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DETECTION OF OCCULT TUMOR CELLS IN RESECTED LYMPH NODES OF PATIENTS WITH STAGE I CARCINOMA AND ITS CLINICOPATHOLOGICAL SIGNIFICANCE

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Objective: A lot of 3715 resected lymph nodes from 350 stage I cancer patients, including 94 NSCLC, 112 breast carcinoma, 115 esophageal carcinoma, and 29 vulvar carcinoma cases, were re-examined by immunohistochemistry. Methods: Using monoclonal anti-cytokeratins (AE1/AE3), anti-EMA, and polyclonal anti-keratin antibodies, the tumor cell micrometastases were detected to obtain more reliable information concerning the nodal status. Results: Nodal occult metastases were observed in 113 of 350 (32.5%) patients and in 203 of 3715 (5.5%) nodes. The positive rates in both patients and nodes were higher in NSCLC than in others ($P<0.05$). The nodal occult metastases were seen in 58% of pulmonary squamous cell carcinoma and 53.8% of adenocarcinoma, while they were seen in 22.5% of esophageal and 10.3% of vulvar squamous cell carcinomas and in 27.7% of breast adenocarcinoma ($P<0.05$). Follow-up of a part of breast carcinoma patients showed that the clinical prognosis was worse in patients with positive nodes than in negative ones ($P<0.05$). Conclusion: The data suggested that the immunohistochemical techniques can significantly facilitate the detection of micrometastatic tumor cells in lymph nodes. The frequency of nodal occult metastases may have important impact on the clinical prognosis of cancer patients.

Key words: Nodal occult metastases, NSCLC, Breast Cancer, Esophageal cancer, vulvar cancer, Immunohistochemistry.

The lymph node status, i.e., presence or absence of metastatic tumor, is the critical basis for staging, and therapy, and evaluating prognosis of patients with various cancers. However, small metastatic tumor cell usually be overlooked in resected lymph nodes by conventional histopathologic examination. Several studies have reported that nodal occult micrometastases of tumor, even single tumor cell, can be found in greatly large proportion of ostensible stage I cancer patients by immunohistochemical detection.[1-5] In order to understand the exact status of lymph node and its clinicopathological significance, we re-examined all the negative nodes, contained no metastasis with various cancers, including non-small cell lung carcinoma (NSCLC), breast adenocarcinoma (BA). Esophageal squamous cell carcinoma (ESCC), and vulvar squamous cell carcinoma (VSCC), by immunohistochemistry. The follow-up data of partial postoperative patients with BA and NSCLC were also presented in this paper.

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MATERIALS AND METHODS

Patients Examined

A series of 350 cancer patients were studied, among them 181 cases, including 34 NSCLC, 32 BA and 115 ESCC cases, were treated at 1st Teaching Hospital, Xinjiang Medical College from 1970 to 1995, while 169 cases, including 60 NSCLC, 80 BA and 29 VSCC cases, were treated at UCLA Medical Center from 1982 to 1988. The clinical data included 94 NSCLC patients, aged 60.5 (range 30–80), with 61 males and 33 females; 112 female BA patients, aged 54 (range 29–87); 115 ESCC patients, aged 52 (range 35–72), with 83 males and 32 females; and 29 female VSCC patients, aged 62 (range 54–88).

Specimens

Both resected primary tumor tissues and lymph nodes were fixed in 10% formalin and embedded in paraffin for routine histopathologic examination, and these paraffin blocks were obtained from the pathologic file. The new sections were cut as close to the original block face as possible. Four sections of 4 μm thick were cut from tumor tissue blocks; 4 sections of lymph node blocks were cut in a serial 50 consecutive sections, and 4 sections from every 10 cut slices were made for HE- and immuno-staining respectively.

Immunohistochemical Staining

All sections allowed to dry at 55 ℃ overnight, and deparaffinized, rehydrated by routine procedure. Monoclonal anti-cytokeratins (AE1/AE3), anti-EMA, and poly-clonal antikeratin antibodies were used as the primary antibodies and performed by ABC (LSAB) immunoperoxidase staining.[2-5]

Statistics

The data were examined by the x^2 test, and $P<0.05$ was accepted as a statistically significant difference.

RESULTS

Immuno-staining product in positive cells was seen mainly in the cytoplasm with condensation and reddish-brown color was present beneath the membrane, but the nucleus was negative. The primary tumor tissues including squamous cell carcinoma and adenocarcinoma revealed similar immunoreactivity after stained with the three primary antibodies. In positive lymph nodes, the occult metastatic tumor cells, singly or in clusters, were readily observed (Figure 1–4), usually located in subcapsular and medullary sinuses.

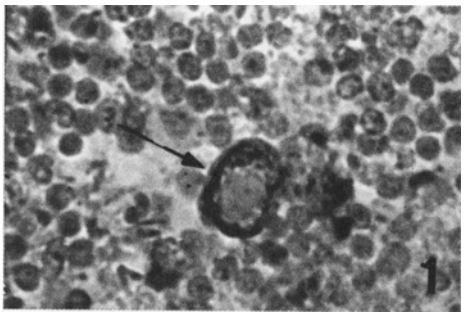

Fig. 1. Single tumor cell in medullary sinus of lymph node, mixed with lymphocytes, was found by immuno-staining (monoclonal anticytokeratins AE1/AE3, 1:80, ABC immunoperoxidase staining, × 400).

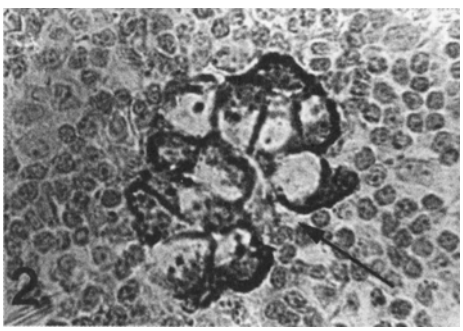

Fig. 2. Micrometastatic clusters of tumor cells in lymph node of BA patient were found by immuno-staining (monoclonal anti-EMA, 1:80, ABC immunoperoxidase staining, × 400).

In all patients with various cancers, the occult metastases of tumor cells were found in resected lymph nodes in 113 of 350 patients (32.3%) and in 203 of 3715 nodes (5.5%). It was found higher in NSCLC patients (56.4%) and detected nodes (16.6%) than in others ($P<0.05$) (Table 1).

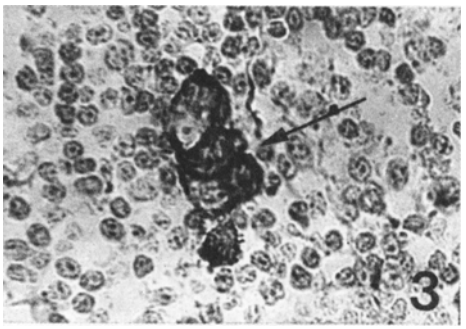

Fig. 3. Small group of metastatic tumor cells in lymph nodes of patient with pulmonary squamous cell carcinoma was found by immuno-staining (polyclonal anti-keratins, 1:1000, ABC immunoperoxidase staining, × 400).

The positive frequency of nodal occult metastasis was 58% in pulmonary SCC and 53.8% in pulmonary adenocarcinoma, and revealed more high than those in esophageal SCC (22.5%), vulvar SCC (10.3%), and breast adenocarcinoma (27.7%) ($P<0.05$) (Table 2).

Follow-up informations were available in 61 patients with breast cancer for a period of 7 years after surgical operation. During the first three years, 4 of 17 (23%) developed distant metastases or recurrence in patients with nodal occult tumor micrometastases. Only 4 of 44 (9%) had metastases or recurrence within last three years in nodal negative patients. There is a statistically significant difference ($P<0.05$) (Table 3).

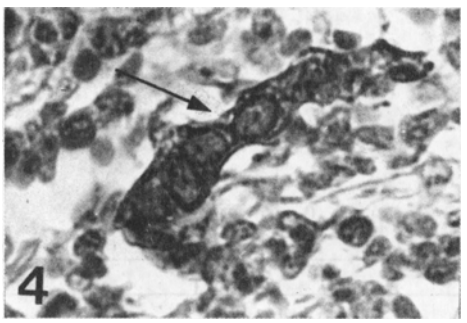

Fig. 4. Micrometastatic foci of adenocarcinoma cells in lymph node of patient with pulmonary adenocarcinoma were found by immuno-staining (monoclonal anti-cytokeratins AE1/AE3, 1:80, LSAB immunoperoxidase staining, × 400).

Table 1. The detected rate of occult tumor cells in lymph nodes of patients with stage I cancers

Type of cancer	No. of patients	Positive patient		No. of LN	Positive nodes	
		N	%		N	%
NSCLC	94	53	56.4	739	123	16.6
BA	112	31	27.7	1840	50	2.7
ESCC	115	26	22.6	380	27	7.1
VSCC	29	3	10.3	216	3	1.4
Total	350	113	32.3	3715	203	5.5

Table 2. Frequency of SCC and adenocarcinoma occult tumor metastases in nodes located at various organs

Location	SCC			Adenocarcinoma		
	N	Positive	%	N	Positive	%
Lung	50	29	58.0	39	21	53.8
Esophageal	111	50	22.5	0	0	0
Breast	112	0	0	112	31.2	27.0
Vulva	29	3	10.3	0	0	0
x^2		$P<0.05$			$P<0.05$	

Table 3. Clinical outcome correlation with the presence or absence of occult tumor in resected lymph nodes

Years after surgery	Status of resected lymph nodes									
	Occult tumor positive					Occult tumor negative				
	No. of patient	Recurrence		Distant metastases		No. of patient	Recurrence		Distant metastases	
		N	%	N	%		N	%	N	%
1	4	1	25	0	0	10	0	0	0	0
2	4	0	0	2	50	7	0	0	0	0
3	4	0	0	1	25	8	0	0	0	0
4	2	0	0	0	0	6	0	0	0	0
5	1	0	0	0	0	7	2	29	1	14
6	2	0	0	0	0	2	0	0	0	0
7	0	0	0	0	0	4	1	25	0	0
Total	17	1	6	3	18	44	3	7	1	2
x^2	$P<0.05$									

DISCUSSION

The identified capacity of occult tumor cells in resected lymph nodes can be greatly increased by immunohistochemistry, using anti-epithelial or other molecular marker reagents. Several studies have reported that detection of occult tumor cells in regional nodes of patients with various cancers revealed a surprising large proportion of ostensibly nodal tumor-free patients by immunohistology. The frequency of nodal occult tumor metastases in patients with early melanomas was 15%,[1] breast adenocarcinomas ranged 9–32%,[2–4,6] lung cancer ranged 56–63%.[5,7] It is noteworthy that the detected rate of occult tumor in resected lymph nodes was 58% in pulmonary squamous cell carcinomas and 53.8% in pulmonary adenocarcinoma, they are clearly higher than those in esophageal SCC (22.5%), vulvar SCC (10.3%) and breast adenocarcinomas (27.7%) ($P<0.05$). It may be related to the facilitated dissemination, poor prognosis, and higher mortality in patients with NSCLC than in others. The clinical data of postoperative follow-up in a part of patients with breast cancer and NSCLC also revealed that the clinical outcome was worse in nodal positive patients than in negative ones ($P<0.05$). Recently Morton, et al.[8,9] have demonstrated that detection of nodal occult tumor in regional lymph nodes of early melanoma patients by intraoperative lymphatic mapping and rapid immunohistochemistry for the best plan was chosen in surgery. Therefore, immunohistochemical detection of nodal occult tumor in cancer patients may be very valuable and useful in cancer research and clinical practice.

REFERENCES

1. Cochran AJ, Wen DR, Motton DL. Occult tumor cells in the lymph nodes of patients with pathological stage I malignant melanoma: an immunohistochemical study. Am J Surg Pathol 1988; 12:612.
2. Trojiani M, DeMascarel I, Boniclion F. Micro-metastases to axillary lymph nodes from carcinoma of the breast: detection by immunohistochemistry and prognostic significance. Br J Cancer 1987; 55:303.
3. Galen MH, Athanassion E, Bell J, et al. Occult regional lymph nodes metastases from breast cancer: immunohistochemical detection with antibodies CAM 5.2 and NCRC-11. J Pathology 1991; 165:221.
4. Chen Zhaolun, Wen DR, Coulson WF, et al. Occult metastases in the lymph nodes of patients with breast cancer node negative by clinical and histologic examination and conventional histology. Disease Markers 1991; 9:239.
5. Chen Zhaolun, Perez S, Holmes EC, et al. Frequency and distribution of occult micrometastases in lymph nodes of patients with non-small cell lung carcinoma.

J Natl Cancer Inst 1993; 85:493.

6. 陈朝伦, 卢晓梅, 黎音, 等. 乳腺癌局部淋巴结内隐匿微小癌转移的免疫组化研究. 中国肿瘤临床 1996,; 23(12):851.
7. Chen Zhaolun, Cochran AJ, Huang Rong, et al. An immunohistochemical study of occult micrometastases in regional lymph nodes of 94 patients with stage I non-small cell lung carcinoma. Chinese J Cancer Res 1993; 5(3):199.
8. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage molanoma. Arch Surg 1992; 172:392.
9. Robert ME, Wen DR, Cochran AJ, et al. Pathological evaluation of the regional lymph nodes in malignant melanoma. Seminars Diagnostic Pathology 1993; 10:102.