Study of microvessel density and the expression of the angiogenic factors VEGF, bFGF and the receptors Flt-1 and FLK-1 in benign, premalignant and malignant prostate tissues

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Summary. Purpose: Vascular endothelial growth factor (VEGF) is an angiogenic factor that stimulates endothelial cell growth and enhances vascular permeability. VEGF exerts its action by binding to the specific cell surface receptors, fms-like tyrosine kinase 1 (Flt-1) and fetal liver kinase 1 (FLK/ KDR). In tumor angiogenesis, Vascular endothelial growth factor stimulates endothelial cells to produce Basic fibroblastic growth factor (bFGF), which further enhances angiogenic activity. Very little information on the expression of VEGF, bFGF, and the receptors Flt-1 and FLK/KDR is available. Herein, we evaluate the expression of these angiogenic factors and receptors in normal prostate, high grade prostate intraepithelial neoplasia (HGPIN) and prostatic cancer (CaP). Materials and Methods: 58 selected surgical specimens exhibiting areas of normal prostate, HGPIN, and CaP were evaluated for microvessel density, and for VEGF, bFGF, Flt-1 and FLK/KDR protein expression by immunohistochemistry. Results were correlated with pathological data. Results: There was a statistically significant increase in the microvessel density and in the expression of the angiogenic factors VEGF, bFGF and the receptors FLK/KDR and Flt-1, in the premalignant and malignant tissues in comparison with normal prostatic glands. Microvessel density also correlated with higher Gleason grade, pathological stage and the expression of the receptors FLK/KDR and Flt-1. Conclusions: The "initiation switch" of angiogenesis was observed to be an early event consistent with the recruitment of new vasculature into high grade PIN lesions and it increased in the progression of prostatic cancer.

Key words: Angiogenesis, VEGF, bFGF, Flt-1, FLK/KDR, PIN, Prostate cancer

Introduction

Prostate cancer is the most commonly diagnosed cancer among men in most western countries, and it is the second leading cause of male death from malignant diseases in Europe and in the USA (Foster et al., 1999). Throughout the western world, the incidence is >30% in men over 50 years of age, rising to 80% by age of 80 years. The overall incidence is of 180,500 new cases diagnosed in the United States in 2000 (Landis et al., 1999).

Prostate cancer is widely known to vary significantly in agressiveness. Given the significant potential morbidity associated with agressive treatment there has been an increasing interest in the development of newer prognostic markers that could potentially distinguish the indolent cases unlikely to progress, from the invasive tumors capable of distant metastases and producing androgen-resistant fatal disease.

Prostatic intra-epithelial neoplasia (PIN), or intraductal dysplasia of the prostate, is considered a premalignant lesion which, according to Bostwick and Brawer (1987), might progress to invasive carcinoma. PIN affects ducts and acinis and is basically defined as proliferation and anaplasia of the lumenal (or secretory) cells. The changes are not abrupt but increasingly greater, the end of the spectrum being invasive adenocarcinoma.

Several chromosomal and molecular genetic studies reaffirm the close relationship of HGPIN to cancer. The

role of the tumor suppressor genes in prostatic carcinoma and PIN has generated considerable interest. p53 mutations have been reported in both and have been shown to be significantly increased compared to benign prostatic epithelium (Tamboli et al., 1998). Of the numerous oncogene studies, c-erbB-2 has consistently shown increased expression in PIN and prostate cancer compared to the expression in benign prostatic cells (Myers et al., 1994). Bcl-2, another oncogene, has been reported to be present in PIN lesions (Colombel et al., 1993).

Today, it is well accepted that the growth of solid tumors beyond 2-3 mm is dependent on the formation of new blood vessels (Folkman, 1971). Angiogenesis plays a fundamental role in neoplastic processes, and it is essential for tumor progression and metastasic spread of solid tumors (Carmeliet and Jain, 2000). The development of the angiogenic phenotype is the result of several genetic changes in oncogenes and tumor suppressor genes that are also responsible for the deregulation of cell growth. Oncogene activation stimulates angiogenesis by increasing the production of different angiogenic factors. Tumor suppressor gene inactivation contributes to the development of the same phenotype by lowering the secretion of inhibitors of angiogenesis (Carmeliet and Jain, 2000). Angiogenesis may be the result of factors produced by the malignant cells that are secreted into the stroma and promote endothelial cell migration and proliferation.

Tumor angiogenesis has correlated with adverse outcome in prostate cancer as measured by microvessel counting studies (Weidner et al., 1993). Significantly higher microvessel counts have been obtained in areas of adenocarcinoma than in benign tissues of radical prostatectomy specimens (Bigler et al., 1993). Increased microvascularity has been found to correlate with the pathologic stage of the disease (Brawer et al., 1994) and has been associated with the presence of metastases (Weidner et al., 1993).

Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor so far detected. It was shown to be highly specific for endothelial cells *in vitro* and *in vivo*. It promotes endothelial cell proliferation and increases vascular permeability (Senger et al., 1983).

VEGF its a 46 Kda heparing-binding, homodimeric glycoprotein that shares sequence homologies with platelet-derived growth factor. Four isoforms of VEGF have been characterized. They are produced by alternative splicing, and consist of 206, 189, 165 and 121 amino acids. VEGF binds to at least two tyrosin-kinase receptors: c-fms-like tyrosin-kinase or Flt-1, and fetal liver kinase-1 or FLK/KDR. FLK/KDR is the principal receptor of VEGF. Both are glycoproteins with seven immunoglobulinic homology domains in their extracellular part and an intracellular tyrosine-kinase signaling domain split by a kinase insert (Houck et al., 1991).

They are expressed mainly in the blood vascular endothelium, but studies on pancreatic and uterine tissues have revealed that specific receptors for VEGF may also be found in epithelial and smooth muscle cells (Brown et al., 1997; Rooman et al., 1997).

Fibroblast growth factors (FGFs) play an important role in the growth and maintenance of the normal prostate (Huss et al., 2003). Expression of bFGF has been demonstrated in the DU145 and PC3 prostate cancer cell lines (Nakamoto et al., 1992). Yan et al. (1993) showed that the expression of bFGF was increased in highly metastatic sublines of the PC3 cell line.

Diverging results regarding location of bFGF in human prostatic tissues have been found. Ittman and cols. (1999) showed immunoreactivity for bFGF in stromal and endothelial cells of normal prostate but not in epithelial cells. Also, they couldn't find staining of neoplastic cells for bFGF using frozen samples of prostatic cancer, although they have demonstrated an increase in the content of bFGF in prostate cancer tissues.

Although expression of VEGF, bFGF and the receptors Flt-1 and FLK/KDR has been examined in various human tumors, information about their distribution in human prostate tissues is scarce (Harper et al., 1998). To date, little is kwown regarding the expression of those angiogenic factors and their receptors in the different steps of human prostate carcinogenesis and its correlation with capillary density and architecture.

The aim of this study was to investigate microvessel density and immunohistochemical expression of VEGF, bFGF and the receptors Flt-1 and FLK/KDR in normal prostate, high grade prostatic intraepithelial neoplasia (HGPIN), and their relationship with the invasive prostatic carcinoma.

Materials and methods

Prostate tissue specimens

Prostate tissue was obtained from 58 patients having undergone radical retropubic prostatectomy, with histologically diagnosed prostatic carcinoma pathologic stage pT2 (26 cases) and pT3 (32 cases). Specimens were collected from the Department of Patothogy files of the Vall d'Hebron Hospital of Barcelona from 1996 to 2002. None of the patients included had undergone hormonal manipulation and the radical prostatectomy specimens were totally embedded. The study was approved by the Local Ethical Committee.

Specimens were selected after initial review of hematoxilin-eosin stained slides and included tumors of low, moderate and high grade. 27 cases had Gleason grade 2 to 6, and 31 cases were high grade carcinomas with Gleason grade from 7 to 10. The formalin-fixed, paraffin-embedded tissue was cut into 4 micrometer sections and mounted on slides for evaluation.

The slides were selected to contain representative Gleason tumor grade in each case, high grade prostatic

intraepithelial neoplasia (HGPIN) and adjacent benign tissue for comparative evaluation.

Immunohistochemical techniques

Immunohistochemistry was performed by indirect immunoperoxidase staining. Briefly, four micrometer paraffin-embedded tumor sections were deparaffined in xylene and rehydrated in graded alcohol (100%, 95%, 70% and 50%). An additional unmasking step using pepsine for VEGF, and heat with the slides immersed in citrate buffer (pH:6) for FLK/KDR, Flt-1 and CD.34, were performed before the initial blocking step. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 for 5 minutes. The sections were then incubated with the primary antibody for 30 minutes at room temperature. The antibodies used are described in Table 1. Then, they were washed with PBS and incubated for 30 min. at room temperature with a dextrane polymer (EnVision +, DAKO) peroxidase specific for the primary antibody. Immunoreactivity was visualized with 3',3'-diaminobenzidine. Sections were counterstained in Mayer's weak hematoxylin and dehydrated prior to mounting for light microscopy.

