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DOCTORAL THESIS

**THE REACTION AND MEANING OF MASTOCYTES
IN INFLAMMATORY AND TUMOR LESIONS
OF THE ORAL CAVITY**

SUMMARY

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SUMMARY

Inflammatory and tumor lesions of the oral cavity are currently a topic of great practical interest. The paper highlights the complexity and importance of the study of oral structures in the context of their reaction to pathogens. Mast cells, which are discussed in detail, influence changes in lymphatic vessels, being involved in inflammatory and tumor lesions. These studies analyze their distribution and density in gum and periodontal lesions, using specific methods of investigation. It also emphasizes the role of salivary glands in maintaining the balance of the oral cavity and examines in detail their characteristics.

Regarding mast cells, the uncertainties and controversies in the literature on these cells are highlighted. It raises questions about their role in the body's immune system, their possible influence on tumor cells, and the viability of blood and lymphatic vessels in tumor environments. The answers to these questions currently remain unsolved or are difficult to determine.

Targeted mast cell therapy is a relatively recent approach, given that previous studies had mostly focused on epithelial cells in inflammatory and tumor conditions. Data on the involvement of mast cells in these pathological conditions are limited or even absent with regard to their relationship with microscopically observed lesions. Oral tumors are less common than other types of malignant tumors, such as breast, prostate, or lung tumors, especially affecting the lips, tongue, and oral plank.

The incidence of these tumors has increased significantly in recent decades, often being detected in advanced stages. Even though their progression is often slow compared to other types of tumors, they can be aggressive, invading local tissues and metastasizing at the systemic level. Most oral cavity tumors are squamous cell carcinomas, with little or no response to standard treatments such as radiotherapy or chemotherapy, which gives an unfavorable prognosis in advanced stages.

Currently, although there is progress in the implementation of molecular techniques, there is still no viable and practical molecular classification for tumors in the oral cavity and the head and neck area. The molecular profile of these tumors is not yet clearly defined in terms of clinical applicability, in contrast to other tumor types.

The research highlights the complexity of increasing oral cavity pathology over the last decades, highlighting various reasons such as late presentation to the dentist and poor oral hygiene. These issues lead to severe injuries that affect all oral tissues and can influence your overall health. It is known that lesions of the oral cavity can have an impact on general health, and systemic conditions can cause significant changes in the oral tissues.

Moreover, in the paper it can be noted that inflammatory lesions of the supporting tissues of the teeth are the subject of discussion and controversy regarding their classification and therapy. Despite extensive debates in the literature, there are still many unclear issues, controversies and unknown data in the field of pathogenesis, classification and therapy of pathologies of the oral cavity.

This study involved surgical biopsy sampling, standardized processing of biological material, and the application of specific coloration techniques to identify mast cells and characterize other tissue elements. Advanced histochemical and immunohistochemical methods were used, and the results were accurately analyzed. It also highlights the importance of clarifying some controversial issues related to diagnosis and therapy, using the results of this investigation as a solid basis for future medical interventions.

Inflammatory and tumor lesions of the oral cavity are currently a topic of great practical interest. There is an increased concern for clinical classification, histopathological diagnosis and appropriate therapies for such lesions. Also, the increase in the incidence of tumors in the oral cavity is discussed, their diagnosis being frequently made in advanced stages, having an unfavorable prognosis.

Mast cells are referred to as being involved in these pathological conditions, but information about them is limited or absent in relation to their relationship with microscopically observed lesions. The uncertain and controversial aspects of mast cells, as presented in the literature, call into question the functions of these cells in the context of oral cavity tumors.

Previous studies and the objectives of the mentioned research focus on assessing the reaction of mast cells in correlation with biopsies taken from the pathological and healthy tissues of the oral cavity. The goal is to clarify the involvement of mast cells in oral pathology, including tumors, and this can benefit both in fundamental research and diagnostic practice.

The general part is 4 main chapters. In the first chapter there is information about the microscopic structure of the soft tissues of the oral cavity; in the second chapter aspects of inflammatory lesions are presented – and microscopic peculiarities; in the third chapter, cancer and precancerous lesions (incidence, pathology and molecular diagnosis) are discussed; and in the fourth chapter, information about mast cells in normal conditions are reported as well as findings of inflammatory and tumors in the oral cavity.

The special part includes the personal research elaborated in four studies. The aim of the research is to evaluate the reaction of mast cells in correlation with microscopically studied biopsies taken from clinically healthy pathological tissues of the oral cavity.

STUDY 1. VALIDATION AND SPECIFICITY OF METHODS FOR IDENTIFYING MASTOCYTES IN NORMAL AND PATHOLOGICAL TISSUES OF THE ORAL CAVITY

Mastocytes do not stand out with common morphological methods of coloring microscopic sections. This is the cause of non-reporting these cells in histopathological bulletins, being assimilated in most cases with other cells of the inflammatory response of the body. For this reason, research on the role of these cells under normal, inflammatory and tumor conditions have been studied less than for other connective cell components, and the data we currently have is unclear or even controversial. For more than 100 years, mast cell identification has been based on highlighting some of the components of specific granules.

