Immunohistochemical evaluation of VEGF in prostate cancer

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ABSTRACT

We evaluated expression of VEGF in prostate cancer specimen in tumorous and non-tumorous tissue in a standardised setting. *Material and Methods:* Immunohistochemical staining of prostate cancer (PCa) specimens for VEGF was performed (n=30). Sections were analysed for growth factor-positive cancer/epithelial cells as well as staining intensity in malignant and histologically benign, non-tumorous tissue. *Results:* The number of VEGF positive cells in PCa was significantly enhanced compared to non-tumorous tissue ($p<0.0001$), whereas there was no difference in staining intensity. In benign glands, VEGF expression is chiefly restricted to basal cells. *Conclusion:* Our results show that VEGF plays an important role in the angiogenesis and carcinogenesis of prostatic adenocarcinoma.

KEYWORDS: *prostate cancer, VEGF, growth factor*

Angiogenesis plays a crucial role in the growth and metastasis of tumors, including prostate cancer [1,2]. Angiogenesis is thought to be regulated by pro-angiogenic as well as anti-angiogenic influences [3]. As previously described, some of the strongest is vascular endothelial growth factor (VEGF) [4]. So far, no trials with standardised experimental set-up, for this pro-angiogenic stimuli in different parts of PCa specimen.

Our objective was to quantify the expression of VEGF in carcinoma and epithelial cells of human PCa specimens. The expression of peptides was evaluated in a standard area (7.9 mm^2) of maximum expression within the carcinoma and in surrounding non-tumorous tissue.

MATERIALS AND METHODS

The study included a total of 30 prostate cancer specimens obtained by radical prostatectomy due to clinically localized prostate cancer (Department of Urology, University Hospital Mannheim, Germany). Specimens were not selected by any particular means. The mean age on the date of surgery was 66.2 ± 6.3 years.

Tissues were fixed in 10% buffered paraformaldehyde (Sigma Chemical, St. Louis, USA) for 24 h and embedded in paraffin. After hematoxylin/eosin staining, routine staging and grading diagnostics were performed in accordance with the UICC TNM classification (1997) and the Gleason method at the Department of Pathology. The major tumor-containing block of each prostate cancer specimen was used for further investigations.

For Immunohistochemical analysis serial sections of 3 µm were stained using the standard ABC method (Vector Laboratories, Burlingame, USA). Specific staining for VEGF was conducted by using monoclonal mouse anti-human immunoglobulin as primary antibody. Primary and secondary immunoglobulins were diluted in PBS and incubated in a humid chamber. In each series, primary antibodies were omitted as a negative control. Diaminobenzidine (DAB, Vector Laboratories) served as a chromogene. The tissue sections were briefly counterstained with Mayer`s H?malaun (Merck). As we menioned above we used monoclonal mouse anti-human VEGF(C-1) (Santa Cruz Biotechnology, Santa Cruz, USA) as the primary antibody, in a dilution of 1:500 used in an overnight staining procedure at 4°C. VEGF(C-1) is directed against human VEGF.

Quantification of VEGF. Tumor sections stained for VEGF was analyzed semiquantitatively by the number of positive cancer/epithelial cells as well as the intensity of immunohistochemical staining in a standard area (7.9 mm^2) of maximum expression within the carcinoma and in surrounding non-tumorous tissue. Briefly, up to 5% positive tumor cells were considered (grade 1), 6% to 49% as (grade 2), 50% to 94% as (grade 3) and 95% or more as (grade 4). Grade 1 and 2 were rated as low expression, grade 3 and 4 as high expression. The staining intensity was graded semiquantitatively as no immunoreactivity, weak reaction, strong reaction and very strong reaction and evaluated in each of the two above-mentioned regions.

STATISTICAL ANALYSIS

For statistical comparison of growth hormone expression in different areas of the section the number of positive cells and intensity of staining were ranked in 4 categories as described. All statistical analysis was performed with SAS for Windows (Version 8.02; SAS Institute Inc., Cary, NC, USA) using Kruksal-Wallis rank sum test. A $p<0.05$ was considered to be significant.

RESULTS

With one exception, positive staining in malignant and histologically benign epithelial cells was observed in 30 VEGF-stained prostate cancer specimens. The expression of VEGF was assessed in two regions of the tissue specimen by two criteria: number of positive cells and staining intensity.

