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**Possible biological and translational significance of mast cells density in colorectal cancer**

Marech I *et al*. Mast cells density in colorectal cancer

Ilaria Marech, Michele Ammendola, Claudia Gadaleta, Nicola Zizzo, Caroline Oakley, Cosmo Damiano Gadaleta, Girolamo Ranieri.

**Ilaria Marech, Caroline Oakley, Cosmo Damiano Gadaleta**, **Girolamo Ranieri,** Interventional Radiology Unit with Integrated Section of Translational Medical Oncology, National Cancer Research Centre Istituto Tumori “Giovanni Paolo II”, 70124 Bari, Italy

**Michele Ammendola,** Department of Clinical Surgery, University of Catanzaro "Magna Graecia" Medical School, 88100 Catanzaro, Italy

**Claudia Gadaleta, Nicola Zizzo,** Department of Pathology, Veterinary Medical School, University of Bari, Valenzano, 70010 Bari, Italy

**Author contributions**: Marech I, Gadaleta CD and Ranieri G ideated the manuscript and performed a critical review of the literature; Ammendola M, Gadaleta C and Zizzo N contributed to literature research and data analysis. Oakley C edited the manuscript. All authors wrote the manuscript.

**Correspondence to: Girolamo Ranieri, MD,** Interventional Radiology Unit with Integrated Section of Translational Medical Oncology, National Cancer Research Centre Istituto Tumori “Giovanni Paolo II”, Via Orazio Flacco 65, 70124 Bari, Italy. giroran@tiscalinet.it

**Telephone:** +39-80-5555561 **Fax:** +39-80-5555563

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**Abstract**

Mast cells (MCs), located ubiquitously near blood vessels, are descended from CD34+ hematopoietic stem cells. Initially, although their role has been well defined in hypersensitivity reactions, the discovery of their sharing in both innate and adaptive immunity has allowed to redefine their crucial interplay on the regulatory function between inflammatory and tumor cells trough the release of mediators granule-associated (mainly tryptase and vascular endothelial growth factor). In particular, in several animal and human malignancies it has been well demonstrated that activated c-Kit receptor (c-KitR) and tryptase (an agonist of the proteinase-activated receptor-2) take pivotal part in tumor angiogenesis after the MCs activation, contributing to tumor cells invasion and metastasis. In this review, we focused on crucial MCs density (MCD) role in colorectal cancer (CRC) development and progression angiogenesis-mediated; then, we will analyze the principal studies that have focused on MCD both as possible prognostic factor. Finally, we will consider a possible role of MCD as novel therapeutic target mainly by c-KitR tyrosine kinase inhibitors (imatinib, masitinib) and tryptase inhibitors (gabexate and nafamostat mesylate) with the aim to prevent CRC progression.

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**Key words**: Tryptase; Mast cell density; Proteinase-activated receptor-2; c-Kit receptor; Vascular endothelial growth factor; Angiogenesis; Colorectal cancer; Tumor progression; Tryptase inhibitors; c-Kit receptor tyrosine kinase inhibitors

**Core tip:** In several malignancies it has been well demonstrated that mast cell (MC) activated c-Kit receptor (c-KitR) and tryptase secreted after MC degranulation play a pivotal role in tumor angiogenesis, helping tumor cell invasion and metastasis. The close relationship between MC density, angiogenesis and tumor progression could suggest a role for MCs as a possible prognostic factor in colorectal cancer (CRC). Moreover, considering MC-mediated CRC development, c-KitR tyrosine kinase inhibitors (imatinib, masitinib) and tryptase inhibitors (gabexate and nafamostat mesylate) could be used to block MC activation/degranulation and the tryptase/proteinase-activated receptor-2 axis respectively, and may be evaluated in future clinical trials in CRC patients.