The purpose of this study is to evaluate the most effective methods of identifying mast cells and their accuracy.

The main objective of the study is to characterize the specific methods of tissue identification of mast cells and calculate the micro density of these cells by applying histochemical and immunohistochemical dyes.

MATERIAL AND METHODS

For testing the value of conventional histological methods in identifying mast cells, we selected five biopsies from the following entities: gum with inflammatory lesions, lip cancer, lingual cancer and major salivary glands associated with pleomorphic adenoma tumor tissue. All specimens were processed primarily by buffered and paraffin-inclusive formalin fixation, the standard technique, sections of 5 thickness being used for photonic microscopy assessment.

Of the histochemical techniques, we applied the quoted methods with the best results from this point of view, namely blue toluidine at pH 2.2, alcian blue at pH 2.5, alcian blue safranin at pH 0.2. At this stage of the research, the evaluation was qualitative.

Conclusion

1. Methods using alcian blue staining at pH 2.5 have moderate sensitivity for mast cells and reduced specificity; toluidine blue staining at pH 2.2 has moderate specificity and high sensitivity by metachromatic reaction from mast cell granules.
2. The blue alcian safranin method has high sensitivity and specificity, being the only one of the histochemical methods with value for numerical applications.
3. Electron microscopy is a useful method for the ultrastructural study of mast cells, but does not provide information on the composition of specific granules.

4. Structurally modified mast cells with inequalities between granules and low or moderate electronic density have been identified in lip and tongue cancer.

Study 2. MASTOCYTES AND LYMPHANGIOGENESIS IN GINGIVAL INFLAMMATORY LESIONS

Lymph angiogenesis is the process by which new lymphatic vessels are formed from postcapillary venules or/or pre-existing lymphatic vessels. Unlike angiogenesis – the formation of new blood vessels, at lymph angiogenesis are not clearly defined at present the characters of this process at the formation of de novo or from preexisting vessels, despite the importance that the lymphatic path has in various pathological processes.

The aim of this study was to evaluate the distribution, number and density of mast cells and lymphatic micro-vessels in patients with gum and periodontal lesions of varying degrees of severity, applying a double immunoclearing method to mast cells (mastocyte tryptase) and lymphatic endothelial cells (Podoplanin).

Specific objectives:

- Assessment of the presence of mast cells in perivascular spaces in inflammatory conditions;
- The numerical correlation between mast cells and lymphatic vessels in inflammatory gingival and periodontal lesions.
- Assessment of the prognostic character of perivascular mastocytes in inflammatory gingival and periodontal lesions.

Conclusion

1. Mast cells and lymphatic vessels can be identified and quantified in the same microscopic fields by double immunocolored mast cell triptase-D2-40.
2. In biopsies taken from the seemingly normal mucosa, no particular relationships between mast cells and lymphatic vessels were identified.
3. The number of mast cells and lymphatic vessels increases with increasing the severity of lesions, except for advanced forms of the disease.
4. The blood vessels in the dermis are dilated, with aspects of stasis, which greatly change the nutrition capacity of the epithelium.

5. The inducing effect of mast cell degranulation and release of biologically active substances on lymph angiogenesis in inflammatory conditions of periodontal disease is clear.

6. Histological changes occur in both epithelial and connective tissue, and are represented by the cellular inflammatory infiltrate, the density of which increases from the early to the serious forms, being populated with neutrophil, eosinophil and plasmacyte granulocytes.

7. The detailed conventional morphological diagnosis brings additional data, which complements the clinical diagnosis, thus contributing to the modeling of the therapeutic strategy.

STUDY 3. THE RELATIONSHIP BETWEEN MASTOCYTE REACTION AND MICROVASCULAR DENSITY IN EPITHELIAL TUMORS OF THE ORAL CAVITY

Malignant tumors have aroused the interest of many researchers and practicing doctors, and the limited resources we currently have, they are mostly due to the etiological mechanism as well as the ways by which normal cells turn into malignant cells. Moreover, there are currently no objective criteria of predictive character for lymph node and remote metastases, which can occur even in the conditions of small or even clinically undetectable tumors. Studies investigating the tumor microenvironment, previously called stroma, are very rare before 2000. Only after the introduction of current molecular analyses in the current period, the different behavior of cancer cells was observed depending on the type of tumor microenvironment.

The purpose of this study is to identify the relationships between the vascular neoformation structures in the tumor and intratumorally and mast cells, evaluated by localization and density.

Specific objectives:

- Numerical correlation between mast cells and angiogenesis.
- Numerical correlation between mast cells and lymph angiogenesis
- The relationship between intra-and perilesional mast cells.

Conclusion

1. The density of mast cells is reduced with increasing differentiation.

2. There is a statistically significant correlation between MVD and MCD for precancerous lesions and well-differentiated squamous cell carcinoma.

