ORIGINAL PAPER

Mast cell reaction in malignant laryngeal neoplasm

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Abstract

Introduction. Mast cells are normal connective tissue residents. Their densities vary from an organ to other, but are constantly well represented in respiratory tract. Mast cell hyperplasia was found in many malignant tumors, but the significance of this phenomenon is still unknown. In the literature, there are few data about mast cell reaction in malignant laryngeal neoplasm. *Material and methods*. We studied archive blocks from 127 laryngeal carcinomas. For histological diagnosis two sections were prepared for Hematoxylin–Eosin staining and Alcian blue–Safranin histochemistry at pH 0.2 for identifying mast cells. Examination has been performed with Nikon Eclipse 600 microscope. Microscopic images were analyzed with Lucia G program. Microvessel density was calculated using the hot spot method. *Results*. Most of the cases were squamous cell carcinoma G1 – 24.4%, G2 – 56.69%, G3 – 18.11%, and 0.78% adenoid cystic carcinoma. Invasive squamous cell carcinoma mast cell microdensity was 2.19 and 4.66 in microinvasive squamous cell carcinoma. Mast cell microdensity in malignant laryngeal papillomatosis was 9.33 and 46.66 in adenoid cystic carcinoma. In carcinoma-associated mast cell hyperplasia, the large majority of mast cells were Alcian blue positive. *Conclusions*. In early stages, the mast cells are numerous (microinvasive squamous cell carcinoma mast cell microdensity 4.66) and rare or even absent in late stages (invasive squamous cell carcinoma mast cell microdensity 2.19). Mast cell microdensity in malignant laryngeal papillomatosis was 9.33 and 46.66 in cystic carcinoma. Alcianophil mast cells are present in tumor area, and safraninophil mast cells are residents of connective and muscular tissue, at a distance from the tumor.

***Keywords*:** carcinoma, larynx, mast cell, Alcian blue–Safranin histochemistry.

Introduction

Mucosal squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common cancer affecting men. Despite diagnostic and therapeutic advances, there has been little improvement in the cure rate over the last three decades [1].

A mast cell (or mastocyte) is a resident cell of areolar connective tissue (loose connective tissue) that contains many granules rich in histamine and heparin. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing and defense against pathogens.

Paul Ehrich first described mast cells in 1878 based on their unique staining characteristics and large granules. These granules also led him to the mistaken belief that they existed to nourish the surrounding tissue, and he named them "mastzellen", meaning "feeding cells". Nowadays, they are considered part of the immune system. Mast cells are very similar to basophil granulocytes (a class of white blood cells) in blood; the similarities between mast cells and basophils have led many to speculate that mast cells are basophils that have "homed in" on tissues. However, current evidence suggests that they are generated by different precursor cells in the bone marrow. Nevertheless, both mast cells and basophils are thought to originate from bone marrow precursors expressing the CD34 molecule.

The basophil leaves the bone marrow already mature while the mast cell circulates in an immature form, only maturing once in a tissue site. The immature mast cell determines its precise characteristics in the tissue site where it settles.

Two types of mast cells are recognized, from connective tissue and a distinct set of mucosal mast cells. The activities of the latter are T-cells dependent. Mast cells are present in most tissues in the vicinity of blood vessels, and are especially prominent near the boundaries between the outside world and the internal milieu, such as the skin, mucosa of the respiratory and digestive tract, as well as in the mouth, conjunctiva and nose.

Among the immune cells (i.e., tumor-associated macrophages, dendritic cells, neutrophils, T-cells and mast cells) in the microenvironment, mast cell has probably received the least attention despite well-established evidence for its roles in carcinogenesis [2].

Our purpose was to calculate microvessel density using the hot spot method.

Material and methods

We studied archive blocks from 127 cases of laryngeal carcinoma. We performed seriate sections at 5 μm following usual histological technique. For histological diagnosis and grading, two sections were prepared for Hematoxylin–Eosin staining.

Because this paper evaluates the diagnosis and prognosis, we also used histochemical Alcian Blue Safranin method at pH 0.2 for mast cell identification. Examination has been performed with Nikon Eclipse 600 microscope, the images were recorded in *.jpeg* format and the microscopic images were analyzed with Lucia G program.

