Chinese Journal of Cancer Research 8(4):260 — 262, 1996

Clinical Observations

MORPHOLOGICAL AND QUANTITATIVE STUDY ON STROMAL MICROVASCULATURE OF NASOPHARYNGEAL PRECANCEROUS LESIONS

Liu Kela 刘克拉 Yun Jingping 云径平 Hou Jinghui 候景辉

Department of Pathology, Tumor Hospital, Sun Yet-sen University of Medical Sciences, Guangzhou, 510060

The stromal microvasculature in 27 cases of nasopharyngeal precancerous lesions (NPP) were studied for morphological features and parameter quantitation by computerized image analysis system. FVIIIR:Ag was used as a vascular endothelium marker to show the blood vessels by immunohistochemistry. The microscopic and quantitative results show that most cases (79%) had hyperplastic abnormal blood vessels similar to the stromal neovascularization of nasopharyngeal carcinoma (NPC). These findings suggest that angiogenesis may be a prerequisite to cancerous transformation of nasopharyngeal epithelium, and that cases of NPP with neovascularization have a high tendency to cancerous transformation.

Key words: Nasopharyngeal carcinoma, Precancerous lesion, Angiogenesis.

It has been demonstrated that angiogenesis, the formation of new capillary blood vessels is an indispensable requirement for growth of tumor and the growing tumors are able to induce a neovascular response in host tissue by releasing tumor angiogenesis factor (TAF) or by other ways. It has also been found that some precancerous lesions have such ability too.[1,2] This suggests that the ability to induce neovascularization may be an important biological trait in some precancerous lesions as well as in carcinoma, and this trait can be used as an index to judge the tendency of precancerous lesions to cancerous transformation[3,4] To probe this problem in nasopharyngeal precancerous lesions (NPP), we studied the microvasculature in the stroma of 27 such cases microscopically and quantitatively.

Accepted September 5, 1996

MATERIALS AND METHODS

According to the morphological criteria of nasopharyngeal precancerous lesions,[5,6] 27 cases of NPP, which included 16 cases of atypical hyperplasia and 11 cases of atypical metaplasia, were selected from formalin-fixed and paraffin-embedded tissue specimens in our Department. All specimens were cut vertically with more stroma under the epithelium. 30 cases of chronic nasopharyngeal inflammation (NPI) and NPC at clinical stages I and II were selected as control groups. The tissue sections were processed with LSAB immunohistochemical procedure to delineate the blood vessels labeled by FVIIIR:Ag. Rabbit anti--human--FVIIIR:Ag polyclonal antibody and LSAB Kit were products of DAKO Company.

The stained sections were studied by light microscope and IBAS-II interactive image analysis system manufactured by the Kontron Co. to quantitate the vascular morphological parameters, including density, area, diameter and shape factor.

RESULTS

Microscopic Vascular Morphology

All capillary endothelial cells showed strongly positive staining by FVIIIR:Ag. Veins stained comparatively darker than arteries. Lymphangial endothelial cells showed a lack of FVIIIR:Ag in our sections. Under the light microscopy, only a few NPI cases (6%) had markedly increased stromal capillaries and those blood vessel branches with small and round lumens were evenly scattered and usually perpendicular to the epithelium. But most NPC cases (93%) had significantly increased capillaries in the stroma. These newly formed blood vessels were characterized by high density, uneven distribution, abundant but tortuous branching and variations of thickness. The vessels lumina were irregular, varying from as narrow as cracks to as dilated as sinusoids, and large, thin-welled vessels were common. Similar to NPC, most of NPP cases (79%) had highly significantly increased stromal vascularity. The newly formed capillaries with similar morphological features to those of the NPC usually distributed near the basement membrane of the epithelium, or surrounded the atypical hyperplastic epithelial cells extended into the stroma (Figure 1,2). It has also been noted that some NPP cases (21%) have no marked differences in vascular morphology from NPI.

