Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 01, pp.64146-64148, January, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EVALUATION OF MAST CELL DENSITY IN ORAL SQUAMOUS CELL CARCINOMA AND NORMAL ORAL MUCOSA – AN IMMUNOHISTOCHEMICAL STUDY

[1]Dr. Anuradha Kote, [2]Dr. Ajinkya Deshmukh and [3,] *Dr. Atul Deshmukh

[1]Junior Research fellow, Center for Interdisciplinary Research, D Y Patil University, Navi Mumbai, India
[2]Scientific Officer, Pre-Clinical Research Center & Oral & Maxillofacial Pathology & Immunohistochemistry Center, Navi Mumbai, India
[3]Director, Center for Interdisciplinary Research, D Y Patil University Navi Mumbai, India

ARTICLE INFO

Article History:
Received 15th October, 2017
Received in revised form
18th November, 2017
Accepted 21st December, 2017
Published online 19th January, 2018

Key words:
Mast cell tryptase,
Oral squamous cell carcinoma,
Immunohistochemistry.

ABSTRACT

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. The prognosis and five year survival rate of OSCC has not improved over a decade. Many factors have been proposed which induce tumor formation, tumor progression and metastasis in OSCC. Mast cells have prominent role in tumor progression and metastasis. Mast cells contains proteases namely tryptase and chymase. These proteases degrade the extracellular matrix. This proteolytic activity promotes angiogenesis, invasion and metastasis. Thus, mast cells can be used as therapeutic targets to improve the prognosis and overall survival of the OSCC cases. We evaluated density of mast cells using anti mast cell tryptase antibody. The density of mast cells in OSCC is significantly greater in OSCC as compared to normal oral mucosa. Localization of mast cells is predominantly seen surrounding vascular channels and infiltrating tumor islands.

Citation: Dr. Anuradha Kote, Dr. Ajinkya Deshmukh and Dr. Atul Deshmukh, 2018. "Evaluation of mast cell density in oral squamous cell carcinoma and normal oral mucosa – An immunohistochemical study", *International Journal of Current Research*, 10, (01), 64146-64148.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide. It is the most common malignancy in Indian males and third most common in Indian females. It is easily accessible for early diagnosis and treatment. However, the prognosis and five year survival of OSCC remain unchanged over a decade (Ferlay *et al.,* 2015). Multiple factors have been proposed for the tumor induction, tumor formation and metastasis in OSCC. Role of mast cells have also been proposed in inflammation and tumor progression in OSCC. Mast cells in human mucosa are of two types based on the type of proteases. One type of mast cells which contain only tryptase in their granules and the other type of mast cells contain both tryptase and chymase. Mast cell degranulation is associated with release of these proteases which are responsible for degradation of extracellular matrix. Thus, degradation of extracellular matrix indirectly promotes angiogenesis. All these events lead to induction of primary tumor in OSCC. It also promotes tumor progression and metastasis in OSCC. Histologically, these mast cells in OSCC are located in sub mucosal region and advancing front of the lesion (Pusa Nela Gaje *et al.,* 2016).

Corresponding author: **Dr. Atul Deshmukh**
Director, Center for Interdisciplinary Research, D Y Patil University Navi Mumbai, India.

These mast cells predominantly surround newly formed and existing vascular channels. Thus, mast cells can be used as therapeutic targets in OSCC to prevent tumor progression and metastasis. In the present study, we have evaluated the density of mast cells in OSCC and normal oral mucosa (NOM). Immunohistochmistry method is used to determine the mast cell density in the present project. Anti mast cell tryptase primary antibody is used to study the mast cell expression.