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**INTRODUCTION**

In 1869 Nettleship and Tay[1] described a particular form of pigmented rash (‘urticaria pigmentosa’), which presented a dermographism entirely similar to some urticaria forms. Mast cells (MCs) were identified by Ehrlich[2] in 1879 and named “mastzellen”(from the German mast = well-fed) because it was believed that they were particularly numerous in overfed animals. It was subsequently shown that cutaneous lesions observed in these animals were characterized by a focal accumulation of some of these mast cells[2]. In 1949 Ellis[3] described a form of systemic mastocytosis characterized by an abnormal infiltration of MCs into extracutaneous organs. Historically, “mastocytosis” is a morbid condition characterized by a marked increase (usually about ten times compared to normal) of the density of tissue MCs in specific anatomical sites[4]. Currently, “mastocytosis” includes a wide spectrum of clinical disorders (with an extremely heterogeneous clinical course and prognosis) sharing particular tyrosine kinase c-Kit receptor (c-KitR) mutations that confer its increased activation, determining Stem Cell Factor (SCF)-independent MC proliferation[5,6].

MCs are the progeny of CD34+ hematopoietic stem cells and require SCF for their differentiation, activation and proliferation[7]. MCs are located throughout the body; on the epithelial surface, in blood vessels, nerves and glands[8]. Classically, MCs are divided into three subgroups according to the protease expression in their granules: the first type of MC contains only tryptase, the second only chymase, and the third tryptase, chymase and other proteases[8,9].

Although the role of mast cells has long been well defined in hypersensitivity reactions, since 1990[10,11] it has been discovered that they also have a role in both innate and adaptive immunity. This has allowed us to redefine their crucial interplay on the regulatory function between inflammatory and tumor cells[12-15] by means of the release of various granule-associated mediators [histamine, serotonin, heparin, tryptase, chymase, thymidine phosphorylase, tumour necrosis factor, vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), platelet-derived growth factor-β (PDGF-β), epidermal growth factor (EGF)]; lipid-derived mediators (leukotrienes, prostaglandins, platelet-activating factor); cytokines (transforming growth factor-β, interleukins, IL-6); and chemokines[16-19].

MCs express many types of receptors allowing them to recognize different stimuli and to respond accordingly[8,9]. For the fragment crystallisable portion of Immunoglobulin (Ig)G and IgE, MCs express various receptors, and in response to several antigens they release preformed (*e.g.,* histamine, tryptase) and synthesized *de novo* mediators (*i.e.,* leukotrienes, prostaglandins)[10,20]. Regarding innate immunity, MCs express some receptors for components of complement (CR3, CR4, CR5), and others belonging to the Nod-like receptors family. The recognition of pathogens by the innate immune cells and the link between innate and adaptive immunity however are *via* Toll-like receptors (TLR type 1, 2, 3, 4, 6, 7 and 9)[21].

Many experimental studies have assessed MCs as protagonists both in inflammation and angiogenesis[20,22,23], processes closely interconnected and related to tumor development and progression[24-27]. Following the above-mentioned synthetic review of the various functions of MCs, in the upcoming sections we focus on the crucial role of MCs in angiogenesis-mediated tumor development and progression and illustrate the most common identification methods of MCs. In particular, as well as playing a role in tumor angiogenesis, it has been demonstrated that the number of MCs, so-called MC density (MCD), increases in several human and animal malignancies, and this increased MCD correlates with increased angiogenesis. On this basis, we analyze the principal studies that have focused on MCD as a possible prognostic factor, considering the MC as a possible novel therapeutic target in colorectal cancer (CRC).

**INVOLVEMENT OF MAST CELLS IN ANGIOGENESIS-MEDIATED TUMOR DEVELOPMENT AND PROGRESSION**

During inflammatory reactions, immune cells (MCs, macrophages, neutrophils, and lymphocytes) synthesize pro-angiogenic factors that induce first neovascularization then the further migration of inflammatory cells to the site of inflammation, amplifying the process[25,28]. At the same time, there is well-established evidence that tumor cells are surrounded by an infiltrate of inflammatory cells, which synergize with stromal cells and malignant cells in a paracrine manner[29-31]. As a consequence, there is a stimulation of endothelial cell proliferation and blood vessel formation[32-34]. It is important to underline that MCs are located near blood vessels and regulate many functions of endothelial cells[35-37].