Within the tumorous area 20 specimens (70.0%) displayed a high number of VEGF-positive cells (positive staining reached 95% or more cells in 9 of these cases). In opposition to tumorous tissue, non-tumorous surrounding tissue showed a statistically significant lower number of positively stained epithelial cells ($p<0.0001$): only 7 specimens (23,3%) of displayed a high number of stained cells. None of the histologically benign regions displayed more than 95% VEGF-positive cells. The relevant data is summarized in Table 1.

The staining intensity was assessed as a secondary characteristic: In standard areas of tumorous regions, 26.4% of specimens were rated as very strong, whereas in non-tumorous regions of the section, only 2 specimens (4.0%) were ranked equally.

Additionally, in most benign appearing acini's, the expression of VEGF was restricted to the basal cell compartment of the gland, whereas in malignant tissue, entire formations of malignant cells stained positive.

Discussion

Tumor growth and metastasis depend on the process of angiogenesis. This process is regulated by inhibiting and stimulating factors (including VEGF). The latter were investigated in this study.

The role of VEGF in angiogenesis is not a matter of debate: several reports have been published on the expression of these growth factors by prostate cancer cells as well as their influence on endothelial proliferation and angiogenesis (5, 6, 7). Recent immunohistochemical investigations on growth factor expression were restricted to the assessment of a single growth factor and do not allow comparative conclusions on pro-angiogenic stimuli in prostate cancer (5, 8, 9, 10, 11).

VEGF and its receptors are already a target for anti-angiogenic therapeutic strategies. In our study, the number of VEGF-secreting cells was statistically enhanced in tumorous tissue when compared to non-tumorous areas of the sections. These results conform with previous reports (10, 11). In over 70% of investigated cases, more than 50% of neoplastic cells secreted VEGF. Remarkably, statistically relevant focal differences in VEGF expression within the tumor were observed: 9 investigated cases showed areas of more than 95% positive cells. The focally enhanced expression of VEGF seems to functionally influence angiogenesis: VEGF hotspots displayed the highest rate of topographical relationship to areas of strongest angiogenesis and can therefore be identified in the investigated specimens as the most prominent stimulus for angiogenesis in PCa. Previously, an increased staining intensity of VEGF in PCa compared to benign tissue was reported (10). In the tissue sections included in this study, no changes were observed in the staining intensity of VEGF, although the number of investigated sections was twice as high.

CONCLUSION

It is obvious that this growth factor is not expressed in a unique pattern neither within the tumour or in surrounding non-tumorous tissue: Statistically relevant changes in malignant tissue were observed. Our results show that VEGF plays an important role in the angiogenesis and carcinogenesis of prostatic adenocarcinoma. Therefore, new therapeutic strategies taking VEGF and its receptors as a target seem to be a valuable treatment option for prostate cancer. These findings support further investigations in the field of anti-angiogenic therapy.

VEGF	**Tumor** n=53	**Non-tumorous tissue** n=50
Low (grade1)/(2)	(30.0%), n=9	(76.7%), n=23
High (grade2)/(3)	(70.0%), n=21	(23.3%), n=7

Staining was assessed in two regions of tissue specimens: within the tumor and in non-tumorous tissue.
Staining of 0-5% positive cells is considered as grade 1, 6-49% as grade 2, 50-94% as grade 3 and 95% as grade 4.

Tab.1 *Number of positive epithelial/cancer cells stained for VEGF.*

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Иммуногистохимическая оценка VEGF при раке простаты

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Р Е З Ю М Е

Исследована экспрессия VEGF в малигнизированных и гистологически доброкачественных участках прераратов, полученных после радикальной простатэктомии. *Материал и методы:* Иммуногистохимически окрашены препараты (n=30) с диагнозом рака простаты с использованием первычного антитела против человеческого VEGF. Слайды проанализированы на наличие VEGF, позитивно окрашенных канцер/эпителиальных клеток, а также на интенсивность окраски в малигнизированных и гистологически доброкачественных участках препаратов. *Результаты:* Количество VEGF - позитивных клеток в опухолевых участках значительно превышало таковые, найденные в немалигнизированных отделах ($p<0.0001$), тогда как различия в интенсивности окраски не выявлены. В гистологически доброкачественных участках експрессия VEGF в основном была отмечена в базальных клетках. *Заключение:* Установлено, что VEGF играет значительную роль в процессе агиогенеза и канцерогенеза рака простаты.

Ключевые слова: *рак простоты, VEGF, фактор роста*