Emerging Evidence of Mast Cell Involvement in Oral Squamous Cell Carcinoma

Simon Basha¹, Nur Mohammad Monsur Hassan*,² Rahena Akhter³, Soichiro Ibaragi², Tatsuo Okui², Stephen Cox³ and Akira Sasaki²

¹School of Dentistry and Health Sciences, Charles Sturt University, Orange, Australia
²Department of Oral and Maxillofacial Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Kita-ku, Okayama, Japan
³Faculty of Dentistry, The University of Sydney

*Corresponding author: Nur Mohammad Monsur Hassan, School of Dentistry & Health Sciences, ²School of Community Health, Charles Sturt University, Orange NSW 2800, Tel: Tel: +61 2 6365 7117; Email: nhassan@csu.edu.au

ABSTRACT

Objective: The purpose of this review is to discuss the current understanding of and concepts surrounding, the involvement of mast cells in Oral Squamous Cell Carcinoma.

Methods: We performed a search in PubMed, limited to English language papers, using the keywords mast cells, oral cancer, Oral Squamous Cell Carcinoma, and supplemented the search results with manual search of relevant cited articles within the search results. Each article was revised and summarised.

Results: Mast cell aggregation is increased around sites of Oral Squamous Cell Carcinoma, and frequently Mast Cell Density increases correlated with increased microvessel density in tumoural stroma, and increased levels of mast cells products promoting Extracellular Matrix remodeling, tumour mitogenicity and modulating host responses.

Conclusion: Mast cells can promote tumour proliferation and aggressiveness via a plethora of secreted molecules and raised levels of such secretions as well as mast cell aggregation at Oral Squamous Cell Carcinoma sites is implicit of their involvement in progression of the pathology. There lacks, however, correlative data in the literature between mast cells and clinicopathological features such as tumour size, regional nodal involvement, or metastasis.

Mast Cells – as Sentinel Cells

Mast Cells (MC) are tissue-resident, bone-marrow derived, granular sentinel cells. They enter circulation from the bone marrow as committed Mast Cell Progenitors (MCP) and undergo terminal differentiation in response to a stimulus. MC and their progenitors are found throughout connective tissues and the mucosae, though the highest MC densities are found in the skin, airways and digestive tract, that is, host-environment interfaces-congruous with their role as sentinel cells [1]. Two distinct subpopulations of MC have been identified in human tissues, classified according to the protease contents of their secretory granules-tryptase-only containing type, referred to as MCT, and chymase- and tryptase-containing type, referred to as MCTC [2]. The latter also contain other proteases, including cathepsin G and carboxypeptidase A. In addition to these proteases, MC possess an arsenal of effector molecules. These include molecules stored in secretory granules, such as heparin, serotonin, and histamine, and those which are synthesised de novo, such as leukotriene C4, prostaglandin D2, platelet activating factor, and an assortment of cytokines [3]. MC activation may precipitate degranulation, or only the release of select effector molecules, and may occur in response to IgE crosslinking, complement activation or certain toxins [4]. MC effectors may contribute to both physiological and pathological events. While these cells are well known as orchestrators of hypersensitivities and immune reactions, more recently the role of...
MC in modulating tumour growth–both positively and negatively–has become a focus.

**Tissue Distribution, Activation and Migration of Mast Cells in Oral Cancer**

The altered tumour microenvironment induces changes in mast cell migration, activation and tissue distribution. Mast Cell Progenitors in peripheral tissue are under the influence of tumour-derived cytokines and chemoeffectors controlling their maturation and migration. Stem Cell Factor (SCF), derived from the tumour cells, and its receptor c-Kit represent possibly the most significant and potent, and best characterised, mast cell chemotaxis and migration pathway. An increase in mast cell infiltration in areas of Oral Squamous Cell Carcinoma (OSCC) development has been demonstrated in a number of studies [5-12], and correlation between Mast Cell Density (MCD) and disease progression has also been reported [13-15]. In a slightly different vein Aromando, et al. [16] noted no change in total mast cell numbers in a hamster cheek pouch tissue during experimental carcinogenesis, but rather a decrease in MC in the adventitious tissue and an accumulation of MC in peritumoural and intratumoural stroma, as well as a reversion of the ratios of active/inactive MC in favour of the former [16]. Furthermore, differences in MCD between intratumoural and peritumoural stroma were evaluated, showing significantly higher MCD values in the peritumoural stroma than intratumoural, probably reflecting the migration of the cells from adventitious tissue as well as functional roles in Extracellular Matrix (ECM) degradation and induction of cell proliferation [16].

