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## Distribution of Mast Cells in Mediastinal Lymph Nodes from Lung Cancer Patients

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### Abstract

**Background:** Mast cells have been documented to have several key functions with regards to malignant neoplasms. However, the functional significance of their accumulation is largely unknown. An analysis of the mast cell profile in mediastinal lymph nodes from lung cancer patients is reported here.

**Methods:** One hundred thirty-four, randomly selected lymph nodes (63 with positive pathological lymph node status) from 39 surgically treated lung cancer patients were examined. All cancer negative nodes were obtained from stage I patients. Mast cells were stained with Alcian blue and safranin O. Metastatic cancer cells were stained using anti-cytokeratin antibody.

**Results:** Immunohistochemical studies with cytokeratin revealed micro metastasis in 9/71 (12.68%) nodes previously diagnosed as histological negative. In tumor-free mediastinal lymph nodes, the mast cell count was significantly higher than in metastatic nodes. In all cases, mast cells were observed primarily in the T-cell area.

**Conclusions:** An inverse relationship was observed between the number of mast cells and the amount of tumor tissue. The presence of mast cells primarily in the T-cell area implies a relationship between mast cells and the T-cell system. From the present study it is not possible to conclude whether mast cells in lymph nodes are for or against tumor spread.

### Introduction

Numerous studies on mast cells have revealed its several key functions with regard to malignant disease process [1]. These include anti tumor functions of natural cytotoxicity [2,3], and release of anti tumor compounds [4,5], while on the other it has also been associated with angio-

neogenesis [6-9]. The functional significance of accumulation of mast cells around tumor is a subject of controversy because of contradictory previous experimental data [10]. It is still not clear whether mast cells are for or against tumor spread [10], however, a correlation

between mast cells and survival in pulmonary adenocarcinoma has been previously demonstrated [11].

Little attention has been paid to the role of mast cells in lymph nodes from patients with malignant neoplasms. Several of these have produced contradictory results with some reporting an increased number of mast cells [12–14], while others reported no change or even a decrease in their number [15,16]. The present study analyzes the mast cell profile in mediastinal lymph nodes from lung cancer patients.

### Materials and methods

Randomly 134 formalin-fixed and paraffin-embedded lymph nodes from 39 primary lung cancer patients (24 adenocarcinoma and 15 squamous cell carcinoma) treated surgically at Miyazaki Medical College during 1994–1995 were selected. Of these, 15 patients were in stage I, 2 in stage II, 13 in stage IIIA, and 2 in stage IIIB. None of the patient was a smoker. Routine pathological examination with hematoxylin and eosin staining revealed 63 nodes to be metastasis positive. All cancer negative nodes were obtained from stage I patients. Using one paraffin block for each node, studies were conducted to determine the number of mast cells and to detect the presence of cytokeratin positivity.

Serialized 4  $\mu\text{m}$ -thick sections were deparaffinized, rehydrated, and stained with Alcian blue (pH 0.3) and safranin O (pH 0.1). The number of mast cell per 5 fields at a magnification of 200 $\times$  was counted under light microscopy. These results were assessed semi quantitatively by two authors, and the average count was used as the final score.

The specific monoclonal antibody against cytokeratin 19 (Santa Cruz Biotechnology, California, U.S.A.) was used to detect micro metastasis. Before staining, serialized 4 $\mu\text{m}$ -thick sections were deparaffinized in three changes of lemosol and rehydrated through a descending series of ethanol. These sections were immersed in 0.6%  $\text{H}_2\text{O}_2$  in methanol for 20 minutes at room temperature to block endogenous peroxidase activity. After blocking non-specific protein bindings by incubating with Block Ace<sup>®</sup> (Dainippon Inc., Osaka, Japan), the sections were incubated overnight at 4 $^\circ\text{C}$  with primary antibodies against human cytokeratin (1:50). Subsequently, sections were incubated with secondary antiserum (1:500) for one hour, followed by incubation with peroxidase anti-peroxidase complexes for 30 minute at room temperature. The sections were visualized with the diaminobenzidine/metal concentration (10x) and stable peroxide substrate buffer (1x) system (Pierce, Rockford, Illinois, U.S.A.). After washing with water, they were counterstained with hematoxylin. Double staining for mast cells and cytokeratin

was also performed to determine the correlation between mast cells and metastasis. Sections were stained with cytokeratin antibody in the same manner as described above the were counter stained with Alcian blue and safranin O. Immunohistochemical results too were assessed semi quantitatively by two authors.

