Assessment of Angiogenesis in Human Cervical Lesions

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Abstract. Angiogenesis has been extensively studied in several
types of invasive carcinomas and has been correlated with tumor
growth and metastasis. In some of these studies it has been
shown that angiogenesis precedes neoplastic transformation.
A
correlation is evident between microvessel density and conditions
that exist much before the onset of tumor formation (i.e.
dysplastic lesions). In this study, tumor vascularity was
quantified in a series of cervical lesions: 92 dysplasias (31 mild,
24 moderate and 36 severe) and 11 infiltrating squamous cell
carcinomas. Microvessels were visualized by a polyclonal
antibody against factor VIII-related antigen (DAKO), using a
streptavidin - peroxidase immunohistochemical method. Vessel
density was quantified in 3 high power fields (hpf) of the most
vascular areas, by two independent observers. Mean vascular
counts were 13 ± 5 vessels per unit area in CIN I lesions, 17 ± 4
in CIN II, 20 ± 6 vessels in CIN III and 17 ± 5 in infiltrating
carcinomas. There was a progressive increase of vascularity in
the dysplastic lesion in the samples with increasing atypia in
relation to controls. No significant differences were noted
between severe cervical dysplasias and infiltrating carcinomas.
Our findings suggest that angiogenesis may be an important
event in tumor initiation and the conversion of the normal
epithelium into cancer.

Angiogenesis is a fundamental phenomenon occurring in
embryogenesis wound healing, immune reactions and
tumorogenesis. Folkman (1) has demonstrated that tumor
cells in organ culture can grow in the absence of
vascularisation only up to nodules in the range of 1 to 2 mm
in diameter. When these nodules are implanted in tissue,
however, they develop a blood supply from the surrounding
host tissues and further growth ensues.

In humans, most tumors persist in situ for months to years
without neovascularization and become vascularized when a
subgroup of cells in the tumor "switches" to an angiogenic
phenotype (2). In the prevascular phase, the tumor is rarely
larger than 3 mm³. Such asymptomatic lesions originate from
epithelial cells, for example in carcinoma of the bladder,
brast, cervix or skin (3).

Tumor angiogenesis not only provides the means for tumor
cell metastasis, but it is also required for growth of the
metastatic foci (4). Recently, numerous studies have shown a
statistically significant relationship between increased
intratumoral microvessel density and the risk of metastasis
and/or decreased survival of patients with solid tumors. This
relationship has been shown for patients with breast carcino-
ma (5,6,7) non-small cell lung carcinoma (8) head and neck
squamous cell carcinoma (9) and prostate carcinoma (10).

However, to our knowledge the correlation between
microvessel density and conditions that exist before the onset
of overt tumor formation has not been studied in any detail.
Squamous epithelium of the cervix is an interesting model
since carcinoma of the cervix arises in a series of incremental
epithelial changes ranging from mild to the progressively
severe dysplasia (CIN) and finally to invasive carcinomas.

In this study we examined the degree of vascularity in a
series of cervical lesions, including cervical dysplasia, with
varying degrees of cytologic atypia and infiltrating squamous
cell carcinomas. Our goals were to determine if there were
any significant differences in vascularity between these
various types of premalignant and malignant conditions and
to identify the stage when angiogenesis is initiated during
umor progression of cervical neoplasia.

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Average values

![Graph showing average values of microvessel counts for normal, CIN I, CIN II, CIN III, and infiltrate](image)

Figure 1. Vessel density in normal squamous epithelium, CIN dysplasia and infiltrating carcinomas. Bars represent mean values (± standard deviation).

![Image of normal squamous epithelium with low microvessel density](image)

Figure 2. Normal squamous epithelium of the cervix with low microvessel density.

![Image of cervical intraepithelial neoplasia (CIN) I and II with relatively high angiogenesis](image)

Figure 3. Cervical intraepithelial neoplasia (CIN) I and II with relatively high angiogenesis (compare with figure 2).

Materials and Methods

Our material consisted of cervical biopsies or tumor specimens showing CIN I (31 cases), CIN II (24 cases), CIN III (36 cases) and infiltrating squamous cell carcinoma (11 cases). Areas representative of each lesion were selected from sections stained with hematoxylin and eosin. All blood vessels were visualized by staining endothelial cells with polyclonal antibody against factor VIII-related antigen (Dako Corporation, Santa Barbara, C.A.) using a streptavidin-peroxidase immunohistochemical method.