Further characterisation of MC subpopulations within the Intratumoural (IT) and Peritumoural (PT) stromal regions has shown that, while both MCTC and MCT counts are significantly increased throughout the tumoural stroma, MCTC type predominates in the PT stroma, while MCT subtype predominates in the intratumoural stroma [17]. The authors hypothesise that the distribution of subpopulations reflects functional requirements: MCTC contain chymase, which plays a role in activation of Pro-Matrix Metalloproteinase-2 (MMP-2) and Pro-Matrix Metalloproteinase-9 (MMP-9) to their active MMP-2 and MMP-9 forms, respectively [18]. Both MMP-2 and MMP-9 possess the capacity to degrade type IV collagen [19], a significant component of the basement membrane and barrier to tumour invasion. Hence, the localisation of MCTC at tumour peripheries suggests an ECM remodelling role for these cells. Similarly, MCT predominance in the IT stroma suggests a role of these cells and their potent angiogenic mediator, tryptase [20], in neovascularization of the tumour. In contrast, some studies of OSCC and other carcinomas have failed to demonstrate a statistically significant increase in MCD in tumour regions, or a decrease in MCD as the degree of differentiation decreases [21-23]. These results have on occasion been attributed to massive degranulation of the MC compromising visualization, or shortcomings in specificity of the toluidine blue staining protocol compared with antitryptase immunohistochemistry [23,25]. Other studies have described a significant reduction in MC in cancers compared with controls [12,25].

Putative causes of this decrease are tobacco exposure, shown in an experimental carcinogenesis model to accentuate a decrease in MC infiltration in tumours caused by 4-NQO [26], or a failure in migration of MC, indicated by the significantly decreased c-kit/MC/tryptase–MC ratio, compared with control, attributable to changes within the tumour microenvironment [25] (the c-kit receptor on MC, together with its ligand SCF, are responsible for the migration, activation and maturation of MC [27]). However other authors have found no significant difference in c-kit/tryptase–MC ratio [25], suggesting no migration failure in such a case. Additionally MC are present in neoplasms irrespective of the presence of inflammatory infiltrate, suggesting chemotactic pathways are selective for MC. Transforming growth factor-beta (TGF-β) is synthesised and released by MC, and is increased in OSCC [5]. Its local roles are pleiotropic, including: its initially cytotoxic, but progression of the pathology. There lacks, however, correlative data in the literature between mast cells and clinicopathological features such as tumour size, regional nodal involvement, or metastasis. Eventually cytokinetic role in tumourigenesis [25] its action as a potent chemotactic factor for MC [28]; its role in angiogenesis; and its supposed part role in mediating a phenotypical change in tumours from CD34+ fibrocytes to alpha-smooth muscle antigen (α-SMA)-myofibroblasts [5].

Mangia et al. [29] report that tryptase can also induce phenotypic shift from CD34+/α-SMA+ fibroblasts to CD34+/α-SMA+ myofibroblasts, lending further credence to the role of MC in this context. CD34+ fibrocytes express Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) [30], which downregulates CD117 (c-Kit) expression in mast cells [31]; hence a phenotypic shift away from CD34+ fibrocytes as they differentiate to alpha-SMA myofibroblasts, decreases repression of CD117 expression and consequently, allows MC migration and infiltration [5].

**Mast Cells in Oral Cancer and Angiogenesis**

Angiogenesis and neoangiogenesis are the processes of formation of new blood vessels from pre-existing blood vessels, and formation de novo, respectively. Tumour proliferation is limited by oxygen perfusion, and tissue oxygen perfusion greater than 2mm has been reported to be prohibitive of tumour growth [32]. Neoangiparation, therefore, is a process central to tumour growth and development, and has been implicated in dissemination and metastasis. Mast cells store and have the capacity to synthetise a number of angiogenic and neoangiogenic mediators, including angiopoietin-1, FGF-2, VEGF, IL-8, TGF-β, TNF-α, histamine, heparin, tryptase and chymase, among others [33]. These mast cell mediators can act at various stages of angiogenesis including degradation of the ECM, migration and proliferation of endothelial cells, formation and distribution of new vessels, synthesis of ECM.
and pericyte mobilization [34,35]. It has been shown that during
the initiation of angiogenesis, mast cell tryptase promotes ECM
degradation through the activation of MMPs and plasminogen
activator [36]. One quantification of the degree of tumour
vascularisation is microvessel density. A number of studies have
related mast cell densities with Microvessel Densities (MVD) in
oral cancers [10,32,37-40].