MATERIALS AND METHODS

Collection of Samples

Total 60 paraffin-embedded tissue blocks were retrieved from departmental archives after obtaining permission from institutional ethics committee. Histologically proven 50 paraffin-embedded blocks of oral squamous cell carcinoma and 10 paraffin-embedded blocks of normal oral mucosa were selected. Two serial sections of 3-5µ thickness were taken from each block using soft tissue microtome. Each tissue was stained employing H & E and anti mast cell tryptase antibody using immunohistochemistry method. H and E stained slides of oral squamous cell carcinoma were examined under a binocular compound microscope and mast cell tryptase expression was determined by immunohistochemistry to measure the density of mast cells.

Histopathology and Light Microscopy

H&E stained sections were observed under compound microscope to determine the degree of differentiation. We found 28 cases of well differentiated oral squamous cell carcinoma and 22 cases of moderately differentiated squamous cell carcinoma.

Immunohistochemsitry

Immunohistochemistry was performed on sections taken on silane coated microscope slides. Anti-mast cell tryptase antibody from Dako (clone name AA1) was used. Sections were subjected to series of xylene, absolute alcohol and water. Sections were thoroughly washed under running water. Antigen retrieval was carried out using citrate buffer at pH 6.2. Heat mediated antigen retrieval technique using microwave at 800 watt for 10 minute, 420 watt for 10 minute and 360 watt for 5 minute was used. Sections were washed in tris buffer at pH 7.4. Sections were transferred to 3% hydrogen peroxide in methanol for 5 minutes. Sections were then incubated with serum from same species in which primary antibody was raised. Sections were washed in tris buffer at pH 7.4 for 5 minutes. Sections were incubated in humidifying chamber at room temperature with anti mast cell tryptase antibody for 45 minutes. Sections were washed in tris buffer solution for 5 minutes. Sections were subjected to poly-HRP super sensitive secondary detection antibody kit from Biogenex. Sections were washed in tris buffer. Sections were incubated with substrate DAB. Sections were washed in tris buffer. Sections were transferred to increasing grades of alcohol and xylene. Sections were mounted using DPX. All the sections were observed under compound microscope. Brown stained mast cells were calculated in ten high power fields. Mast cells were expressed as per square mm.

RESULTS

The study was conducted on 50 OSCC and 10 NOM paraffin embedded tissue blocks. Histological study of H&E sections revealed 28 well differentiated oral squamous cell carcinomas and 22 moderately differentiated squamous cell carcinomas. Mean density of mast cell tryptase positive cells in OSCC was 179.34 and the mean density of mast cell tryptase positive cells in NOM was 60.90. Thus, p value is 0.0001 which is considered as extremely significant. Mast cell tryptase positive cells were seen surrounding the vascular channels and infiltrating tumor islands. Mast cell tryptase positive cells were also noted surrounding newly formed vascular channels.

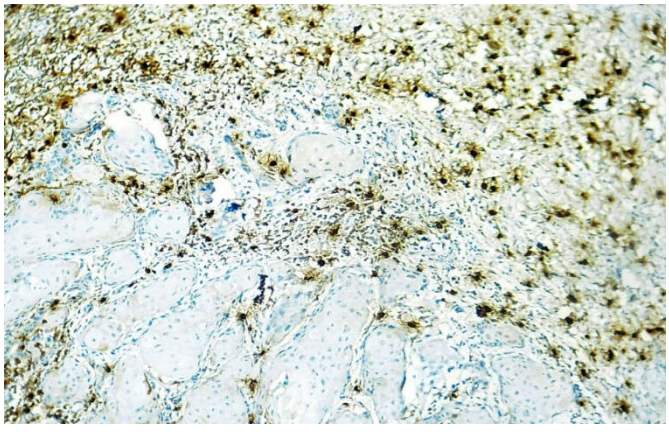

Figure 1. 10X magnification showing brown stained mast cells in oral squamous cell carcinoma

