Clinicopathological significance of expression of Estrogen Receptor-Beta (ERβ), Progesterone Receptor(PR) and Vascular Endothelial Growth Factor-A (VEGF) in colorectal cancer.

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Abstract

Background: Estrogen-receptor-beta (ER β), Progesterone-receptor (PR) and Vascular-endothelial-growth-factor have been implicated to have a role in colorectal cancer. The expression of these markers and the clinicopathological significance of the same remain unclear.

Purpose: The purpose of this study was to determine the expression of these biological markers: ERβ, PR and VEGF in colorectal cancer cells and their prognostic significance.

Methods: An immunohistochemical assay of ER β, PR and VEGF was performed in 72 patients with colonic adenocarcinoma. Normal colonic tissue from the same cases was assessed as a control. The correlation of the presence of these markers with other clinicopathological features as well as survival was then determined.

Results: None of the cases showed expression of ERβ or PR by the malignant cells of the tumor. Whereas, in two of the cases, ERβ was detected in the normal colonic mucosa. VEGF was expressed strongly in the malignant colonic cells in 64 cases, in comparison with 10 cases of normal colonic mucosa. VEGF expression was observed in 37/40(92%) patients with colorectal cancer metastatic to nodes or distant organs, whereas 24/27 (88.8%) patients with disease limited to the colon expressed the factor. Strong VEGF expression did not correlate with tumor grade, angiolymphatic-involvement, stage, disease-free and overall survival. 23/26(88.4%) patients with recurrence had strong VEGF expression, whereas 41/47(87.2%) patients with no recurrence had strong VEGF expression.

Conclusions: Colorectal cancers do not express ERβ or PR. Absence of ERβ expression by normal mucosa contests the previous reports of a protective effect of ERβ with decreased levels associated with colorectal tumorigenesis in females. VEGF expression in colorectal cancer compared to normal mucosa indicates that it may have a role in tumorigenesis. However VEGF expression cannot be used as a prognostic marker.

Introduction

Colorectal cancer is the third most frequently diagnosed cancer and the second most leading cause of cancer related deaths in both men and women in the United States[1]. Steroid hormones may have a role in the development of colorectal cancer. Epidemiological studies show that incidence of colorectal cancer is lower in women than men at all ages[2]. Hormone replacement therapy has been shown to have a protective role with estimated risk reduction of 30-40% in post menopausal women [3-7].

However, unlike the well established role of estrogen receptor (ER) and progesterone receptor(PR) in breast cancer, the prevalence and prognostic significance of these receptors in colorectal cancer is undetermined. Reports have been conflicting regarding the expression of these receptors in normal colonic mucosa and in colorectal cancer[8-11]. Estrogen effects can be mediated via ERα and ERβ members of the steroid receptor superfamily and ERβ has been demonstrated as the predominant subtype in colonic mucosa with lower expression being associated with carcinogenesis [12,13].

Vascular endothelial growth factor (VEGF) plays a role in endothelial cell proliferation and migration and hence in angiogenesis. VEGF is upregulated in adenomas and persists in carcinoma[14]. The reports on correlation of VEGF expression with lymphovascular invasion and metastases is inconsistent [14,15].The purpose of this study was to define the prevalence of these biological markers: ERβ, PR and VEGF in colorectal cancer cells and determine their prognostic significance.

Materials and Methods

Study Population:
Our study group was comprised of 72 patients who underwent colonic resection for colorectal cancer at Providence Hospital, performed by a single surgeon over a two year period (1/2002 – 12/2004). A retrospective review of medical charts was performed. Age, gender, race, tumor grade, location, lymph node status, stage, and clinical outcomes were noted.

Immunohistochemistry:
Immunohistochemical assays of ERβ, PR and VEGF were performed on formalin-fixed paraffin-embedded colonic adenocarcinoma tissue. Normal colonic tissue from the same cases were assessed as a control.

Specimens were cut into 4 µm sections, deparaffinized and rehydrated. The slides were then immersed in 3% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity. Antigens were retrieved in steam for 30 min. using Target Retrieval Solution pH 6.0 (Dako, Carpinteria, CA). Slides were allowed to cool for 20 min. and then placed in blocking solution (1.5% normal goat serum in phosphate-buffered saline) for 20 minutes to block nonspecific binding sites. For VEGF expression immunostaining was performed using a rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:100 (2 µg/ml) in Tris-HCl buffer overnight at 4° C. The sections were then treated with biotinylated goat anti-rabbit immunoglobulin followed by an avidin-biotin peroxidase complex (Vectastain ABC Elite kit, Vector Laboratories). The peroxidase

staining was visualized with 3,3' diaminobenzidine (Dako, Carpinteria, CA), and the sections were counterstained with Mayer's hematoxylin. A rabbit IgG was used for the negative controls (Dako). ERα and PR expression were assessed using mouse monoclonal antibodies (Dako) at dilutions of 1:25 and 1:100, respectively. Sections were incubated for 30 min. at room temperature, followed by detection with the Dako Cytomation Envision + System – HRP (DAB) (Dako, Denmark A/S, Glostrup, Denmark).