Moreover, mast cell densities have been shown to increase with
MVD as disease progresses, or degree of tumour differentiation
decreases [41]. The distribution of MC within tissue is also indicative
of functional roles. As described in section 2, Rojas et al. [42]
characterized MC subpopulations in OSCC and determined
that MCT were the predominant subtype in the intratumoural
stroma, while MCTC were in the peritumoural stroma of OSCC.
Separate studies have shown increases in MVD in the intratumoural
stroma, while no significant increase was observed in peritumoural
regions. This supports the hypothesis of Rojas et al. [42], that
MCT are so localised for an angiogenic role via the known potent
angiogenic factor, tryptase [41]. It has been shown that during
the initiation of angiogenesis, mast cell tryptase can also promote
ECM degradation through the activation of MMPs and plasminogen
activator [36]. Data reporting the colocalisation of MC and blood
vessels in oral cancer also suggest an intimate relationship between
the pair and role in angiogenesis for MC in tumourigenesis [10].
While MC are implicated in neovascularization and are known to
contain angiogenic factors, mechanisms are uncertain. The role of
the potent angiogenic cytokine Vascular Endothelial Growth Factor
(VEGF) contained in MC and released from tumour cells is not
straightforward.

Several studies correlate MC with VEGF expression. Release
of VEGF from MC is mediated in part by the interleukin-33/ST2
signalling axis [44]. IL-33 is upregulated in OSCC, and a correlation
between IL-33 and MVD, as well as IL-33 and MCD has been
reported, suggesting VEGF may be the intermediary [39]. However,
the specific role of VEGF in MC-mediated angiogenesis is not clear.
Artese et al. [46] showed that, in OSCC, while MVD was significantly
increased in tumours and correlated with tumour grading, VEGF
expression did not vary between OSCC and tumour-free controls,
and no significant correlation between VEGF expression and MVD
was observed. Carlile et al. [47] produced similar results in a
similar study, noting that vascularity increased in OSCC compared
with control, though this increase did not correlate with VEGF
expression. Other studies have found significant VEGF expression
increase in OSCC vs control, although VEGF expression did not
directly correlate to MVD [32]. MCD did, however, correlate with
MVD. A single-linkage cluster analysis on these three variables
(MCD, MVD, VEGF expression) grouped VEGF expression and MVD,
implying an indirect link between the two variables. Whereby,
VEGF may recruit mast cells [48] which contain angiogenic factors [32,49]. Hence, VEGF indirectly induces angiogenesis.

More prominent and directly acting role for tryptase is therefore
suggested [50,51], seems to be confirmed by the results of Rojas et
al. [42]. Yet interrelationship between MC and angiogenesis is not
however a universal finding and we must state while the evidence
is significant there remain many inconsistencies. Some authors,
while finding a significant increase in MVD in SCC vs control, did
not observe a correlation between MVD and MCD [52]. Other data
as much as show the converse of the above – that is, increasing MVD
inversely and significantly correlated with MCD [52].

**Mast Cells in Extracellular Matrix Remodelling in OSCC**

An important feature of cancer progression is the ability to
degrade the Extracellular Matrix (ECM), and consequently permit
proliferation and migration of cells, invasion of surrounding
tissues and dissemination. Matrix Metalloproteinases (MMP)
are endopeptidases responsible for degradation of the ECM. The
MMPs can be categorized according to their preferred substrates,
i.e. the collagenases (MMP-1, -8, -13), gelatinases (MMP-2, MMP-
9), stromelysins (MMP-3, MMP-10, MMP-11). Human mast cells
interact with several MMP, both stimulatory and inhibitory, in
ECM homeostasis [53]. Gelatinases A and B (MMP-2 and MMP-9,
respectively) are secreted by MC [54,55], or indirectly activated by
MC-secreted chymase [54]. MC tryptase itself has also been shown
to directly exert gelatinase-like activity [56], and tryptase is also
involved in the processing and activation of MMP-3 and MMP-1,
the latter being dependent on the activation of the former [57,58].
Chymase is also capable of directly activating MMP-1 and MMP-3
[59]. Further MC chymase, but not tryptase, may directly cleave
procollagen to fibril-forming collagen [60]. Hence MC contribute
both directly and indirectly to processes which degrade the ECM.