For all the immunohistochemical analysis performed in this study, a set of controls was run to verify the specificity of the antibody reaction. Positive controls included tissue sections of psoriasis, previously shown to react with anti-VEGF, bFGF, Flt-1 and FLK/KDR. Primary antibody was replaced with non-immune sera for negative controls.

Slide analysis

Two certified pathologists evaluated in a blind fashion the immunoexpression of VEGF, bFGF, Flt-1 and FLK/KDR in epithelium, vessels and stroma of normal tissue, HGPIN and carcinoma. The interobserver variability was 5% of the cases.

Immunoreactivity was graded semi-quantitatively in the whole slide using the following scores: 0: absent staining, 1: light staining, 2: moderate staining, 3: strong staining.

In each specimen the scoring was applied for areas of normal glands, HGPIN and prostatic carcinoma, and with regard to the percentage of cells with positive immune reactions. To reduce the subjectivity we applied the formula:

Hscore = 1X (% light staining) + 2X (% mod.staining) + 3X (% strong staining) We obtained for each case and area a Histoscore ranging from 0 (no immunoexpression) to 300 (maximum immuno-expression).

Measurements of the vessel density

The Chalkley counting procedure was applied for estimating angiogenesis in the prostatic carcinoma, HGPIN and normal areas. This technique was suggested

as a standard method for quantification of angiogenesis in solid human tumors in an International Consensus (Vermeulen et al., 1996).

Briefly, a 25 point Chalkley eyepiece graticule (Olympus X250, Chalkley grid area 0.196 mm^2) was applied to the ocular of the microscope.Then we scanned the tumor, HGPIN and normal areas at low magnification to identify the areas giving the impression of containing the maximum number of vessel profiles (hot spots). The microvessels were immunohisto-chemically stained by antibodies against CD.34, wich is a very specific marker for endothelial cells.

At higher magnification (200-250x), the eyepiece graticule containing 25 randomly positioned dots is rotated so that the maximum of points are on or within the vessels of the vascular hot spot. Instead of counting the individual microvessels, the overlying dots are counted.

From each section we studied the three most vascular areas of the tumor, and the interglandular stroma of the HGPIN and normal glands. The Chalkley count for each area was obtained as the mean value of the three graticule counts.

All the Chalkley counts were performed by two observers with a satisfiying reproducibility of the Chalkley assay and coutings.

Statistical analysis

Calculations were performed with SPSS 10.0 (SPSS, Inc.). For all the tests we considered statistical power $\alpha=0.8$ and a significance level of $p=0.05$.

The Student's t test was used to assess association between continuous and categorical variables.
We used the Pearson rank correlation coefficient to examine the relationship between the variables of the study.

Results

Microvessel density (MVD)

We observed statistically significant differences in the number of capillaries and the distribution of the microcirculation in the different morphologic patterns. The study demonstrated a very statistically significant increase in the capillary density in the prostatic carcinoma (mean: 8.81) compared with the benign

Table 1. Antibodies used in the study.

PROTEIN	CLONE	SOURCE	DILUTION
VEGF	A20	Santa Cruz Biotech.	1:200
bFGF	147	Santa Cruz Biotech.	1:200
Flt-1	C-17	Santa Cruz Biotech.	1:100
FLK/FDR	A3	Santa Cruz Biotech.	1:100
CD34	BI-3C5	DAKO	1:100

glands (mean: 2.95) (p<0.001) (Student's t test). On the other hand, in the adenocarcinoma, no apparent orientation of capillaries with respect to the malignant glands and cells was observed. The capillaries were variable in size, with very small or inconspicuous lumens and irregular shapes (Fig. 1A).

The HGPIN areas and benign tissue had similar distribution of the microcirculation. The smooth muscle stroma contained few venules and arterioles, with very few capillaries. In contrast, the stroma immediately adjacent to the epithelial basement membranes contained a rich network of capillaries surrounding each of the glands and ducts (Fig. 1B).

There was a statistically significant higher vascular density in HGPIN areas (mean: 5.39) compared to the benign tissue (mean: 2.95) (p<0.001) (Student's t test). Finally, the prostatic carcinoma had higher vascular

density than the HGPIN, and also those differences showed to be statistically significant (p<0.001) (Student's t test) (Fig. 2).

Microvessel counts significaly increased with increasing Gleason's score (p=0,006) (Student's t test). The highest vascular density was observed in poorly differentiated carcinomas (Gleason's grade 7, 8, 9 and 10) with a mean of microvessel count of 9 (range, 4 to 16). In moderately and well differentiated carcinomas (Gleason's grade 4, 5 and 6) the mean microvessel count was 7 (range, 3 to 13).

An increase in MVD was also associated with pathological stage. Tumors with pathologic stage T3 showed significantly higher MVD (mean: 9) than pT2 tumors (mean: 7) (p=0.009) (Student's t test).