In particular, the c-KitR activated by SCF and tryptase after its degranulation from activated MCs plays a pivotal part in tumor angiogenesis[38,39].

The increased activation of the c-KitR pathway leads to MC activation, which induces pro-angiogenic cytokines (such as VEGF, PDGF, FGF-2) and tryptase degranulation[38,39]. MC c-KitR activation induces cross-talk between MCs and the tumor cell microenvironment (endothelial and other cells), leading consequentially to the strengthening of pro-angiogenic signaling[6].

Tryptase is also an agonist of proteinase-activated receptor-2 (PAR-2)[40],which is expressed in epithelial and endothelial cells with proteolytic activities. It belongs to the unique superfamily of G-protein-coupled receptors and is activated by tryptase. Tryptase activation leads to cell proliferation and the release of IL-6 and granulocyte-macrophage colony-stimulating factor, which act as pro-angiogenic molecules[41]. Moreover, tryptase degrades extracellular matrix components[42], activating in its stored matrix metalloproteinases[43] and plasminogen activators that together help the invasion and metastasis of tumor cells[44] (Figure 1). *In vitro* studies on matrigel and *in vivo* studies on the chick embryo chorioallantoic membrane displayed the capillary growth induced by tryptase and, conversely, suppressed by tryptase inhibitors[45,46].

Apart from the above biological background, the role of MCs in tumor development has emerged from observation of a strong correlation between an increase of MCD and an increase of microvascular density (MVD) in many human and animal malignancies such as oral squamous carcinoma[13,47], breast cancer[11,12,16], gastrointestinal cancer[26,48-50], hepatocarcinoma[51], pancreatic adenocarcinoma[52], renal cell carcinoma[53], non-small cell lung cancer[54,55], melanoma[56], endometrial carcinoma[27,57], non-Hodgkin’s lymphomas[58], and multiple myeloma[59]. With particular reference to hematological disorders, some evidence suggests that high MCD infiltration is directly correlated with tumor progression and worse disease outcome[60-62].

Conversely, a few studies have shown that high MCD is linked to good prognosis[63,64].

To further emphasize that MC activation plays a pivotal role in tumor progression, it was shown in breast cancer that degranulated MCs (MCs-Try) are mainly present in peri-tumoral tissue (to strengthen the hypothesis that they are tumor-reactive), unlike those rich in granules MCs (MCs-TB) which are especially present in tumor infiltration and contribute to stromal remodeling and differentiation of myofibroblasts (through tryptase released in stromal microenvironment)[11].

The close relationship between MCD, angiogenesis and tumor progression could suggest a role for MCs and the pro-angiogenic factors released from them as novel therapeutic targets in cancer. In particular, it is possible to block MC activation/degranulation by means of c-KitR tyrosine kinase inhibitors (TKI) such as imatinib and masitinib, and also to block the tryptase released from MCs by means of tryptase inhibitors (gabexate and nafamostat mesylate)[12,65-67].

**PRINCIPAL METHODS FOR IDENTIFICATION OF TISSUE MAST CELLS**

MCs can be classically or conventionally identified by means of histochemical methods. Among these, Toluidine blue histochemistry (Undritz Stain) metachromatically stains MC granules, making them appear red or blue-red due to the presence of sulphated proteoglycans (heparin)[68]. With the above histochemistry, MCs appear as rather large oval or elongated cells (diameter of 20-30 μm) containing numerous basophilic granules in their cytoplasm that can hide the nucleus[12,69].