In the context of oral cancer, MMP-9 expression has been
shown to be upregulated in OSCC compared with healthy tissues,
and significantly correlated with MCD [61]. Another study showed
lip SCC samples that expressed higher MC counts also showed
increased collagen degradation, assayed by picro-sirius staining
[7]. MMP-9 has been associated with aggressive tumour growth,
proteolytic processing of the ECM and activation of cytokines (such
as TGF-β) [10]. MMP-9 is capable of processing type IV collagen
of the basement membrane [62] and other ECM components, which
are key events in tumour invasion and metastasis (see Fig. 1).
However, evidence supports a fluctuating role of MMP-9 in OSCC.
High MMP-9 expression has been shown to correlate with nodal
involvement and metastasis, and poor prognosis in OSCC [63].
Meanwhile, Guttman et al. [64] reported no correlation between
MMP-9 and tumour size or nodal involvement. Similarly, other
authors reported that MMP-9 expression was not associated with
clinical variables, such as tumour stage, recurrence rate, etc. [65].
Other data suggest that MMP-2 and MMP-2 expression significantly
correlates with collagen degradation and local invasiveness, though
this was not related to metastatic potential of the disease [66].
Meanwhile, it has been suggested that although MMP-2 and MMP-
9 expression is high in OSCC, the ratio of active/inactive MMP-9 is low, suggesting MMP-2 is the gelatinase of greater importance in OSCC [67]. Conversely, MCs have also been implicated in collagen deposition. Vidal et al. [10] observed the accumulation of MC in areas of fibrosis surrounding malignant minor salivary gland tumours and proposed the hypothesis that ECM remodelling, specifically collagen synthesis, may be mediated by MC.

A similar hypotheses have been made regarding odontogenic tumours [68] and breast cancers, in which it was suggested that tryptase played a role in collagen deposition [69]. Additionally, an association between MC and fibroblasts in the potentially malignant condition, oral submucous fibrosis, has been inferred [70,71]. Supporting these observations, MC products TGF-β and tryptase have been shown to stimulate collagen deposition, fibroblast migration and fibroblast proliferation [20,72-77]. Hence, ECM homeostasis is a delicate balance between factors, which promote degradation, and proliferation. The tumour microenvironment may lead to unpredictable disruption of this balance.

Mast Cells and Tumour Proliferation, Invasion and Dissemination

Mast cells can precipitate mitogenicity in tumour cells directly through mediators, and indirectly through microenvironment modulation. The most abundant mast cell protease, tryptase, is implicated in promoting neoplastic cell proliferation via the Receptor Tyrosine Kinase (RTK) protease activated receptor 2 (PAR-2), expressed on the surface of neoplastic cells. Tryptase cleaves and activates PAR-2, stimulating proliferation of receptor-bearing cells, as well as inducing the expression of cyclooxygenase-2 (COX-2) [16,77]. The proliferative consequence of tryptase-mediated PAR-2 activation has been reported in lung tissue, colon cancer and breast cancer [76,78], but few studies exist correlating MC with tumour cell proliferation in OSCC, and those that fail do to demonstrate a significant correlation [7]. A study pertaining to the potentially malignant oral condition actinic cheilitis has, however, quantified COX-2, PAR-2, MC and tryptase in human actinic cheilitis tissues. COX-2 is responsible for eicosanoid biosynthesis from arachidonic acid, and among the metabolites is Prostaglandin E2 (PGE2), which is also capable of promoting tumour proliferation [79]. The authors reported a significant correlation between tryptase-positive MC and PAR-2 expression, as well as COX-2 overexpression, inferring a role for tryptase in PAR-2 activation and COX-2 overexpression. Increased MC counts have also been associated with higher levels of DNA synthesis in an experimental hamster oral carcinogenesis model, again implicating tryptase-mediated PAR-2 activation [16].

Conclusion

Mast cells are influenced by, and influence, malignant tumours. They commonly accumulate in tumour-associated stroma and can promote tumour proliferation and aggressiveness via a plethora of secreted molecules. However, the literature is divided on the clinical significance of these local effects, and if or how they may represent or open up therapeutic possibilities. This variability in evidence suggests that a more complex set of interactions exists and overbear the net outcome and effects of mast cells in tumours. That is, while the mechanistic role of mast cells in cancer is becoming clearer and is seemingly becoming important, it is still incompletely understood. As such, conclusions about the role of mast cells in oral cancer would be fallacious to draw despite evidence correlating their accumulation with factors correlated with tumour progression.

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