Scoring:
We studied both the intensity of staining (IS) and the percentage of positive staining (PS) cells. The IS was scored as 0 (absent), 1+ (weak), 2+ (moderate), and 3+(strong). The percentage of tissue with PS was scored 1 (<25%), 2+ (25-50%), 3+ (50-75%), 4+ (75-100%). A Final score (FS) was calculated using the formula: Final Score (FS) = IS x PS.

Statistics:
Statistical analysis was performed using Chi-Square tests of association, and survival analyses were performed using Kaplan-Meier. p values of less than 0.05 were considered significant

Results

The demographics of the population and the characteristics of the tumor are described in Table 1. Mean age was 68 years and the age ranged from 38-94 years. 71% were Caucasians and just over a quarter were African-Americans. The group comprised of 30 females and 42 males.

Majority of the tumors were located in the distal colon and rectum (42/72; 58.3%). 54 tumors were well or moderately differentiated and 10 were poorly differentiated. Venous involvement was seen in 26.4% and lymphatic involvement was seen in 29%. Metastasis to lymph nodes was seen in 37 (51.4%) and distant metastasis was seen in 10(14%). Mean follow up was for 54.5 months. Twenty-five patients had recurrence of disease. The median time to recurrence was 43 months. The most common site of recurrence was local recurrence (n=9) followed by lungs(n=7) and liver (n=6).

All the tumors were ERβ and PR negative. ERβ was detected in the normal colonic tissue in only two cases. PR was negative in normal colonic tissue. All tumors expressed VEGF (Fig 1). VEGF was expressed strongly in the malignant colonic cells in 64 cases, with scores of 8 in thirty cases (41.7%), 9 in eighteen cases (25%) and 12 in sixteen cases (22.2%). In comparison 10 cases of normal colonic mucosa had strong expression of VEGF with scores of 8 in five cases, 9 in 1 case and 12 in four cases. VEGF- A expression was observed in 37/40(92%) patients with colorectal cancer metastatic to nodes or distant organs, whereas 24/27 (88.8%) patients with disease limited to the colon expressed the factor. Strong VEGF expression did not correlate with tumor grade, angiolymphatic-involvement, stage, disease-free and overall survival. 23/26(88.4%) patients with recurrence had strong VEGF expression, whereas 41/47(87.2%) patients with no recurrence had strong VEGF expression.

Discussion

Unlike the well-established role of steroid receptors in breast cancer, the same in colon cancer has been debatable. Colorectal cancer has been shown to be associated with cancers of breast and ovary in women [16,17]. However studies have also suggested the decreased incidence of colon cancer in post-menopausal women treated with hormone replacement therapy [3-7].Similarly the preclinical data for the role of steroid hormones in colon cancer is unclear. Estradiol has been shown to play a role in stimulating growth of gastric and colorectal cancer cell lines[18]. High expression of sex steroid receptors have been seen in dimethylhydralazine (DMH) induced colon cancer in rats[19] and tamoxifen has been shown to reduce incidence of DMH induced colon cancer in cell lines[20]. On the other hand, studies have also shown estrogen to be inhibitive of receptor positive colorectal adenocarcinoma xenografts [21].

Studies have varied regarding the expression of estrogen and progesterone receptors in colorectal cancer. This could be due to differences in the techniques. Earlier studies used dextran coated charcoal assay and showed high concentrations of ER and PR proteins in nucleus and cytoplasm of normal colonic and colorectal cancer [22,23]. Recent studies using paraffin embedded tissue and immunohistochemistry for ER and PR have had opposing results [8,10]. This could be due to the presence of a particular subtype of Estrogen receptor. ERβ has been shown to be the predominant subtype present in colonic mucosa by assessment of mRNA expression levels of same with decreased levels in malignant cells[12].

We therefore postulated that expression of ERβ may correlate with stage. The data from this study showed no expression of ERβ or PR in normal colonic or malignant mucosa. This is in accordance with the findings of others [10,24]. One reason for the difference in the results between all the studies could be due to the techniques used. As mentioned before, previous studies used dextran coated charcoal assay and some studies used fresh specimens. The antibodies used in this study for detection of estrogen and progesterone receptors are used clinically to detect the presence of these receptors in breast cancer specimens and have been used in paraffin embedded tissues. One limitation of this study is the small sample size.

Vascular endothelial growth factor (VEGF) plays a role in endothelial cell proliferation and migration and hence in angiogenesis. VEGF is up regulated in adenomas and persists in carcinoma[14]. The reports on correlation of VEGF expression with lymphovascular invasion and metastases is inconsistent[14,15]. This study did show a high expression of VEGF in the malignant cancer cells. The expression was stronger in the malignant cells compared to normal colonic mucosa although this was not statistically significant. This increased expression in colon cancer cells has been shown using immunohistochemistry and Northern analyses[25,26]. As angiogenesis is necessary for tumor growth, VEGF does have a role in tumor growth and metastasis. A challenge in the management of colorectal cancer patients is to identify those patients who may develop metastasis. From this study we can deduce that VEGF is not a reliable marker as we found no correlation between VEGF expression and stage of disease, recurrence or survival.

Conclusions

Colorectal cancers do not express ERβ or PR. Absence of ERβ expression by normal mucosa contests the previous reports of a protective effect of ERβ with decreased levels associated with colorectal tumorigenesis in females. VEGF expression in colorectal cancer compared to normal mucosa indicates that it may have a role in tumorigenesis. However VEGF expression cannot be used as a prognostic marker.

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Sex	
Male	42(58.3)
Female	30(41.7)
Race	
Caucasian	51(70.8)
African-American	19(26.4)
Others	2(2.8)
Location of tumor	
Proximal colon	28(38.8)
Distal colon	42(58.3)
Unknown	2(2.7)
Histological grade	
Well differentiated	3(4.2)
Moderately differentiated	51(70.8)
Poorly differentiated	10(13.9)
Unknown	8(11.1)
Venous involvement:	
Positive	19 (26.4)
Negative	27 (37.5)
Unknown	26 (36.1)
Lymphatics involvement	
Positive	21 (29.2)
Negative	26 (36.1)
Unknown	25 (34.7)
Lymph node status	
Negative	33(45.8)
Positive	37(51.4)
Unknown	1(1.4)
Stage	
I	9(12.5)
II	11 (15.3)
III	28 (38.9)
IV	10 (13.9)
Unknown	14 (19.4)
Recurrence	**24(34.7)**

Table 1: Characteristics of 72 patients with colorectal adenocarcinoma.

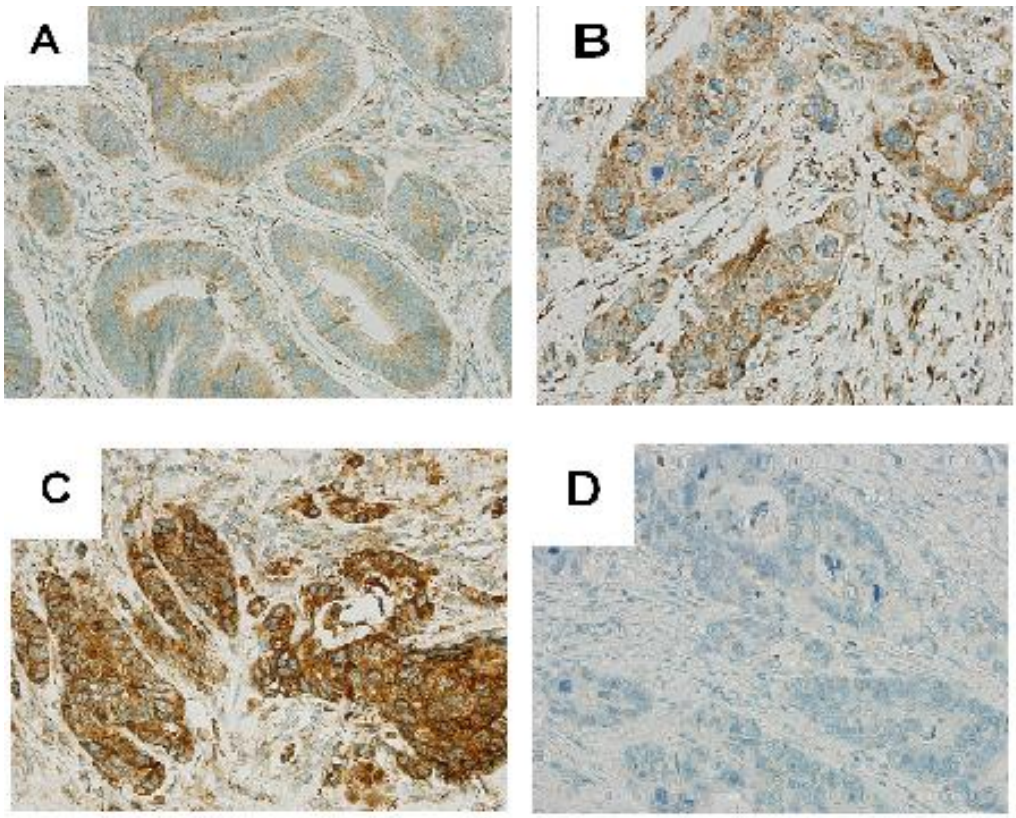

Figure 1: VEGF Immunohistochemical Expression. Figure 1A shows a tumor with 1+ staining for VEGF, Figure 1B shows a tumor with 2+ staining for VEGF, Figure 1C shows tumor with 3+ staining for VEGF and Figure 1D is a negative control slide.