Finally, MVD correlated with the expression of the angiogenic receptor FLK/KDR in the tumor cells (r=0,353) (p=0.007) (Pearson correlation), and with the vascular expression of Flt-1 (r=0.326) (p=0.013) (Pearson correlation). Tumors with high vessels density also had more expression of the vascular receptor Flt-1 and FLK/KDR in the tumor cells.

VEGF expression in normal prostate, HGPIN and prostate carcinoma

All prostate cancer specimens (n=58) were positive for VEGF (mean: 160, min: 100, max: 290). Immunoreactivity was heterogeneous in prostate cancer specimens with different Gleason grades and within individual tumor foci. The staining was diffuse and cytoplasmic, stronger at the cellular apex or luminal aspect of prostate cancer cells. Strongly positive VEGF immunoreactivity was detected in vascular endothelial of
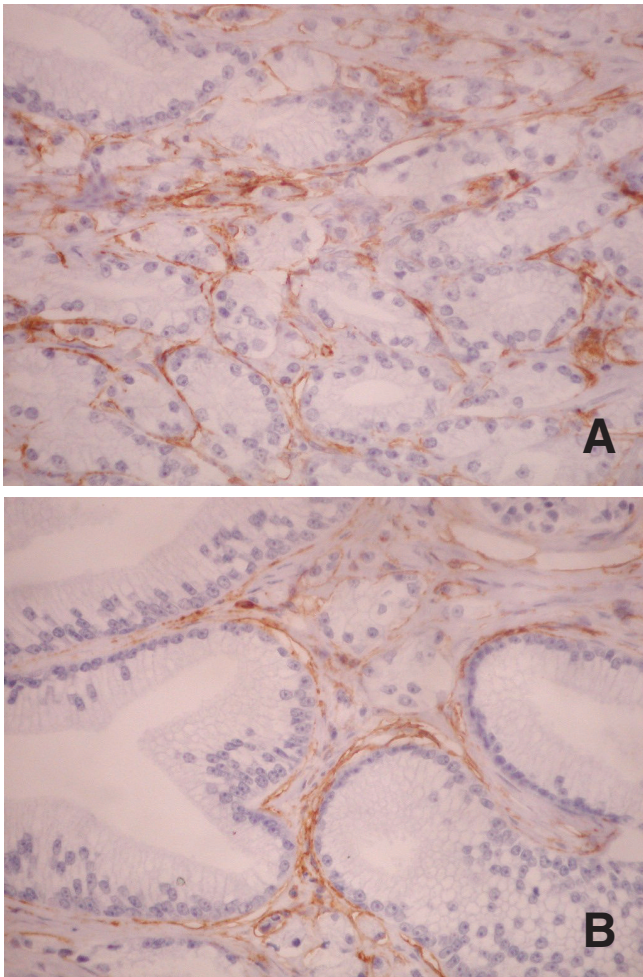

Fig. 1. A. Prostatic adenocarcinoma. The capillaries are numerous and irregularly distributed. **B.** In the HGPIN there is a high density of capillaries closely adjacent to the basal epithelial cells. CD-34, x 40
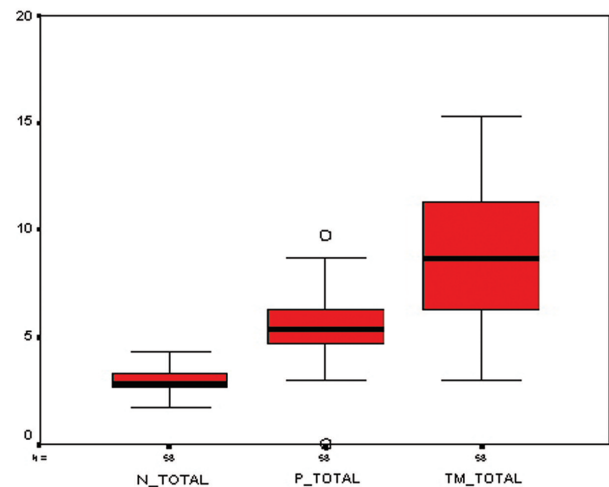

Fig. 2. Box-plot of microvessel density for normal glands, HGPIN and prostatic adenocarcinoma demonstrating an increase in the MVD in the premalignant and malignant lesions of the prostatic gland.

blood vessels, and with paler staining in the stromal cells surrounding them (Fig. 3A).

HGPIN showed strong immunoexpression of VEGF. The staining was diffuse and cytoplasmic in the secretory cells. Vascular endothelial cells surrounding the glands were also stained positive (mean: 140, min.: 0, max.: 230) (Fig. 3B).