Mast cell reaction

We analyzed mast cell microdensity and malignant laryngeal neoplasm proliferation type. We performed Alcian blue–Safranin staining, which assess two major types of mast cell, of medullar origin (alcianophil) and connective tissue origin (safraninophil).

To evaluate mast cell number we performed Hot Spot method choosing three fields with maximum mast cell density, the results being arithmetic average at ×400 magnification.

Results

We encountered invasive squamous cell carcinoma, G3, with relative extensive necrosis area, tumor cells in plaques and islands separated by a conjunctive stroma, rich in lymphocyte inflammatory infiltrate. Tumor cells presents wide size variations, the majority are polygonal, with large euchromatic nucleus (smooth granular chromatin), big and multiples nucleoli and low acidophil cytoplasm in Figure 1.

In Figure 2, we present a keratinized squamous cell carcinoma, G2, with islands shape and compact area malignant cell proliferation pattern, in stroma there is an abundant inflammatory infiltrate; tumor cells are large, with vesicle-like nucleus, with large and unequal nucleoli, frequent atypical mitosis. Cytoplasm is moderate acidophilic and between tumor cells, we may find degenerate granulocyte. We also found complete keratinization in some areas, intensive acidophilic cytoplasm and keratosic and parakeratosic corpuscles.

Most of the cases were squamous cell carcinoma, which presents a wide variety of grading: G1 – 24.4%, 31 cases, G2 – 56.69%, 72 cases, G3 – 18.11%, 23 cases and in one case, we encountered adenoid cystic carcinoma 0.78%. In Table 1, we present case selection criteria: growing type, grading and TNM classification.

Table 1 – ***Case selection criteria: growing type, grading and TNM classification***

No.	Growing Type	Grading	TNM classification
1.	Vegetant (25.98%, 33 cases) Ulcerative (0%, 0 cases) Infiltrative (11.81%, 15 case) Ulcer-infiltrative (0%, 0 cases)	G1 (7.08%, nine cases) G2 (21.25%, 27 cases) G3 (9.44%, 12 cases)	T1b N0 Mx Stage I (37.79%, 48 cases)
2.	Vegetant (26.77%, 34 cases) Ulcerative (0.78%, one case) Infiltrative (8.66%, 11 cases) Ulcer-infiltrative (0%, 0 cases)	G1 (9.44%, 12 cases) G2 (20.47%, 26 cases) G3 (6.29%%, eight cases)	T2 N0 Mx Stage II (36.22%, 46 cases)
3.	Vegetant (0.78%, one case) Ulcerative (0%, 0 cases) Infiltrative (2.36%, three cases) Ulcer-infiltrative (0.78%, one case)	G1 (0.78%, one case) G2 (2.36%, three cases) G3 (0.78%, one case)	T2 N1 Mx Stage III (3.93%, five cases)
4.	Vegetant (10.23%, 13 cases) Ulcerative (0%, 0 cases) Infiltrative (5.51%, seven cases) Ulcer-infiltrative (0%, 0 cases)	G1 (5.51%, seven cases) G2 (9.44%, 12 cases) G3 (0.78%, one case)	T3 N0 Mx Stage III (15.74%, 20 cases)
5.	Vegetant (1.57%, two cases) Ulcerative (0%, 0 cases) Infiltrative (0.78%, one case) Ulcer-infiltrative (0%, 0 cases)	G1 (0.78%, one case) G2 (1.57%, two cases) G3 (0%, 0 cases)	T3 N1 Mx Stage III (2.36%, three cases)
6.	Vegetant (0.78%, one case) Ulcerative (0.78%, one case) Infiltrative (1.57%, two cases) Ulcer-infiltrative (0.78%, one case)	G1 (0.78%, one case) G2 (1.57%, two cases) G3 (0.78%, one case) Cystic adenoid carcinoma (0.78%, one case)	T3 N2 Mx Stage IVA (3.93%, five cases)

From 127 patients, 48 were in stage I, T1b N0 Mx (37.79%), 46 in stage II, T2 N0 Mx (36.22%), 28 patients in stage III (22.04%, T2 N1 Mx – five cases (3.93%), T3 N0 Mx – 20 cases (15.74%), T3 N1 Mx – three cases (2.36%), and the rest, five patients (3.93%) were in stage IVA T3 N2 Mx.

