of grade 5. Within the areas greater than or equal to grade 4, the tumor epithelium was human CD31 negative, whereas the vasculature was human CD31 positive (data not shown). However, additional studies using a basement membrane marker such as antilaminin antibody and a prostate tumor cell marker such as AMACR along with human CD31 would be necessary to evaluate the contribution of tumor cells to the lining of the neovasculature in the CaP tumor xenografts.

A marker specific for angiogenic vessels in human tumors has not been identified, and may be tissue specific. Three markers commonly used for evaluation of vessel density in morphometric and topographic studies are CD31 (PECAM-1), CD34 (myeloid progenitor cell antigen), and vWF (factor VIII-related antigen; Refs. 31, 35, 37–41). Our observation that the MVD values were similar when quantitated by CD34 and CD31 vascular staining in benign (uninvolved) prostate and kidney tissue, CaP, and RCC specimens indicated that both antigens have merit as vascular markers. However, the observation that CD31 stained more small caliber vessels suggested the use of huCD31 antibody as the most appropriate IHC marker of angiogenic human vessels in the human xenografts (48). In addition, the observation that the number of vessels stained with vWF was consistently lower than the number of vessels stained with CD31 or CD34 suggested that many of the studies that quantitated MVD in human prostate cancer based on vWF-positive vessels may have underestimated the level of tumor vascularization, particularly vessels undergoing active angiogenesis (31, 34, 39, 59, 60).

Contrary to the expectation that host angiogenesis would provide the neovasculature to perfuse the implanted xenografts, after 30 days in the mouse host, at least 80% of the vasculature in the human prostate xenografts was of human origin. The presence of viable RBCs in the human vessels indicated anastomosis of the angiogenic human vessels with the host vasculature. Host vessels were confined generally to the cuff of compressed mouse tissue on the periphery of the human xenograft tissue. Additionally, perfusion via cardiac puncture before harvesting the xenograft tissue cleared RBCs from the xenograft vasculature (data not shown). Furthermore, benign prostate xenografts from 10 patients, which were maintained in the host for 2 months, contained 61% ± 13.4 human vessels (data not shown). A possible explanation for the retention of human vasculature is that there is a species-specific difference in vascular endothelial growth factor molecules or other pro-angiogenic signals, and their differential specificities for host versus human receptors could produce two separate angiogenic microenvironments to explain the retarded or lack of invasion by host vessels in the xenograft. Such a dual response could be differentially mediated if angiogenesis in the host tissue were elicited as a wound response, whereas angiogenesis in the xenograft developed in response to hypoxia induced by surgical excision and transplantation. Ultimately, the host and xenograft microenvironments might act cooperatively with pro-angiogenic signals, resulting in a host angiogenic response that provides complete perfusion to long-term xenografts (12, 20, 61). We analyzed the species of origin of the vasculature in the serially transplanted CWR22 human prostate cancer xenograft and observed that all of the vasculature was of host (mouse) origin. Vasculature of mouse host origin is predictable in models in which human tumor cells without stroma are injected into a mouse tissue before harvesting the xenograft tissue cleared RBCs from the xenograft vasculature (data not shown). Furthermore, benign prostate xenografts from 10 patients, which were maintained in the host for 2 months, contained 61% ± 13.4 human vessels (data not shown).
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host. Similarly, long-term serially transplanted human prostate xenografts contain mouse host vessels (62). However, this may occur due to a serial dilution of human angiogenic signaling molecules and the cells that produce them as the xenografts are harvested, fragmented, and re-implanted (63). Consequently, the human vasculature present in the human prostate primary xenograft model may reach equilibrium with the mouse host vasculature when left in the initial host animal longer than 1 month but may be replaced over time if the xenografts are serially transplanted.

The applicability of currently available in vivo models of prostate cancer to the study of tumor angiogenesis has been reviewed in the literature (42, 45, 62, 64–68). Models of serially transferred human CaP that are composed of cell lines or xenografts established from a single tumor and that produce tumors with a consistent grade and/or pattern of metastasis, often requiring many months of incubation, develop in the absence of a human stroma and are vascularized with vessels of host origin. The human prostate primary xenograft model presents many advantages by allowing in vivo modeling of human angiogenesis in a human tissue-signaling microenvironment. In addition, the human prostate primary xenograft model allows rapid (1-month) analysis of human angiogenesis in a sampling of tumors at various points during cancer progression in xenografts established from a large number of patients with diverse human genetic backgrounds. The ability to cryopreserve specimens before transplantation provides additional flexibility by allowing specimens to be collected and grouped for study based on disease stage/grade, androgen status, ethnicity, geographic location, or environmental exposure. The primary xenograft model also provides the ability to modulate the host environment of the xenografts through genetic and/or investigational manipulation of the host and to address the response of the human vasculature in the human tissue microenvironment to exogenous factors such as androgen ablation, anti-angiogenic therapies, and antitumor therapies. In summary, short-term human prostate primary xenografts are a unique new model with wide applicability to the study of human prostate cancer angiogenesis in a human tissue microenvironment.

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