In contrast, normal prostate tissue immunoreactivity of the secretory cells ranged from negative to low positive (mean: 90, min.: 0, max.:100). Very few basal cells stained for VEGF. Positively stained stromal cells were evident surrounding both positively and negatively stained ducts and acini.

There were statistically significant differences between the morphologic patterns studied ($p < 0.005$) (Student's t test). Figure 4 shows that the highest expression of VEGF was seen in prostactic carcinoma (mean: 160). HGPIN had a moderate expression of VEGF (mean: 140) and the lowest expression was noted in the normal prostatic glands (mean: 90).

FLK/KDR expression in normal prostate, HGPIN and prostate carcinoma

All prostate cancer specimens were positive for the angiogenic receptor FLK/KDR (mean: 230, min.: 10, max.: 300). The staining was cytoplasmic ranging from moderate to strongly positive. It was seen in the tumor and the endothelial cells of the tumor vessels (Fig. 5A).

HGPIN showed strong immunoexpression of FLK/KDR in the cytoplasm of the secretory cells and the vessels of the interglandular stroma (mean: 180, min.: 0, max.: 280).

Interestingly, in benign prostatic glands a consistent pattern was seen, in which the basal cell layer and isolated glandular epithelial cells expressed FLK/KDR (Fig. 5B).

Stastistically significant differences were observed between the expression of the receptor in these different morphologic patterns. The expression of FLK/KDR was significantly higher in prostatic carcinoma (mean: 230) than in the HGPIN (mean: 180). And the HGPIN showed more expression of the angiogenic receptor than normal glands (mean: 45) ($p < 0.005$) (Student's t test).

Flt-1 expression in normal prostate, HGPIN and prostate carcinoma

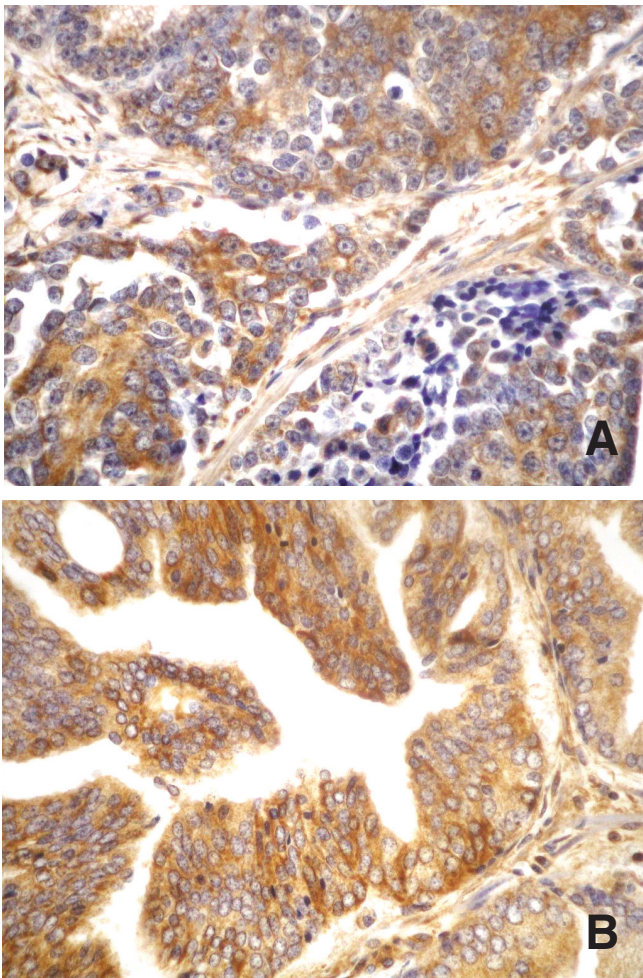The immunoexpression of the angiogenic receptor Flt-1 was slightly higher in the HGPIN lesion than in the

Fig. 3. A. VEGF staining in a case of poorly differentiated prostatic adenocarcinoma (Gleason 10 (5+5)). Note the intense staining of the tumor cells. **B.** In the HGPIN the VEGF immunoexpression was also heterogeneous, cytoplasmic and granular. x 400
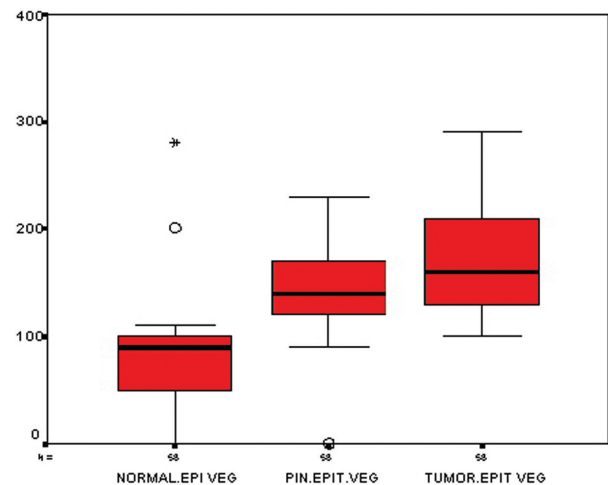