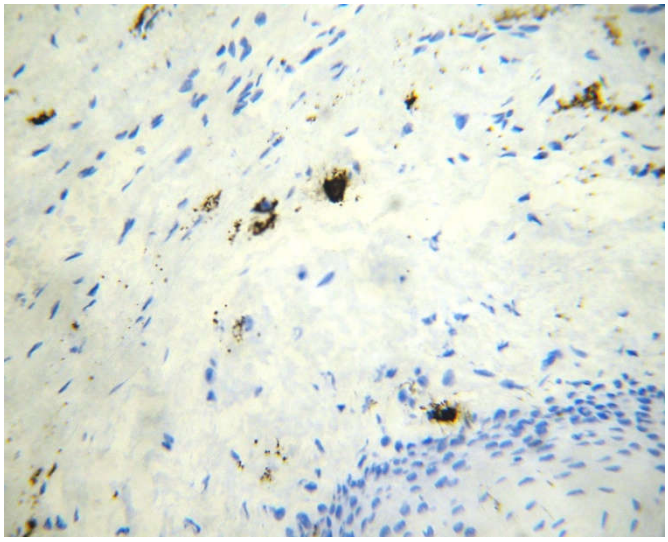

Figure 2. 10X magnification showing brown stained mast cells in normal oral mucosa

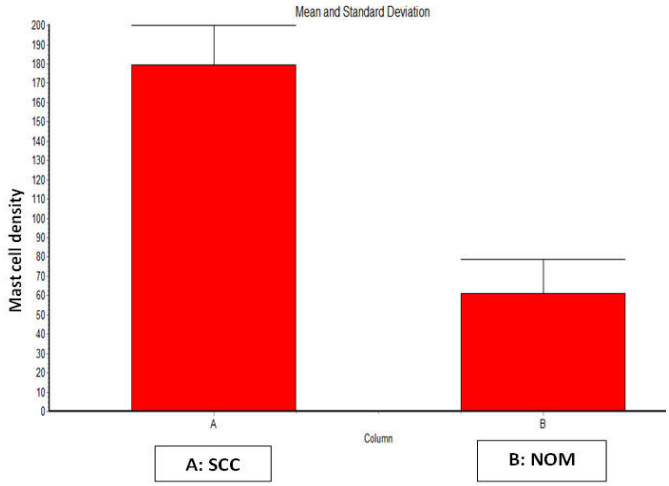

Figure 3. Graph illustrating the mean and standard deviation of mast cell density in oral squamous cell carcinoma and normal oral mucosa

DISCUSSION

Mast cells are a hematopoietic progenitor cell. They are multipotent in nature. They migrate to peripheral tissues via blood vessels and go through terminal differentiation. They play a part in regulating the immune response. Local environment and mast cell growth factor influence the migration process of mast cells (Deshmukh Ajinkya *et al.,* 2017; Kim *et al.,* 2009). They are capable to degrade extracellular matrix and also act in the innate and acquired immune response. Mast cells release specific products like tryptase, chymase, heparin, histamine, matrix metalloproteinase, basic fibroblast growth factor, interleukins like (IL-3,4,5,6,8,10,13,16), lipidic mediators and chemokines (Batista *et al.,* 2005; Guidolin *et al.,* 2009; Iamaroon *et al.,* 2003; Metcalfe *et al.,* 1997; Walsh, 2003). Mast cell granules are rich in tryptase serine proteinase (Zhao *et al.,* 2002). Tryptase contribute to inflammation, tissue remodeling, and extracellular matrix destruction and it is measured to be an essential angiogenic factor (Walsh *et al.,* 2003; Caughey, 2007; Okayama *et al.,* 2007). Angiogenesis and neovascularization are responsible for the various biological processes. Angiogenesis aid in progression and metastasis of different malignant tumors including oral cavity. Mast Cells encourage