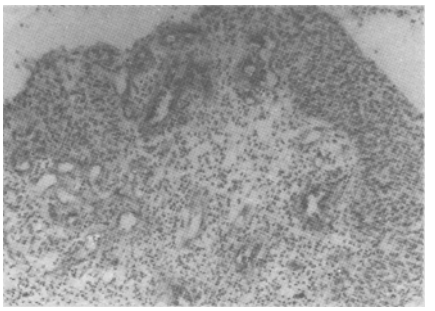

Fig 1. Atypical epithelial hyperplasia, showing increased capillaries near the epithelium (LSAB×100)

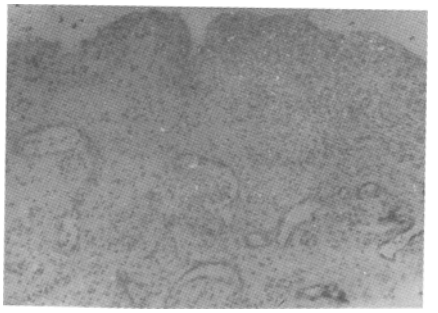

Fig 2. Atypical epithelial metaplasia, showing newly formed blood vessels characterized by uneven distribution, tortuous branching and varied thickness.

Quantitation of Vascular Morphology

Five vascular morphological parameters were measured by computerized image analysis system. The means were summarized in the Table 1.

*Table 1. Means of vascular morphological parameters by Image Analysis System**

	Number of case	Number of vessles (/100,000 μm²)	Percent vessel area (PVA)(%)	Diameter (μm)	Perimeter (P, μm)	Figure factor (FF)
NPI	30	12.9 ± 2.67	1.47 ± 0.57	11.08 ± 2.14	42.3 ± 5.25	0.71 ± 0.07
NPP	27	20.56 ± 4.06	6.97 ± 2.77	18.78 ± 3.41	68.40 ± 14.25	0.61 ± 0.07
NPC	30	24.55 ± 6.37	6.54 ± 2.68	17.13 ± 3.03	66.18 ± 14.75	0.63 ± 0.07

* At least four fields of stroma just under the epithelium were measured on all sections. PVA stands for the ratio of total vessel area to the field area. Diameter is the perimeter equivalent area calculated by the formula P/π. Figure factor stand for the geometrical 4×area/P, which is equal to 1 when the figure is round, otherwise it is less than 1.

All NPP parameters were compared with those of NPI and NPC using the Student-Newman-Keuls's analysis of differences between NPP or NPC and NPI ($P<0.01$) but no significant differences between NPP and NPC ($P>0.05$).

DISCUSSION

It is well established that severe atypical hyperplasia or metaplasia of nasopharyngeal epithelium are NPC precancerous lesions. But it has also been found that not all such cases develop into cancer and some may even reverse to normality. So it is of value to study if the stromal vascularity is related to the epithelial pathological changes. Our study of the stromal microvasculature of 27 NPP specimens shows that most cases (79%) have abnormal hyperplastic features of the capillaries, similar to the neovascularization of early stage NPC. Only a few cases (21%) had no significant differences from NPI in vascular morphology. As to the epithelial morphology, there are no marked differences between the two, but both differ in stromal vascularity. This may demonstrate that the stroma in most NPP cases has been affected by the cytobiological traits of atypical hyperplastic cells, which may released some angiogenic factors to induce neovascularization as in NPC. Our results also show that the newly formed capillaries distributed near the epithelial basement membrane or the atypical hyerplastic epithelia stretching to the stroma. This suggests that these capillaries are closely related to the nutrition and metabolism of epithelial cells. Due to the increased abnormal blood vessels, the proliferative rate of atypical epithelia cells rise sharply and their potential malignant biologic traits are gradually enhanced until cancerous transformation occurs. On the basis of this knowledge, we consider that the presence of increased stromal abnormal blood vessels appears to be a prerequisite for the carcinous transformation of epithelia, and that cases of NPP with neovascularization have a high tendency to cancerous transformation.

In order to compare the vascular morphology of three kinds of tissue more objectively and accurately, 5 geometrical parameters were measured by image analysis system, which has been used to study the microvasculature of the brain, esophagus and skin.[7,8] The quantitative data showed that there was significant statistical difference between NPP or NPC and NPI in all parameters, but not between NPP and NPC. This demonstrates that there are resemblance between NPP and NPC in vascular morphology, and conforms our microscopical findings and conclusions.

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