Fig. 4. Box-plot of the VEGF expression. There is a significant increase in the staining in the adenocarcinoma and the HGPIN compared to the normal glands.

prostatic carcinoma. The mean in the HGPIN was 122.50 and in the prostatic cancer 110. Also, the expression of the receptor decreased in the normal glands (mean: 30). Those differences were statistically significant (p=0.012) (Student's t test). The staining was also cytoplasmic and granular in the carcinoma and in the secretory cells of the HGPIN and the normal glands (Fig. 6).

bFGF expression in normal prostate, HGPIN and prostate carcinoma

The angiogenic factor bFGF was expressed in the cytoplasms of the tumor cells and in endothelial cells and prostatic stroma (Fig. 7). However, we only observed differences in the bFGF expression in the epithelial compartment of the carcinoma, premalignant and benign glands. The results showed that bFGF immunoexpression was significantly higher in the adenocarcinoma and the premalignant lesion of HGPIN compared to the normal glands. The mean of bFGF expression in the prostatic carcinoma and HGPIN was 90 and 10 in the normal glands (p< 0.005) (Student's t test).

Finally, there was a significant increase in the bFGF expression in the high grade prostatic adenocarciomas compared to low grade tumors (p= 0.024) (Student's t test).

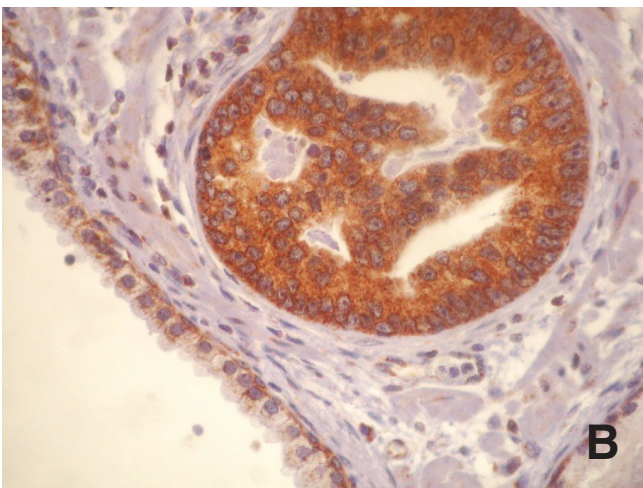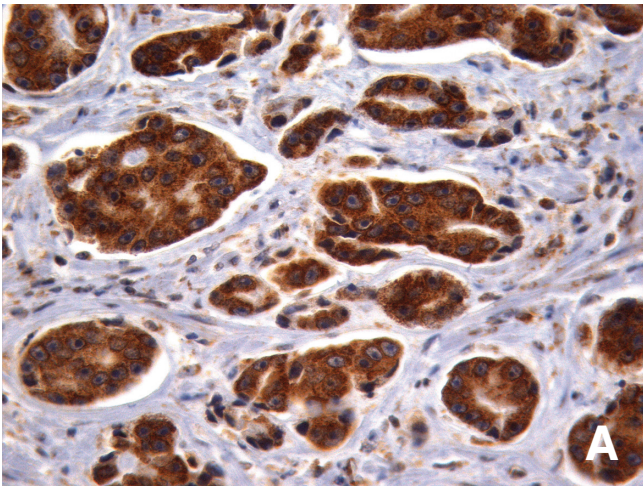**Fig. 6.** Flt-1 staining in poorly differentiated prostatic adenocarcinoma. x 400