neovascularization by the release of angiogenic factors, such as VEGF, or different substances with angiogenic properties, such as tryptase, IL- 8, tumor necrosis factor (TNF), basic fibroblast growth factor (bFGF), heparin, and histamine. Heparin present in the mast cells induces vessel proliferation as well as increases half-life of angiogenic substance like basic fibroblast growth factor. Thus they promote tumor angiogenesis and facilitate the local invasion and interleukins are responsible for epithelial proliferation. Degradation of extracellular matrix through the proteolytic activity of proteases like tryptase and chymase stimulate angiogenesis and aid in invasion and metastasis through extracellular matrix remodeling (Blair *et al.,* 1997). In the current project, we used anti-mast cell tryptase antibody to locate the mast cells in paraffin embedded tissue sections of oral squamous cell carcinoma and normal oral mucosa. In OSCC, mean density of mast cell tryptase positive cells was 179.34 which is much higher as compared to NOM of 60.90. Thus, our study agreed with the study conducted and published in the scientific literature on mast cell tryptase and OSCC. In our study, we found the mast cells at the advancing front of the tumor islands. It is significant in terms of promoter of angiogenesis and degradation of extracelluar matrix with the help of proteases present in their granules. It also helps in induction of primary tumor due to proteolytic action of proteases.

Conclusion

Mast cells are key players in the development of inflammation of various malignant lesions like oral squamous cell carcinoma. Proteases present in mast cell granules degrade the extracellular matrix, promotes angiogensis, tumor development, tumor progression and metastasis in oral squamous cell carcinoma. Thus, mast cells can be used as therapeutic target to prevent tumor progression and metastasis in oral squamous cell carcinoma.

REFERENCES

Batista, A.C., Rodini, C.O., Lara, V.S. 2005. Quantification of mast cells in different stages of human periodontal disease. *Oral Dis.,* 11:249-54.

Blair, R.J., Meng, H., Marchese, M.J., Ren, S., Schwartz, L.B., Tonnesen, M.G., et al. 1997. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. *J Clin Invest.,* 99:2691-700.

Caughey, G.H. 2007. Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev.,* 217:141-54.

Deshmukh Ajinkya A., Deshmukh Atul and Buva Kirti B. 2017. Expression of mast cell tryptase in periapical cyst- an immunohistochemical study, *DYPJHS*, Volume 5, Issue 1, pp 10-11.

Ferlay, J., Soerjomataram, I., Dikshit, R., et al. 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer,* 136(5):E359-E386.

Guidolin, D., Nico, B., Crivellato, E., Marzullo, A., Vacca, A., Ribatti, D., et al. 2009. Tumoral mast cells exhibit a common spatial distribution. *Cancer Lett.,* 273:80-5.

Iamaroon, A., Pongsiriwet, S., Jittidecharaks, S., Pattanaporn, K., Prapayasatok, S., Wanachantararak, S., et al. 2003. Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma. *J Oral Pathol Med.,* 32:195-9.

Kim, M.S., Kim, Y.K., Lee, D.H., Seo, J.E., Cho, K.H., Eun H.C., et al. 2009. Acute exposure of human skin to ultraviolet or infrared radiation or heat stimuli increases mast cell numbers and tryptase expression in human skin in vivo. *Br J Dermatol.,* 160:393-402.

Metcalfe, D.D., Baram, D., Mekori, Y.A. 1997. Mast cells. *Physiol Rev.,* 77:1033-79.

Okayama, Y., Ra, C., Saito, H. 2007. Role of mast cells in airway remodeling. *Curr Opin Immunol.,* 19:687-93.

Pusa Nela Gaje et al. 2016. Mast cells: key players in the shadow of oral inflammation and in squamous cell carcinoma of oral cavity. *BioMed Research International*.

Walsh, L.J. 2003. Mast cells and oral inflammation. *Crit Rev Oral Biol Med.,* 14:188-98.

Walsh, L.J. 2003. Mast cells and oral inflammation. *Crit Rev Oral Biol Med.,* 14:188-98.

Zhao, Z.Z., Savage, N.W., Sugerman, P.B., Walsh, L.J. 2002. Mast cell/T cell interactions in oral lichen planus. *J Oral Pathol Med.,* 31:189-95.

****