From mast cell reaction morphologic description, we observed squamous cell carcinoma with negative tumor cells, moderate alcianophil stroma due to presence of collagen, intense positive gland and caliciform cells mucus. Safraninophil mast cells are very rare in tumor area being localized at distance from tumor area (Figure 3.)

Degranulated mast cells were rare, appeared constantly in cases of safraninophil mast cells (Figure 4). Invasive squamous cell carcinoma mast cell density was 2.19.

In muscular tissue, at distance from tumor, in cases of microinvasive carcinoma there are predominantly safraninophil mast cells with variable degree of degranulation, aspect which is considerate to be normal (Figure 5). Mast cell density is 4.66.

In the case of adenoid cystic carcinoma, mast cells were numerous; all were alcianophil type, localized in stroma, and in vicinity of glands (Figure 6). Mast cell microdensity is 46.66.

In the case of malignant laryngeal papilloma, in stroma we identified numerous mast cells, all were safraninophil and majority presented degranulation phenomena (Figure 7), and constantly we encountered between epithelial cells isolated mast cell (Figure 8). Mast cell microdensity is 9.33.

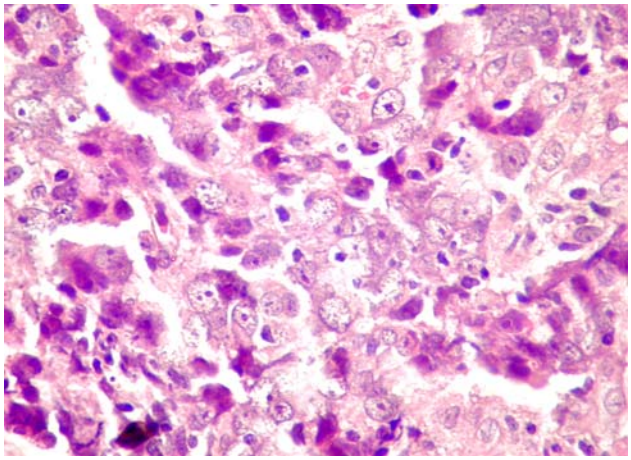

Figure 1 – ***Squamous cell carcinoma G3. Tumor cells with wide size variations (HE staining)***

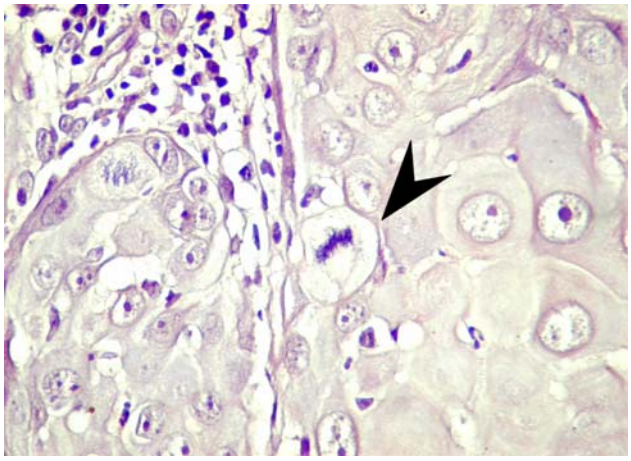

Figure 2 – ***Squamous cell carcinoma G2. Tumor cells are large, with vesicle-like nucleus, with large and unequal nucleoli, frequent atypical mitosis (arrow) (HE staining)***

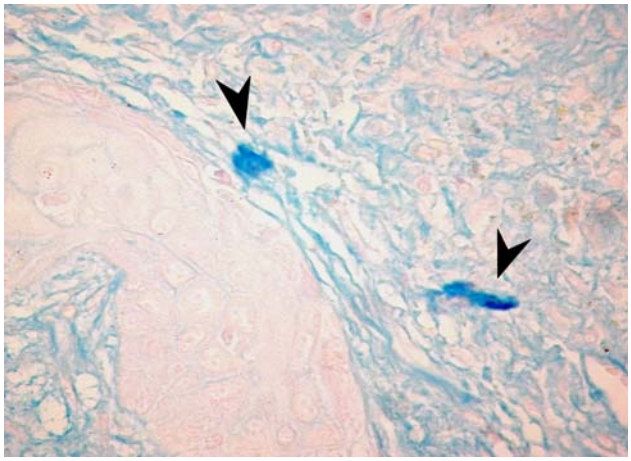

Figure 3 – ***Squamous cell carcinoma. Alcianophil mast cells (arrows) (Alcian blue–Safranin histochemistry)***