Fig. 5.A. Expression of FLK/KDR in prostatic adenocarcinoma (Gleason 9 (4+5)). **B.** Strong staining of FLK/KDR in HGPIN. Notably, a consistent pattern of basal cell staining was seen in normal glands. x 400
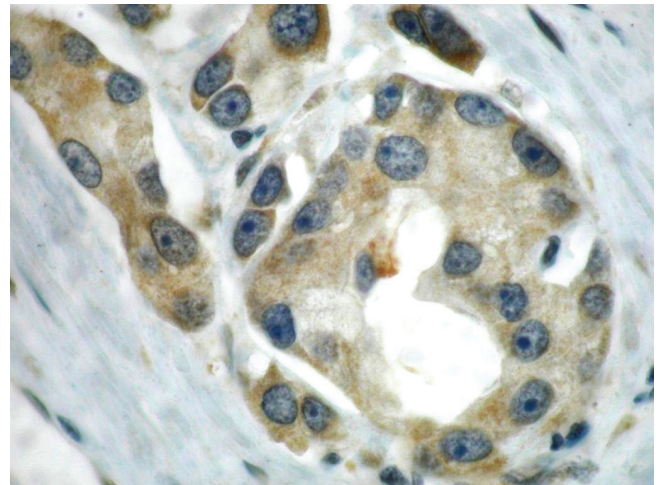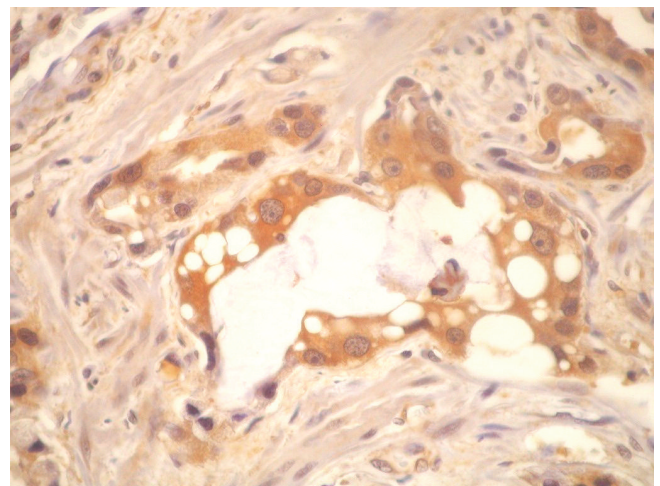

Fig. 7. bFGF immunoexpression in poorly differentiated prostatic cancer x 400

Discussion

Vascular density

Our study showed that the preneoplastic and the neoplastic lesions of the human prostate are associated with increasing qualitative and quantitative changes in the capillary architecture. In agreement with our findings Bigler et al. (1993) and Weidner et al. (1993), also observed an increased capillary density of prostate adenocarcinoma compared with HGPIN areas and benign prostate tissue.

Previous studies observed increased MVD in different preneoplastic lesions of the breast (Weidner et al., 1991), bladder (Brown et al., 1993a), and uterine cervix (Smith-McCune et al., 1993), as well as in apparently normal mucosa and epithelium taken from areas near the cancer cells.

In prostatic cancer Montironi et al. (1993) demonstrated an increased capillary density in the PIN areas compared to the adenocarcinoma. Also, in breast cancer Weidner et al. (1991) observed a rim of "intense angiogenesis" in ducts with carcinoma *in situ*. They termed this as "carcinoma *in situ* angiogenesis".

Tumors have been shown to be heterogeneous in the ability of their individual tumor cells to be angiogenic. So the vascular density is variable in different tumors, and even in different foci of the same tumor. Our results are in agreement with Weidner et al. (1993), showing that poorly differentiated adenocarcinomas had the highest vascular density and in contrast, well differentiated tumors had lower vascular density and the capillary architecture was similar to areas of benign prostatic tissue. This suggests a change of poorly differentiated neoplasms towards an aggressive angiogenic phenotype, with an increment in the incidence of metastasis.

It is very likely that increased capillary density represents only one specific aspect of a complex pattern of changes in the supporting stroma of prostate cancer when compared to benign tissue.

VEGF immunoexpression

In the prostate contradictory observations have been reported on the expression of VEGF in normal, hyperplastic, and carcinomatous glands.

In agreement with our findings Jackson et al. (1997) observed VEGF staining in glandular epithelial and stromal cells and vessels of normal glands, BPH and in the epithelial cells of prostate carcinoma with different Gleason scores.

On the other hand, Ferrer et al. (1997) reported a lack of immunoreactivity for VEGF in 20% of adenocarcinomas and in all the BPH specimens. The different results could be due to the characterictics of the antibody used. The rabbit polyclonal antibodies against VEGF used in this study and by Jackson et al. (1997) were raised against N-terminal 1-20 aminoacids of human VEGF. The characteristics of the antibody used by Ferrer et al. (1997) are not described.

The present study shows that the immuno-histochemical expression of VEGF in HGPIN is stronger that in normal prostate tissue. Mazzucheli et al. (2000) studied 45 cases of prostatic adenocarcinoma containing areas of HGPIN. They also observed VEGF immnuoexpression in all the HGPIN areas, showing two patterns of staining. Pattern A, with low to moderate intensity, and pattern B characterized by a strong and diffuse cytoplasmic staining similar to that seen in foci of poorly differentiated prostatic cancer. In our study HGPIN immunostaining was variable, but no different patterns of VEGF expression were seen. The results suggest that the process of angiogenesis is triggered independently of invasion, due to an increase in the production of inducers of angiogenesis by the secretory epithelium of HGPIN, that is surrounded by a rim of neovascularization.