3. The increased mast cell density correlates in particular with immature and intermediate vessels in both the tumor and peritumoral areas.
4. MCD increases significantly from the normal mucosal stage by going through hyperplasia and dysplasia and well-differentiated carcinoma, then the values decrease significantly.
5. The marked reduction in MCD correlated with the increase in MVD signals the unfavorable prognosis of squamous cell carcinoma of the lip and tongue and the rapid evolution of the tumor.

STUDY 4. INTRAEPITHELIAL MASTOCYTES IN WARTHIN TUMOR: DIAGNOSTIC AND PROGNOSTIC ELEMENTS

Tumors in the oral cavity are of particular structural, evolutionary complexity and pose major diagnostic and therapeutic problems. Heterogeneity of these tumors, which can occur in all normal structures at this level, it is signaled by microscopic elements that in this form are not found in other systems and organs of the human body. This aspect could also be generated by the particular embryological origin of the tissues in the head and neck area.

The purpose of this study is to evaluate intraepithelial mast cells from the Warthin tumor, and the main objective of this study is to identify the accumulation of intraepithelial mastocytes intensely tryptase – positive mastocyte.

Conclusion

1. Study of Warthin tumor-associated mast cells signals massive accumulation of intraepithelial positive mast cell tryptase.
2. The density of intraepithelial mast cells is 10-12 times that of those in lymphoid tissue.
3. Most intraepithelial mast cells are defective, showing depletion of glycosaminoglycans, which limits their identification with conventional histochemical methods.
4. The expression peculiarities of intraepithelial mast cells in all studied cases are positive and for CD117, it can be considered a useful marker for molecular classification.

GENERAL CONCLUSIONS, ORIGINAL FINDINGS AND FUTURE RESEARCH DIRECTIONS

Morphological, histochemical and immunohistochemical studies on normal, inflammatory and tumor tissues in the anatomical area of the oral cavity highlight the general conclusions that we present below:

1. Methods using alcian blue staining at pH 2.5 have moderate sensitivity for mast cells and reduced specificity; toluidine blue staining at pH2.2 has moderate specificity and high sensitivity by metachromatic reaction from mast cell granules.
2. The blue alcian safranin method has high sensitivity and specificity, being the only one of the histochemical methods with value for numerical applications.
3. Electron microscopy is a useful method for the ultrastructural study of mast cells, but does not provide information on the composition of specific granules.
4. Structurally modified mast cells with inequalities between granules and low or moderate electronic density have been identified in lip and tongue cancer.
5. Mast cells and lymphatic vessels can be identified and quantified in the same microscopic fields by double immunocolored mast cell triptase-D2-40.
6. In biopsies taken from the seemingly normal mucosa, no particular relationships between mast cells and lymphatic vessels were identified.
7. The number of mast cells and lymphatic vessels increases with increasing the severity of lesions, except for advanced forms of the disease.
8. It is obvious the inducing effect of mast cell degranulation and release of biologically active substances on lymph angiogenesis in inflammatory conditions in advanced inflammatory lesions.
9. The blood vessels in the dermis are dilated, with aspects of stasis, which greatly alter the nutrition capacity of the epithelium.
10. The detailed conventional morphological diagnosis brings additional data, which complements the clinical diagnosis, thus contributing to the modeling of the therapeutic strategy.
11. The density of mast cells is reduced with increasing differentiation.
12. There is a statistically significant correlation between MVD and MCD for precancerous lesions and well-differentiated squamous cell carcinoma.
13. The increased mast cell density correlates in particular with immature and intermediate vessels in both the tumor and peritumoral areas.

14. MCD increases significantly from the normal mucosal stage by going through hyperplasia and dysplasia and well-differentiated carcinoma, then the values decrease significantly.
15. The marked reduction in MCD correlated with the increase in MVD signals the unfavorable prognosis of squamous cell carcinoma of the lip and tongue and the rapid evolution of the tumor.
16. Study of Warthin tumor-associated mast cells signals massive accumulation of intensely positive intraepithelial mast cells at tryptase-mastocyte.
17. The density of intraepithelial mast cells is 10-12 times higher than those in lymphoid tissue.
18. Most intraepithelial mast cells are defective, showing depletion of glycosaminoglycans, which limits their identification with conventional histochemical methods.
19. The peculiarities of expression of intraepithelial mast cells in all studied cases are positive and for CD117, it can be considered a useful marker for molecular classification.

Original aspects and future research directions

We consider as original the aspects that we mention below, namely:

1. Comparison of specific methods of identification of mast cells
2. Morphological demonstration of the role of mast cells in the initiation and maintenance of lymphangiogenic in particular in tumor lesions.
3. Reporting the mast cell relationship/neofunction vessels in early angiogenesis, with the identification of at least two new therapeutic targets in case of tumor lesions.
4. The peculiarities of expression of intraepithelial mast cells in all studied cases are positive for CD117, it can be considered a useful marker for molecular classification.

Future research directions:

1. Integration of immunohistochemical results for a molecular classification of oral squamous cell carcinomas.
2. Development of an experimental model demonstrating the direct involvement of mast cells.
3. Testing the efficiency in experimental model of mast cell inhibitors and degranulation with effect on blood and lymphatic vessels.