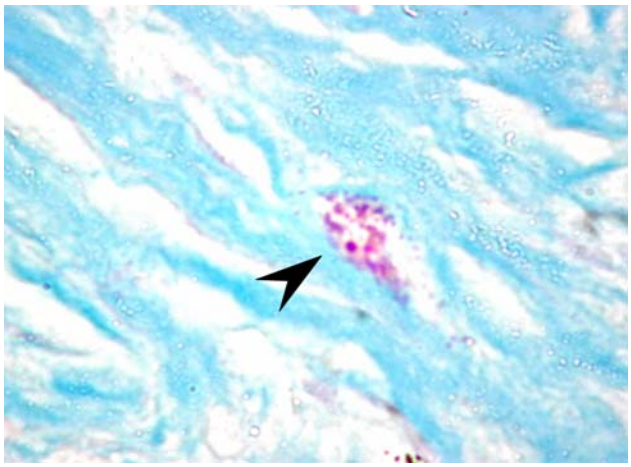

Figure 4 – ***Squamous cell carcinoma. Degranulated safraninophil mast cell (arrow) (Alcian blue–Safranin histochemistry)***

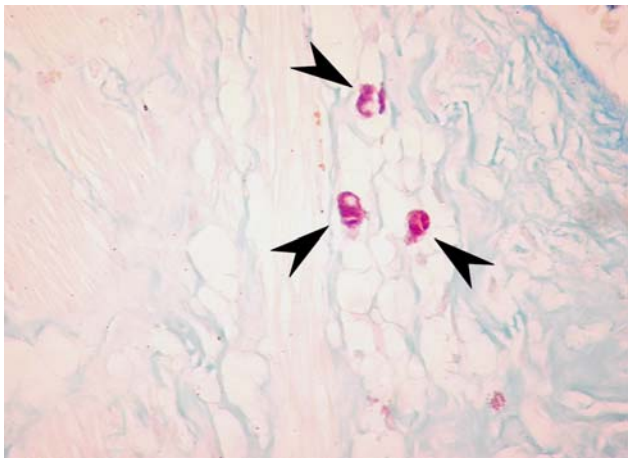

Figure 5 – ***Microinvasive carcinoma. Safraninophil mast cell in muscular tissue (arrows) (Alcian blue–Safranin histochemistry)***

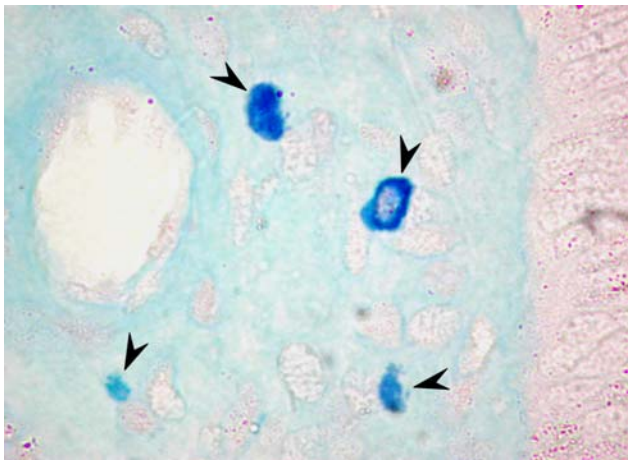

Figure 6 – ***Adenoid cystic carcinoma. Alcianophil mast cells, 2nd field (arrows) (Alcian blue–Safranin histochemistry)***

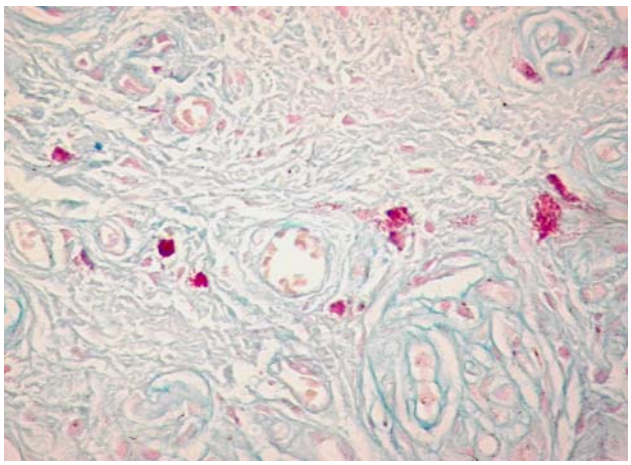

Figure 7 – ***Malignant laryngeal papilloma. Numerous mast cells with degranulation phenomenon (Alcian blue–Safranin histochemistry)***

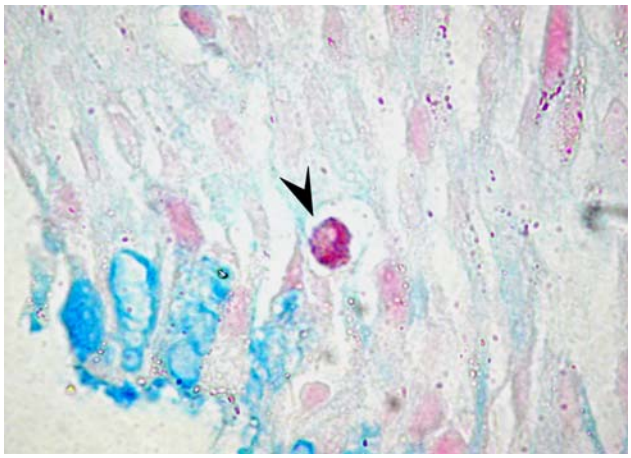

Figure 8 – ***Malignant laryngeal papilloma. Constantly between epithelial cells isolated mast cell (arrow) (Alcian blue–Safranin histochemistry)***

Discussions

Like other inflammatory cells, mast cells are attracted to tumors by various factors, including hypoxia, cellular damage, tissue ischemia and tumor-derived chemo-attractants, including stem cell factor, interleukin-3 (IL-3) and IL-4 [3].

They in turn produce various cytokines, such as tumor necrosis factor-α (TNF-α), IL-1, IL-4 and IL-6, which can induce apoptosis of tumor cells. Mast cells are also known to stimulate anti-tumor lymphocytes through IL-8 and RANTES [4].

Given the plasticity and versatility of mast cells, their phenotype may change, being inhibitory or stimulatory to tumor development, depending on the microenvironment [5].

It is also possible that mast cells are initially recruited to the tumors as part of the host defense system, but subsequently become enmeshed within the stroma participating in carcinogenesis [5].

Flynn E *et al.* demonstrate a direct correlation between sequential mast cell infiltration and activation, and distinct stages of hyperkeratosis, dysplasia, carcinoma in-situ and invasive squamous carcinoma in the oral cavity *in vivo* [6].

Mast cells have been implicated in conferring the angiogenic phenotype in pre-malignant lesions, and contributing to neovascularization during squamous epithelial carcinogenesis [7].

The effects of mast cells on carcinogenesis are likely to be mediated through multiple pathways, including immunosuppression, enhancement of angiogenesis, disruption of the extracellular matrix, and promotion of tumor cell mitosis [2].

A series of 18 head and neck squamous cell carcinoma biopsies, 6 primary and 12 recurrent, were investigated for tumor-infiltrating mononuclear cells with monoclonal or polyclonal antibodies. Our results suggest that the number of T-cells at the tumor edge in vivo correlates well with their ability to expand *in vitro* in the presence of high-dose IL-2 (2000 U/mL).

High MHC class I antigen expression on tumor cells was found to be positively correlated with p53 over-expression, suggesting that p53-derived peptides. Wild type or mutated ones, presented by MHC class I antigens, are potential targets for MHC-restricted cytotoxic T-cells in head and neck squamous cell carcinomas.

However, lack of correlation between peritumoral T-cell infiltration *in vivo* and T-cell expansion *in vitro* on the one hand, and p53 over-expression on tumor cells, on the other hand, suggests absence of p53-peptide-specific T-cells in the patients. Eight out of 10 expanded tumor-infiltrating lymphocyte (TIL) cultures showed T-cell-mediated cytotoxicity [8].

It is becoming accepted that multiple cell types in stromal microenvironment are involved in tumorigenesis. Oliveira-Neto HH *et al.* purposed to evaluate density and migration of MCs in OSCC (*Oral Squamous Cell Carcinoma*) and pre-malignant oral hyperkeratosis (leukoplakia) as well as their relationship with clinical and microscopic parameters.

They found no correlation between MC populations with clinical and microscopic characteristics of OSCC. These findings suggest that the decrease in MC numbers in pre-malignant and malignant oral lesions may be related to the migration failure of these cells, possibly reflecting an important modification in the microenvironment during tumor initiation and progression [9].

Lip squamous cell carcinoma (SCC) is the most common form of oral cancer (Rojas IG). Human mast cells (MCs), which are increased in lip SCC, are classified by their protease content in tryptase-positive (MC(T)) and tryptase/chymase-positive (MC(TC)). MC proteases are associated with tumor progression and angiogenesis.

The aim of Rojas IG *et al.* study was to quantify and characterize MC subpopulations in lip SCC. Their results suggest that MC subpopulations may contribute to lip SCC progression. While intratumoral MC(T) may stimulate angiogenesis, peritumoral MC(TC) may promote extracellular matrix degradation and tumor progression at the invasion front [10].

The MC and microvascular counts were significantly higher in oral SCC than in hyperkeratosis and normal oral mucosa ($P<0.05$). A significant correlation between MC and microvascular densities was observed in oral SCC ($r = 0.5$, $P = 0.012$), suggesting that MCs may up-regulate tumor angiogenesis in oral SCC, perhaps via MC tryptase [11].

In Ranieri G *et al.* work on 50 cases of SCCOC T1-3 N0-1 M0 they examined the microvessel density (MVD), mast cell density (MCD), relationship between these two parameters and their relationship with the pathological clinical features. The data obtained suggest that mast cells play an active role in angiogenetic processes in SCCOC and indicate that MVD is a favorable prognostic factor for SCCOC patients [12].

Tataroglu C *et al.* investigated the association between inflammatory cells, including tumor associated macrophage (TAM), mast cell (MC) and eosinophil leucocyte (EL) densities and angiogenesis, as well as the relation of TAM, MC and EL densities and angiogenesis to tumor stage in specimens of 63 non-small cell lung carcinoma (NSCLC). The absence of correlation between MCs, ELs and TAM counts and angiogenesis and absence of any relation between ELs and TAMs and tumor stage are discordant with the results of some of the previous studies in NSCLCs and in other tumors. The differing results may be due to wide variations in methodologies, which were used for demonstration of inflammatory cells and vessels, and variations in the degree of activation and complexity of functions of these cells [13].

Domagala-Kulawik J *et al.* analyzed the BALF cell profile in peripheral lung cancer. They found a significantly lower proportion of macrophages (60%), and significantly elevated proportions of lymphocytes (24%) and neutrophils (13%) in cases with cancer when compared with controls. The proportion of eosinophils was higher in the cancer patients (2.4%), though not significantly.

These observations confirm the possible participation of lymphocytes (activated and suppressor subtypes) and eosinophils in the response against tumor in peripheral airways [14].

Liu YL *et al.* investigated the relationship between mast cell infiltration and the development and metastasis of gastric carcinoma. Alcian blue–Safranin O staining and image analysis system were employed to observe, classify and quantify the mast cells in 74 gastric carcinoma specimens. In their results, the mast cells resided predominantly in the marginal area of the tumor and most of them were mature type, active and capable of discharging granules into the cytoplasm.