By immunohistochemistry MCs can be stained with antibodies towards c-KitR (*e.g.,* human-specific monoclonal antibodies anti-CD117), towards the content of their granules, *i.e.,* tryptase or chymase[68]. With a primary anti-c-KitR antibody, a membrane, cytoplasmic or mixed staining is observed[68]. With primary anti-chymase and anti-tryptase antibodies a diffuse cytoplasmic staining is observed[68].

Under the electron microscope MCs present a small, round nucleus, few mitochondria, some meandering tanks of rough endoplasmic reticulum and a small Golgi complex. The numerous specific granules (some hundreds) measure 0.3-0.8 μm in diameter and appear bordered by a membrane showing a variable fine granular or lamellar structure[70,71].

Following their activation, MCs degranulate and exocytose the content into the surroundings. Piecemeal degranulation is typified by variable losses of the granule content[71-73].

**MAST CELL DENSITY INVOLVEMENT IN COLORECTAL CANCER AND ITS POSSIBLE ROLE AS PROGNOSTIC FACTOR**

Normally, MCs are present in the mucosa and submucosa of the gastrointestinal tract in humans and mice[74].

In a preclinical study in mice, MCs played a crucial role in epithelial tumorigenesis, appearing in early dysplastic tissue and expanding in polyps[75]. However, when analysing the potential role of MCs in tumor development in several mice studies, Heijmans *et al*[76] were unable to draw certain conclusions due to a lack of a suitable animal model to study CRC. In fact, in IL-10-deficient mice with MCs Chichlowski *et al*[77] showed a reduced risk of development of inflammatory bowel disease (IBD) compared to in that of IL-10-deficient mice without MCs. Thus, this result emphasizes the protective role of MCs within the colonic microenvironment by enhancing the efficacy of the mucosal barrier. In reality, these data suggest that MCs can play a dual and opposite function, and this is probably due to the presence in the intestinal tract of different types of MCs, each with a specific role, with specific granules, and expressing various receptors[74].

It is noted that patients affected by IBD have an increased cumulative incidence of CRC than the general population and that this incidence increases with the duration of the bowel disease[78]. In particular, it was found that high MCD in intestinal adenomatous polyps[75,79-81] could drive a cascade of events to boost the progressive growth of adenomatous polyps, the immediate precursors of CRC[75].

In this regard, Taweevisit, considering 192 CRC patients, displayed a direct correlation between MCD, tumor development and grading[82].

With the aim to find a correlation between MCD and stage/prognosis in CRC patients, many studies (summarized in table 1) have been conducted with mixed results. One Author showed no correlation between MCD and prognosis[83,84]. Other Authors have shown a direct and significant correlation between high MCD and improved prognosis[85-87]. The majority of studies however have shown that high MCD is related to tumor aggressiveness[48-50] and reduced survival[88-90].

Xia *et al*[83] studied MCD in 39 patients with colon adenoma and in 155 colon cancer (CC) patients of all TNM stages, evaluating a relationship between MCD (positive to both tryptase and chymase) and tumor progression. Interestingly, a significant increase of MCD localized in adjacent normal colon mucosa in CC patients was noted compared to those with colon adenomas (*P* < 0.05)[83]. Moreover, MCD located in adjacent normal colon mucosain CC patients was significantly related to pathologic classification (*i.e.,* papillary plus tubular or other), depth of penetration (*i.e.,* high T according to TNM), distant metastases (*i.e.,* M1 according to TNM), and hepatic metastases (*P* = 0.029, *P* = 0.054, *P* = 0.008, *P* = 0.027)[83]. Instead, there is no correlation between MCD located in the invasive margin or in adjacent normal colon mucosaand survival (*P* = 0.092 and *P* = 0.003)[83]. Similarly, in 93 CC patients only in stage IIIB (according to TNM staging), the same Author observed a higher MCD positive to tryptase in non-metastatic regional-draining lymph nodes than in metastatic lymph nodes (*P* = 0.000)[84].