Areas containing the most capillaries and small venules (areas of the most intense neovascularization) were found by scanning the tumor sections at low power (40x, 100x). After these areas were identified, individual microvessels were counted on three consecutive 400 x fields (40x objective lens and 10x ocular lens, 0.1885 mm²/field) using an occult grid. The mean value for total number of microvessels was recorded from these three counts. These measurements were made at three sites: at the junctional zone between epithelium and underlying stroma where most newly formed microvessels were confined, at the adjacent normal dermis and in the tumor tissue (invasive carcinomas).

All measurements were performed by two independent observers. Cases with the greatest variability in vessel counts were reviewed using a double headed light microscope simultaneously by the two observers.
Results are expressed as means of all three areas evaluated from each patient ± standard deviation.

Results

Microvessels were more abundant immediately beneath the basement membrane of the squamous epithelium. The mean vessel counts for non-lesional cervix and for each type of cervical lesions are summarized in Table 1. Intraepithelial neoplasia is nourished by an increased number of microvessels. There was a general trend to increasing vascularity with progressively greater atypia in cervical epithelium. [Mean vascular counts were 13 ± 5 per unit area in CIN I lesions, 17 ± 4 in CIN II and 20 ± 6 vessels in CIN III. Statistically significant differences in vascular density were recorded for CIN I versus CIN II (p < 0.001) and CIN II versus CIN III (p < 0.005)]. The mean values in the dysplasias were all significantly higher when compared with the corresponding values at the adjacent normal dermis (p < 0.001). No significant differences were evident between severe cervical dysplasia and invasive carcinomas. However, most vessels that are present in carcinomas are characterized by increased coarseness and irregularity. Characteristic structural organization of tumor vessels is evident (Figures 1 and 4).

Discussion

The formation of new microvessels is a complex multistep process involving extracellular matrix remodelling, endothelial cell proliferation and migration, capillary differentiation and anastomosis. The process is regulated by several growth factors, such as transforming growth factor alpha and beta, vascular endothelial growth factor and platelet-derived endothelial cell growth factor. These factors may be released by tumor, stroma, or epithelial cells or the extracellular matrix (11).

Previous studies have shown that the initiation of angiogenic activity and neovascularization precedes tumor formation (3).

Most preneoplastic lesions lack obvious neovascularization, as compared to neoplasias which are typically highly angiogenic. Whether capillaries will grow or not towards a tumor may depend on one or more events. In one model the switch to the angiogenic phenotype is via a cancer suppressor gene that codes for an angiogenesis-inhibitor protein (thrombospondin), which is down regulated when the cells undergo malignant transformation and become angiogenic (12). In another model the switch to the angiogenic phenotype involves a change in the local equilibrium between positive and negative regulators of the growth of microvessels.
Tumor cells may overexpress one or more of the positive regulators of angiogenesis, or may cause the mobilization of an angiogenic protein sequestered by the extracellular matrix (2).

As Folkman suggested, the angiogenic phenotype does not always correlate with malignancy and this may precede, coincide with, or follow malignant transformation (1). One goal of the present study was to investigate at which point the increase in vascular density is first evident in the tumor progression of cervical intraepithelial lesions.

Our observations indicated that in intraepithelial neoplasia an increased number of horizontally proliferated new microvessels beneath the basement membrane are present. A statistically significant gradual increase of vascularity was also noted with progressively greater atypia. The vascular density of infiltrative squamous cell carcinomas was comparable with that of highly dysplastic lesions (CIN II-CIN III). Reports by other investigators are in agreement with our findings that angiogenesis may be expressed by cervical epithelium, before the appearance of morphologic changes of malignancy (13). Furthermore, certain preneoplastic lesions of the human urinary bladder have been found to possess angiogenic capacity (14). In addition, preneoplastic lesions, in situ carcinomas and invasive carcinoma have been found in human breast biopsy specimens to possess angiogenic activity, while normal tissues did not (15). This led to the suggestion that an assay for angiogenesis might be used to distinguish patients who are at high risk of developing mammary carcinoma.

Our results demonstrate that angiogenic activity, as evident by vascular density counts, is a property of malignant and premalignant human squamous epithelium. These findings, taken together with similar results for other human organs (breast, urinary bladder, etc.), suggest that "normal" epithelium may also express angiogenic activity prior to their conversion to malignancy.

The relationship between the appearance of angiogenic activity and malignant transformation is difficult to establish. Obviously, the ability to induce new vessel formation does not indicate that the epithelium is neoplastic or that it will necessarily become neoplastic. However, when a cell population acquires angiogenic capacity, it has available one of the requirements for continuous growth. Therefore, angiogenesis could be used to screen for neoplastic potential of hyperplastic epithelium in cervical biopsy tissues.

References

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