The mast cells in the marginal area of the tumor were significantly more numerous than those in the tumor matrix ($P<0.01$) and there was a positive correlation between them ($r = 0.303$, $P<0.01$). The density of the mast cells was inversely correlated to the depth of tumor invasion and the number of lymph node metastasis. They concluded that the mast cell infiltration in the matrix of the tumor plays an important role in preventing the invasion and metastasis of gastric carcinoma possibly by release of heparin and other bioactive substances. Mast cell quantity in the marginal area of the tumor may serve as an indicator for prognostic assessment of gastric carcinoma [15].

Growth of solid tumors requires angiogenesis. Evidence indicates that mast cells (MCs) play an important role in tumor angiogenesis (Tuna B). The aim of Tuna B *et al.* study was to investigate the possible effects of angiogenesis and the presence of MCs on the prognosis of renal cell carcinoma (RCC).

The correlation between microvessels and MC counts was evaluated and compared with tumor stage, grade, and other clinico-pathologic parameters. The results suggested that MCs in RCC and peritumoral areas were observed to be greater than non-neoplastic kidney tissue. No correlation was found between MC number and various clinico-pathologic features such as tumor size, stage, grade, and patient survival. No association was noted between angiogenesis and clinico-pathologic features. On the other hand, significant correlation was found between the number of MCs and microvessel density ($r = 0.295$, $P = 0.034$) [16].

The aim of Esposito I *et al.* study was to characterize the inflammatory infiltrate in pancreatic ductal adenocarcinoma and to analyze its contribution to angiogenesis and its prognostic relevance. There were significantly more mast cells and macrophages in pancreatic cancers than in normal pancreas and the number of mast cells directly correlated with the presence of lymph node metastases. However, there was no relation between the number of infiltrating inflammatory cells and the presence of chronic pancreatitis (CP)-like changes in the parenchyma surrounding the tumor. Mononuclear inflammatory cells of the non-specific immune response are recruited to pancreatic cancer tissues independent of the presence of CP-like changes, may influence the metastatic capacity of the cancer cells, and may contribute to the development of tumors with high angiogenic activity [17].

Conclusions

Mast cell reaction in malignant laryngeal neoplasm reveals:

1. In the tumor area alcianophil mast cell are present, and in the connective and muscular tissue safraninophil mast cells are present.

2. In invasive squamous cell carcinoma mast cell density was 2.19.

3. In microinvasive squamous cell carcinoma mast cell density was higher comparative with invasive squamous cell carcinoma (4.66 *vs*. 2.19).

4. Malignant laryngeal papilloma presented a mast cell microdensity of 9.33 *vs.* 46.66 of adenoid cystic carcinoma.

5. Our results reveal that mast cells, which are identified with Alcian blue–Safranin, are numerous in laryngeal carcinoma evolution early stages and rare or even absent in late stages.

Tumors are endowed with an angiogenic capability and that their growth, invasion and metastasis are angiogenesis dependent. Neoplastic cells are influenced by their microenvironment and vice versa. The specific organ microenvironment determines the extent of cancer cell proliferation, angiogenesis, invasion and survival. Tumor cells are surrounded by an infiltrate of inflammatory cells.

Inhibition or destruction of tumor cells has long been the prime goal of cancer therapy. Treatment of these cells and modulation of their microenvironment may prove a better approach. The balance or imbalance of inducers and inhibitors of angiogenesis secreted by inflammatory cells may favor a tumor's progression or regression.

The circumstances in which MCs are a critical source of angiogenic factors *in vivo*, and in such cases, what signals regulate their production and secretion are all matters that need to be determined as a prelude to the elaboration of new therapeutic strategies associated with their presence and activation.

Finally, a more accurate insight into the role of MCs may be obtained from the use of activation markers, along with consideration of local, microenvironmental stimuli in the regulation of their functions.

References

[1] CROMER A., CARLES A., MILLON R., GANGULI G., CHALMEL F., LEMAIRE F., YOUNG J., DEMBÉLÉ D., THIBAULT C., MULLER D., POCH O., ABECASSIS J., WASYLYK B., *Identification of genes associated with tumorigenesis and metastatic potential of hypopharyngeal cancer by microarray analysis*, Oncogene, 2004, 23(14):2484–2498.

[2] CH'NG S., WALLIS R., YUAN L., DAVIS P. F., TAN S. T., *Mast cells and cutaneous malignancies*, Mod Pathol, 2006, 19(1):149–159.

