Journal of Cellular Biochemistry, Supplement 16H:62-64 (1992)

Quantitative Morphometric Analysis of the Microcirculation in Prostate Carcinoma

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Abstract Many neoplasms have been shown to induce capillary neovascularization and this may correlate with aggressive behavior. We investigated the phenomenon of neovascularity in benign and malignant prostatic tissue. Microvessel profiles and tissue sections were visualized by antibodies to Factor VIII and standard immunohistochemical techniques, and quantified utilizing the Optimas computerized image analysis system.

Microvessel density was compared in benign and cancerous portions of 15 radical prostatectomy specimens. Fourteen of 15 cases demonstrated significantly higher vascular density in the area of carcinoma as compared with benign tissue (ratio = 2.02, $p < 0.001$). Distribution of microvessels within malignancy was random, whereas it was restricted primarily to the periglandular space in benign tissue.

Among 20 men undergoing radical retropubic prostatectomy, there was a correlation between the vessel density and the pathologic stage. No patient with organ-confined carcinoma or cancer penetrating (but not perforating) the capsule had microvessel density greater than 156 microvessels/mm^2. In contrast, six of 15 men with more advanced pathologic stage exceeded this arbitrary threshold. These data demonstrate both increased vascularity of prostatic carcinoma as compared with benign tissue, and a further correlation between pathologic stage and vascularity. Microvessel density may be useful as a prognostic indicator.

Key words: prostate carcinoma, neovascularity

The progression of a neoplasm is dependent upon induction of a blood supply and, in the absence of this, tumors are severely limited in their growth potential [1–3]. In a number of carcinomas, including breast, bladder, cervix and melanoma, there is evidence suggesting that vascularity may be an indicator of aggressive behavior [4–7]. A number of growth factors have been associated with induction of neovascularity, and in general many of the factors related to tumor progression may also be involved with induction of new vessels [8–11].

Prostatic carcinoma represents the number one malignancy in men and the second highest cause of male cancer deaths in the United States [12]. Despite the high clinical significance of this neoplasm, the histologic prevalence of disease far exceeds clinically manifest carcinoma. This then gives rise to the great paradox in prostatic carcinoma today—the identification of carcinomas which have little likelihood of decreasing the quality or quantity of the patient's life. This has been exacerbated by the increased public awareness and interest in early detection of this neoplasm.

Our ability to assign malignant potential to a given prostate cancer is severely limited. Such factors as tumor grade, estimate of tumor volume and stage, prostate specific antigen level, cytomorphometric features, DNA ploidy and expression of various tumor markers have been and are continuing to be investigated for their role in providing prognostic information. However, in general, these have shown little promise. In the present study we investigated vascularity in benign and malignant prostate tissue.

* Recipient of a career development award from the American Cancer Society

Supported in part by a merit review grant from the Veterans Affairs Administration

MATERIALS AND METHODS

Fifteen radical prostatectomy specimens were selected to compare microvessel density in benign and malignant tissue. Cases were selected to represent a spectrum of histologic patterns.

A second series of twenty radical prostatectomy specimens (including some of the previous 15 specimens) in which careful pathologic analysis had been performed were employed to study the phenomena of microvessel density relative to stage. Pathologic staging was defined as organ-confined (OC), capsular penetration without perforation (C_1), positive margin as evidenced by tumor on the inked surface (C_2), seminal vesicle extension (C_3) and pelvic lymph node metastases (D_1).

Grading and staging were performed on hematoxylin and eosin stained sections. Microvessels were visualized utilizing antibodies to Factor VIII; immunohistochemistry utilizing the ABC technique was performed as follows on material routinely fixed in 10% neutral buffered formalin and embedded in paraffin. Predigestion with pronase (Sigma, St. Louis, MO) for 12 minutes was followed by 90 minute incubation with a 1:600 dilution of antibody to human von Willebrand (Dako, Santa Barbara, CA). The reaction product was visualized with biotinylated goat antibodies to rabbit immunoglobulins and the ABC biotin peroxidase complex (ABC Elite, Vectro Laboratories, Burlingame, CA). Slides were developed using diaminobenzidine with 0.01% nickel chloride and H_2O_2 and lightly counterstained with methyl green. Image analysis was performed utilizing a compound microscope (40×) and an external image controller coupled to a 386 personal computer. The Optimas image analysis software package (Bioscan, Edmonds, WA) was employed for interactive manipulation of the image and for data collection. A full description of the image analysis technique was previously reported [13].

The computer-aided counting affords the ability to discriminate between immunoreactive vessels and the pale counterstain. A threshold level of optimal distinction between positive immunoreactivity and background counterstain was converted to a binary black and white image. Validation of the computer counting method was performed by comparing manual counts obtained by two investigators with those obtained with the computer system. Twenty fields from eight different cases were compared which included both benign and malignant regions. There was no significant difference between the two data sets ($p = 0.05$); however, there was a slight positive bias for the image analysis method with a mean error of +2.8%. Standard deviation (SD) was 7.9.

RESULTS

The quality of the immunohistochemical reaction product for Factor VIII was judged to be quite good. Visual inspection revealed no identifiable endothelial cells which were nonimmunoreactive. In benign tissue, the microcirculation is quite uniformly distributed. The bulk of the stroma contain few venules, arterioles or capillaries. In the stroma immediately adjacent to the epithelial basement membrane, there was a rich capillary network investing each of the benign acini and ducts. In contrast, the microcirculation in carcinomas demonstrated no vessel pattern; however, there was a generalized increase in the number of capillary profiles. No obvious orientation of vessels and malignant glands and cells was observed. In 14 of the 15 prostatectomy specimens examined, image analysis revealed significantly higher microvessel density in carcinoma as compared with the benign counterpart ($p = <0.001$). The mean number of microvessels per mm^2 in the benign tissue was 72 (SD, 30.9) with a range of 34–109. The carcinomas had a mean microvessel density of 136 (SD, 49.1) with a range of 66 to 205 vessels/mm^2. The ratio of malignant to benign density was 2.02 (SD, 0.65).

The microvessel density in 20 radical prostatectomy specimens with careful staging demonstrated a trend toward higher vessel density with more advanced stage. If a threshold of 156 vessels/mm^2 was utilized, it was noted that no surgically extirpable malignancy exceeded this vessel density. In contrast, cancers of advanced pathologic stage (C_2, C_3 or D_1) exceeded this vessel density in 6/15 men.

DISCUSSION

These studies demonstrate increased vascularity of prostatic carcinoma as compared to the benign counterpart. There is a different distri-

bution of microvessels in the malignant phenotype. The correlation of pathologic stage with increased microvessel density suggests that this feature may have prognostic significance. The pioneering work of Folkman and associates demonstrated the critical role of angiogenesis in tumor progression [1–3]; this phenomenon is deserving of further investigation in the prostate.

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