In 1999, Nielsen *et al*[85] analysis in a large cohort of CRC patients (*n* = 584) of all Dukes’ stages displayed a significant correlation between high MCD positive to tryptase and good prognosis (*P* = 0.02); 50% of all patients with high MCD positive to tryptase were still alive at 3 years.

Subsequently, Tan *et al*[86] observed that high MCD (positive to tryptase and chymase) is also related to a significantly higher 5-year survival rate (SR). In their study on 60 CRC patients of all TNM stages, a 59% SR was recorded for patients with high MCD compared to 33.3% in those with low MCD (*P* < 0.01). Curiously, low MCD was significantly related to deeper depth of invasion, but also to low rates of lymph node and distant metastases[86].

Recently, Elezoğlu and Tolunay[87] displayed a significant correlation between MCD positive to tryptase, MVD, and survival in 204 CRC patients of all TNM stages. In the MC group, for values < 10, the five-year SR was 48%, whereas for values > 10 it rose to 58% (*P* = 0.035). In the MVD arm for values < 10, the five-year SR was 46%, while for values ≥ 10 it was 58%(*P* = 0.042)[87].

In 1989 Fisher was one of the first researchers to identify high MCD as an unfavorable prognostic factor independent from disease stage or lymph nodal status in 331 rectal cancer patients of all Dukes’ stages[88].

In 60 patients with CRC of all TNM stages Acikalin *et al*[49] showed that MCD (evaluated by means of the Giemsa stain) was higher in patients with disease recurrence compared to those patients who had been disease free for at least 24 months (*P* < 0.001), and that it was correlated to short disease-free survival (*P* = 0.0013), vascular invasion (*P* = 0.06), depth of penetration (*P* = 0.05), lymph nodes metastases (*P* = 0.05), liver metastases (*P* = 0.05) and high TNM stage (*P* = 0.05).

Yodavudh *et al*[89] confirmed Elezoğlu and Tolunay’s report[87] of a strong correlation between MCD positive to tryptase, MVD, and survival in 130 CRC patients of all TNM stages. Contrarily however, they showed that low MVD (hypovascular tumor tissue) and low MCD are related to significantly longer survival rates (*P* < 0.0001).

Gulubova and Vlaykova[50] also confirmed a significant correlation between MCD positive to tryptase, MVD, and survival in 106 CRC patients of all TNM stages. Patients with low MCD had a significantly better prognosis compared to those with high MCD (*P* = 0.038)[50]. In the same way, hypovascular tumor tissue was related to highly significantly longer survival than hypervascular tumor tissue (*P* < 0.0001)[50].

In a recent series of 41 gastrointestinal cancer patients (of whom 22 had CRC of TNM stage IIIC), Ammendola *et al*[30] showed a significant correlation between MCD positive to tryptase and the number of metastatic lymph nodes harvested (*P* = 0.01), and between MCD in primary tumor tissue and in metastatic lymph node tissue (*P* = 0.02). These data suggest that MCD in primary tumor tissue could be a useful prognostic marker[30,49], surrogating the number of postoperative metastatic lymph nodes after surgical treatment in gastrointestinal cancer patients[91-94].

Even more recently, Malfettone *et al*[90] showed in 115 CRC patients of all TNM stages that high MCD positive to tryptase correlates with the advanced stages of CRC (*P* = 0.025). In particular, the expression of PAR-2 (especially at the sites most infiltrated by MCs) is related to MCD expression[90]. Due to the pro-angiogenic activity of tryptase, which stimulates PAR-2 on endothelial cells, it is possible to suggest an involvement of tryptase in CRC angiogenesis[90].

**MAST CELLS, c-KIT RECEPTOR AND PRO-ANGIOGENIC FACTORS FROM MAST CELLS RELEASED AS POSSIBLE THERAPEUTIC TARGETS IN COLORECTAL CANCER**

Ducroc *et al*[95] demonstrated a pivotal role of MC tryptase in inducing PAR-2 activation in several human CC cell lines (T84, Caco-2, HT-29, