Angiogenic properties of carcinoma in situ and microinvasive carcinoma of the uterine cervix

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Summary

Neovascularization is a critical step in the growth ,progression and metastasis of tumors. The degree of angiogenesis may correlate with disease stage and provide prognostic information in various neoplasms. Microvessel density was studied in 24 patients with severe cervical intraepithelial neoplasias, 15 patients with microinvasive carcinomas (International Federation of Gynecology and Obstetrics IA1) and 15 healthy controls who had undergone hysterectomy for benign conditions. The microvessel density (MVD) in microinvasive squamous cell carcinomas was 40 ± 2.42 (mean ± SD) and in squamous carcinomas in situ (CIS) 20.41 ± 2.29 ($p < 0.05$). Among patients with CIS and controls (13.33 ± 1.59) there was also a significant difference in the number of vessels ($p < 0.05$). No significant correlation was found in relation to depth of invasion and histological grade of the microinvasive carcinomas .

It is concluded that microinvasive squamous cell cervical carcinoma is an angiogenetic disorder and it seems that the onset of angiogenesis is an early event, usually in a preinvasive stage.

Key words: Cervix; Angiogenesis; Squamous carcinoma in situ; Microinvasion.

Introduction

It is now well established that invasive tumors depend on neovascularization for their continued growth, expansion and possible metastasis [1]. Several angiogenic molecules have been identified and have been shown to be produced by tumors. Angiogenesis is a complex multistep process that arises from an imbalance between positive and negative stimuli [2]. The new capillaries that are produced adjacent to malignant tumors are similar structurally to the capillaries growing during physiologic neovascularization. The tumor microvessel density (MVD) in breast and prostate cancers is correlated with the clinical outcome, suggesting that angiogenic properties depend on the aggressiveness of the tumor clone [3]. Cervical intraepithelial neoplasia (CIN) is a precursor to invasive squamous cell carcinoma. The malignant potential of the serious cervical intraepithelial neoplasia (CIN3) and carcinoma in situ (CIS) is thought to be approximately 36% over 20 years [4]. Studies have demonstrated that microvessel density increases progressively with grade of CIN [5-7]. In microinvasive squamous cell carcinoma MVD was found increased compared with the preinvasive status [8, 9].

The aim of this study was to investigate the process of neoangiogenesis in normal squamous cell epithelium, and, dysplastic and microinvasive carcinoma of the uterine cervix. We also studied the correlation between MVD, inflammation and depth of invasion.

Materials and Methods

Angiogenesis was studied in cervical cone biopsies and in removed uteri of women with CIS and microinvasive squamous cell carcinoma. Three groups of patients were studied: 24 with severe CIN or intraepithelial carcinoma (CIN 3 - CIS), 15 microinvasive squamous cell carcinomas and 15 controls who had undergone hysterectomy for benign lesions. Microinvasive disease was diagnosed according to the International Federation of Gynecology and Obstetrics (FIGO - 1A1), (depth of invasion 3 mm or less from the basement membrane , horizontally spread 7 mm or less) [10]. In this study we excluded material from women with endometriosis, malignancies of the uterus or adnexae, and women who take hormonal replacement therapy or undergo radiation therapy. Tissues were fixed in buffered formalin (10%) and specimens embedded in paraffin. Sections of 4 μm were cut and stained with hematoxylin-eosin for diagnosis. The estimation of microvessels was performed immunohistocemically with an avidin-biotin-alkaline phosphatase method for CD31 antibody (the binding site, clone PH611, dilution 1:50). At low magnification the slides were scanned for two areas of high vascularization (hot spots) below the basement membrane in the CIS and controls and adjacent to the greatest depth of invasion in microinvasive cancers. The counting of the microvessels was done using a Nikon microscope at 400x magnification (field size 0.216 mm^2). Any red staining endothelial cell or cluster of cells was considered one microvessel. Capillaries, small venules and arterioles (with lumen less than 10 red blood cells in diameter) were included while vessels with muscular coats were excluded. Statistical analysis was performed with the Student's t-test and significant differences were defined as $p < 0.05$.

Results

The mean age of the control group was 47.4 years, patients with CIS 41.5 years and patients with microinvasive disease 49.5 years (8 years difference between in situ

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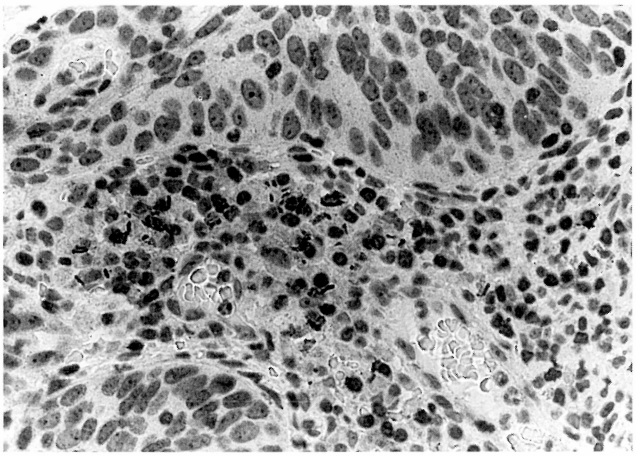

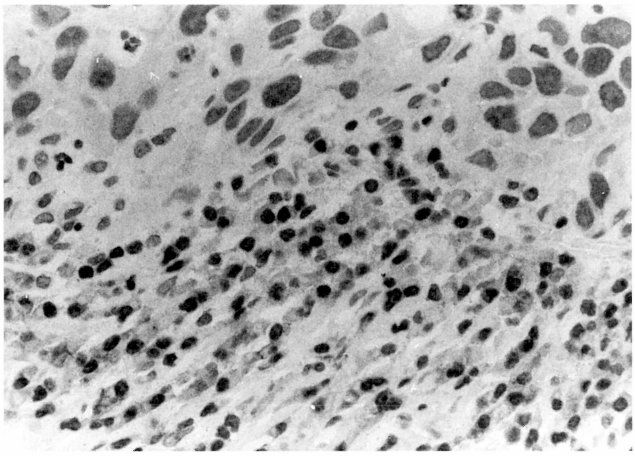

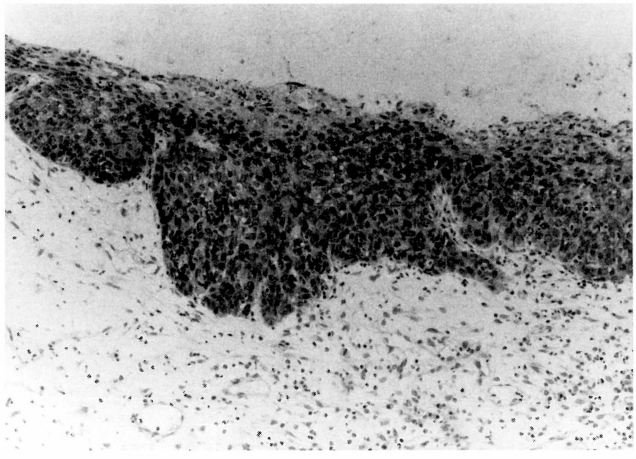

Figure 1. — Immunohistochemical stain CD 31: High microvessel density in the stroma in CIS (x 400).
Figure 2. — Microinvasive cervical carcinoma with numerous new vessels (immunohistochemical stain CD31 x 400).
Figure 3. — Microinvasive squamous cell carcinoma of the cervix stroma with dense neovascularization (Haematoxylin-eosin x 100).

and invasive cancers). The immunohistochemical stain CD31 revealed endothelial cells allowing identification of blood vessels in CIS (Figure 1) and in microinvasive cancer (Figure 2). There was a progressive increase in MVD from controls to microinvasive disease (Figure 3). The MVD in microinvasive squamous cell cancinoma was 40 ± 8.44 (mean ± standard deviation SD), in the CIS 20.41 ± 2.29 and in controls 13.33 ± 1.59. There was a significant difference in microvessel count between the groups of CIS and microinvasive carcinoma ($p < 0.05$). The MVD per high power field in CIS also differed significantly from the controls ($p < 0.05$). No correlation was noted according to depth of invasion, while the high-grade malignancy cancers (3 in our study) had higher MVD (no significant difference because of few samples). The degree of stromal inflammatory reaction among the three study groups was more severe in the microinvasive one. The microvessel count was not related to age.

Discussion

Onset of angiogenesis, the creation of new vessels from pre-existing ones, is a critical step in the growth and metastasis of tumors [11]. Solid tumors are unable to grow larger than 1-2 mm^3 in the absence of neovascularization and need a more developed vessel network, so more tumor cells have a chance to enter in the circulation [1, 11]. In addition the hyperplastic endothelial cells produce growth factors and kinases which stimulate the growth of tumors. The prognostic value of angiogenesis in cervical carcinoma is unclear. Some studies suggest that high grade MVD predicts improved survival when associated with radiotherapy [12] or chemotherapy [13], while Rutgers *et al.* [14] and Schlenger *et al.* [15] found that angiogenesis is not an independent prognostic factor related to stage of disease and patient outcome. The few studies that correlate the number of vessels between high grade intraepithelial neoplasia (CIN3-CIS) and microinvasive squamous cell cancer agree that the last has statistically higher angiogenic activity [7-9]. Several in vivo and in vitro studies have supported that the onset of angiogenesis is before of invasion phenomenon in a variety of tumors as breast and colon cancers [16, 17]. Chronic inflammation is itself angiogenic given that the inflammatory response consists of infiltration by mononuclear inflammatory cells, proliferation of fibroblasts and small vessels. Chronic inflammation and infection by human papilloma virus (HPV) and other sexually transmitted diseases perhaps have cumulative activity. However, because of the common association of these findings with cervical cancer they have to be regarded as an indirect rather than a direct causative factor of angiogenesis [5, 8]. A potential link between HPV infection, mainly subtypes 16 and 18, and angiogenesis may rest with the p53 tumor – a suppressor gene – and its relation-

ship to VEGF expression [7, 18]. Invasive cervical cancer, on the other hand, promotes an inflammatory reaction in the stroma of the host-patient. A study has shown that the inflammatory response is more dense in microinvasive status of cancer than advanced stages [9]. Abulafia *et al.* and Leug *et al.* did not find a statistically significant difference in angiogenesis between CIS and normal controls [8, 20]. However, recent studies have demonstrated that microvessel density increases progressively with grade of CIN [5-7]. Abulafia *et al.* responded with a letter and support that CIS has no angiogenic properties and the differences in the various studies depend on factors like the method of vessel estimation and the proper inclusion and exclusion criteria in the selection of cases [21]. In our study we demonstrated that there was a statistical difference in the MVD between CIS and normal controls ($p < 0.05$). Noteworthy is that the methodology and patient selection were similar to the criteria used in Abulafia *et al.'s* study [8]. Our results support the theory that the development of carcinoma is associated with two phases of growth [2, 11]. Intraepithelial neoplasia can be considered as a prevascular phase of angiogenesis without metastatic ability and once the tumor has penetrated the basement membrane and has invaded the stroma, the second phase has started [2, 22].

In conclusion our study pointed out that angiogenic changes occur in the whole course of carcinogenesis of squamous cell epithelium. Neovascularization may be essential for growth, maintenance and progression not only for infiltrative neoplasms but also for preinvasive cervical lesions. The study of angiogenesis, except for understanding the way of tumor expansion, contributes in clinical trials of angiogenic compounds as a complimentary or alternative therapeutical approach for the above lesions.

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