Reports of upregulation of VEGF expression in other solid tumors, including breast (Yoshiji et al., 1996), ovarian (Boccock et al., 1995), and gastro-intestinal (Brown et al., 1993b) cancers indicate a potential role for VEGF in the establishment and/or progression of malignant neoplasms. Overexpression of VEGF in tumor cells, which may result from the gene amplification, local upregulation of its expression or altered VEGF turnover, presents a potential target in the management of cancers.

FLK/KDR and Flt-1 immunoexpression

We found that FLK/KDR was consistently expressed in both tumor cells and vascular endothelial cells of all prostate carcinoma specimens, and in HGPIN lesions. Few studies have focused on the immunoexpression of angiogenic receptors in prostatic cancer. Hanh et al. (2000) and Ferrer et al. (1999) observed FLK/KDR and Flt-1 immunoexpression in HGPIN and prostatic adenocarcinoma. The angiogenic receptor was expressed in the epithelium and the endothelial cells, suggesting a potential dual role for angiogenic factors involving vascular endothelial cells and tumor cell regulation. Moreover, FLK/KDR immunostaining was related to tumor grade showing that high-grade tumor cells expressed little or no FLK/KDR. In contrast, we observed stronger FLK/KDR immunostaining in poorly-differentiated tumors, although this tendency was not statistically significant.

Studies on pancreatic and uterine tissues have revealed the expression of the angiogenic receptors FLK/KDR and Flt-1 in epithelial and smooth muscle cells (Brown et al., 1997; Huss et al., 2003). It seems that at least in some tissues, the effects of VEGF are unlikely to be endothelial cell-specific.

VEGF has also been shown to be a growth factor for human Kaposi's sarcoma cells. Specifically, VEGF antisense oligonucleotides have been shown to inhibit growth of Kaposi's sarcoma in a nude mouse model

(Masood et al., 1997). Human melanoma cell lines have been shown to express VEGF receptors, and VEGF has been shown to stimulate growth of certain melanoma cell lines (Liu et al., 1995). So, it seems that FLK/KDR expression is not specific of endothelial cells ; it is expressed also in HGPIN epithelium where VEGF can act as an independent promoter of tumor cell proliferation.

Finally, there was an increment in FLK/KDR expression in tumors with high vascular density. We hypothesized that VEGF act both as an autocrine and paracrine factor, activating both vascular endothelial and tumor cells through VEGF receptors. The presence of these receptors in both endothelial and carcinomatous cells supports the hypothesis that VEGF may have both paracrine and autocrine functions in the prostatic carcinoma microenvironment.

bFGF immunoexpression

The epithelial and stromal cells in the prostate use various FGF ligands and their cognate receptors (FGFR) to transduce signals required for the processes such as branching morphogenesis, differentiation, and survival (Huss et al., 2003). b-FGF is a very potent angiogenic factor and plays an important role in the regulation of prostatic development, being abundantly expressed in normal prostate and prostate cancer.

In our study we have shown that bFGF is significantly overexpessed in the premalignant lesion of HGPIN and in the adenocarcinoma. Huss et al. (2003) have demonstrated by using a transgenic mouse model of prostatic cancer (TRAMP) that the expression of bFGF protein was observed concomitant with the emergence of PIN. The "initiation switch" was observed to be an early event consistent with the recruitment of new vasculature into high-grade PIN lesions.

On the other hand, in our series, bFGF was significantly overexpressed in the high-grade prostatic adenocarcinoma. Previous studies observed an increased bFGF expression in the metastatic carcinomas compared to the organ confined tumors (Yan et al., 1993). bFGF increases the synthesis of the urothelial plasminogen activation factor (uPA) and collagenases that promote tumor progression and metastasis (Saksela et al., 1988).

Conclusions

The results presented here indicate a continuum of genotypic and phenotypic characteristics from benign glands to high-grade PIN and carcinoma, suggesting that angiogenic factors like VEGF and bFGF may contribute to a sequential angiogenic pathway with establishment and progression of prostate neoplasia.

The widespread distribution of FLK/KDR and Flt-1 receptors in cells of different prostate tissue components suggest that the function of VEGF is not limited to angiogenesis. Future therapies targeting VEGF receptors may have a direct effect on prostate cancer tumor cells,

as well as VEGF-driven angiogenesis.

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