Data was analyzed using the Student's t-test and the Mann-Whitney U test. Differences were considered significant when the *p* value was less than 0.05.

### Results

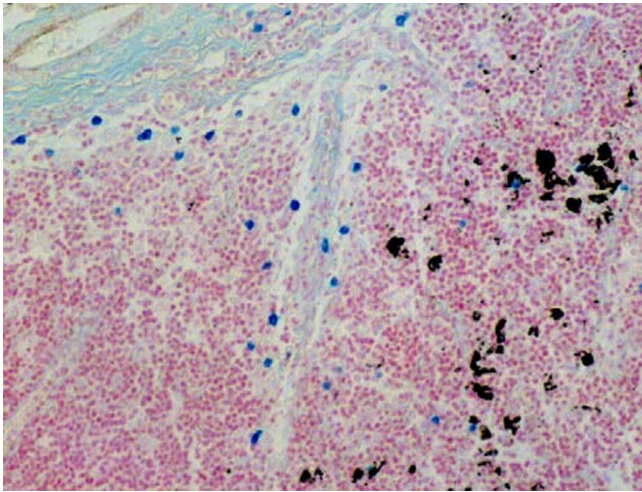
Immunohistochemical staining with cytokeratin revealed micro metastasis in 9/71 (12.68%) nodes that were previously diagnosed as histologically negative. In the 63 metastasis-positive nodes, all samples showed positive staining with cytokeratin. Based on these results, samples were divided into three groups: group A, metastasis negative by both routine pathological study and cytokeratin staining; group B, micro metastasis (malignant cells negative by pathological study, but positive by cytokeratin staining); and group C, metastasis positive by both techniques.

Mast cells were observed in the subcapsular sinus, paracortical areas, medullary cords, and medullary sinus (Figure 1) and were rarely seen in the B cell area. The mast cell count in tumor-free mediastinal lymph nodes ( $33.23 \pm 24.58$ ) of lung cancer patients was significantly higher than in lymph nodes showing metastasis ( $19.78 \pm 12.76$ , and  $9.69 \pm 9.37$  in group B and C respectively; group A vs. B:  $P = 0.0155$ ; group B vs. C:  $P = 0.0064$ ; group A vs. C:  $P = 0.0019$ ). Metastatic lymph nodes had very few mast cells. This trend was observed in patients with both adenocarcinoma and squamous cell carcinoma. However the groups are too small to have meaningful separation.

In cancer-positive nodes, mast cells were observed primarily around tumor deposits with an occasional mast cell seen within the metastatic foci.

### Discussion

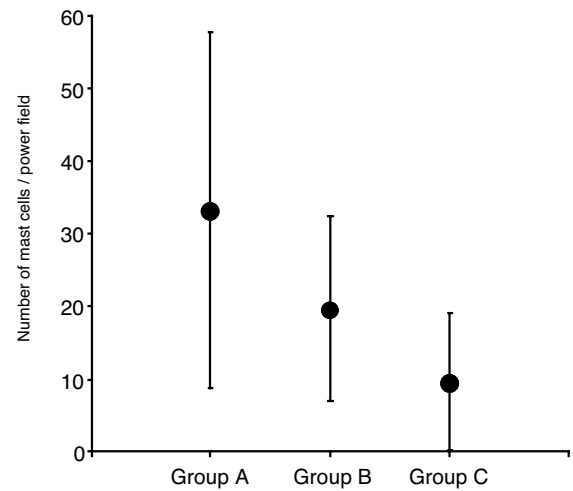
It is not clear till date, whether mast cells are for or against the tumor growth [10]. The functional significance of accumulation of mast cells around tumor areas is a subject of controversy because of contradictory previous experimental data [10]. The results of the present study demonstrate significant differences in the number of mast cells among group A and C in mediastinal lymph nodes. However there is no additional data to interpret the meaning of this phenomenon. Mahapatro *et al.*, [17] reported similar distribution of mast cells in the metastatic lymph nodes of breast cancer patients who survived or did not survive 5-year. Bowers *et al.*, reported high mast cell count in the axillary lymph nodes of breast cancer patients



**Figure 1**  
Mediastinal lymph nodes showing abundance of mast cells in the subcapsular and medullary sinus. (Alcian blue and safranin O staining.  $\times 100$ )

surviving for 5 years compared to non-survivor [14]. However, Naik *et al.*, stated that Bowers' results might be due to no metastasis in survivors, or less number of metastatic nodes compared to non-survivors [16]. Taken together, it is possible that the patient's survival is not related to the number of mast cells in lymph nodes, and the role of mast cells in metastatic lymph nodes remains unclear.

Two theories were considered while evaluating our results: (i) metastasis occurs when cancer cells inhibit mast cell functions either directly or indirectly by producing an inhibiting activator that works against mast cells, resulting in a decrease in the number of mast cells as the cancer spreads, and (ii) because mast cells cannot be stained histologically when complete mast cell degranulation occurs, a decrease in the number of mast cells observed may be the result of degranulation rather than exit. Based on this view, cancer cells may induce mast cell degranulation, thereby resulting in some type of interaction between mast cell degranulation products and cancer cells. The exact relationship between mast cells and cancer cells remains unclear. Further studies are required not only to determine whether mast cells are for or against tumor growth and metastasis, but also to establish that the decreased observed is true decrease and not because of inability to stain the mast cells due to its degranulation. We hope to address this question in our next investigation designed using monoclonal antibodies against mast cells.



**Figure 2**  
Mast cell counts (mean  $\pm$  SD) in mediastinal lymph nodes. Group A vs. B:  $P = 0.0155$ ; Group B vs. C:  $P = 0.0064$ ; Group A vs. C:  $P = 0.0019$  (Group A: metastasis negative, Group B: micro metastasis, Group C: metastasis positive)

The standard deviations of mast cell count of our results are relatively large. There might be other factors influencing mast cell distribution, thereby resulting in the large range of the standard deviation. For example, mast cell counts in the sinuses of axillary nodes from breast cancer patients had been reported to vary with the type of sinus reaction present [18].

We noted an almost complete absence of mast cells in the follicle of the "virgin" B cell area of lymph nodes and observed a significant number of mast cells in the paracortical or thymus dependent (T-cell) area. These results are consistent with an earlier study of axillary lymph nodes from breast cancer patients [17]. Furthermore, Burnet speculated that mast cells and basophilic leukocytes or some subpopulation of such cells are derivatives of thymus-derived lymphocytes [19]. The predominance of mast cells in the T-cell area in mediastinal lymph nodes in our study also supports the theory that the origin of mast cells is related to the T-cell system. McKinnon *et al.*, suggested that a large number of cytotoxic T-cells are activated by tumor-draining reactive lymph nodes, thereby resulting in an increased secretion of interleukins and an expansion of the mast cell population [20].

As reported previously, the number of mast cells stained in formalin-fixed specimens is consistently less than in specimens fixed in Carnoy's solution [21,22]. In the

present study, we used formalin-fixed specimens because we could not obtain specimens fixed in other solutions. Therefore, the possibility remains that our results would be different if our specimens had been preserved in Carnoy's solution.

## Conclusions

In our study, mast cells were seen primarily in T-cell areas, thereby suggesting a positive relationship between mast cells and the T-cell system. Although we observed an inverse relation between the number of mast cells and the amount of tumor tissue, the reasons for this remains unclear. Further studies using enzyme specific staining and CD antigens are required to determine exact relationship of mast cells and cancer cells in the metastatic lymph nodes.

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