[3] LEEK R. D., HARRIS A. L., *Tumor-associated macrophages in breast cancer*, J Mammary Gland Biol Neoplasia, 2002, 7(2):177–189.

[4] AOKI M., PAWANKAR R., NIIMI Y., KAWANA S., *Mast cells in basal cell carcinoma express VEGF, IL-8 and RANTES*, Int Arch Allergy Immunol, 2003, 130(3):216–223.

[5] THEOHARIDES T. C., CONTI P., *Mast cells: the Jekyll and Hyde of tumor growth,* Trends Immunol, 2004, 25(5):235–241.

[6] FLYNN E. A., SCHWARTZ J. L., SHKLAR G., *Sequential mast cell infiltration and degranulation during experimental carcinogenesis*, J Cancer Res Clin Oncol, 1991, 117(2):115–122.
[7] COUSSENS L. M., RAYMOND W. W., BERGERS G., LAIG-WEBSTER M., BEHRENDTSEN O., WERB Z., CAUGHEY G. H., HANAHAN D., *Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis*, Genes Dev, 1999, 13(11):1382–1397.
[8] HALD J., RASMUSSEN N., CLAESSON M. H., *In vivo infiltration of mononuclear cells in squamous cell carcinoma of the head and neck correlated with the ability to expand tumour-infiltrating T cells in vitro and with the expression of MHC class I antigens on tumour cells*, Cancer Immunol Immunother, 1994, 39(6):383–390.
[9] OLIVEIRA-NETO H. H., LEITE A. F., COSTA N. L., ALENCAR R. C., LARA V. S., SILVA T. A., LELES C. R., MENDONÇA F. E., BATISTA A. C., *Decrease in mast cells in oral squamous cell carcinoma: possible failure in the migration of these cells*, Oral Oncol, 2007, 43(5):484–490.
[10] ROJAS I. G., SPENCER M. L., MARTÍNEZ A., MAURELIA M. A., RUDOLPH M. I., *Characterization of mast cell subpopulations in lip cancer*, J Oral Pathol Med, 2005, 34(5):268–273.
[11] IAMAROON A., PONGSIRIWET S., JITTIDECHARAKS S., PATTANAPORN K., PRAPAYASATOK S., WANACHANTARARAK S., *Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma*, J Oral Pathol Med, 2003, 32(4):195–199.
[12] RANIERI G., ACHILLE G., FLORIO G., LABRIOLA A., MARZULLO F., PARADISO A., GRAMMATICA L., *Biological-clinical significance of angiogenesis and mast cell infiltration in squamous cell carcinoma of the oral cavity*, Acta Otorhinolaryngol Ital, 2001, 21(3):171–178.
[13] TATAROGLU C., KARGI A., OZKAL S., ESREFOGLU N., AKKOCLU A., *Association of macrophages, mast cells and eosinophil leukocytes with angiogenesis and tumor stage in non-small cell lung carcinomas (NSCLC)*, Lung Cancer, 2004, 43(1):47–54.
[14] DOMAGALA-KULAWIK J., GUZMAN J., COSTABEL U., *Immune cells in bronchoalveolar lavage in peripheral lung cancer – analysis of 140 cases*, Respiration, 2003, 70(1):43–48.
[15] LIU Y. L., ZHAO M. Q., HOU G., MIAO F., LIU W., *Effects of mast cell infiltration on the development and metastasis of gastric carcinoma,* Di Yi Jun Yi Da Xue Xue Bao, 2005, 25(7):809–811.
[16] TUNA B., YORUKOGLU K., UNLU M., MUNGAN M. U., KIRKALI Z., *Association of mast cells with microvessel density in renal cell carcinomas*, Eur Urol, 2006, 50(3):530–534.
[17] ESPOSITO I., MENICAGLI M., FUNEL N., BERGMANN F., BOGGI U., MOSCA F., BEVILACQUA G., CAMPANI D., *Inflammatory cells contribute to the generation of an angiogenic phenotype in pancreatic ductal adenocarcinoma*, J Clin Pathol, 2004, 57(6):630–636.

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Received: April 5th, 2007

Accepted: July 5th, 2007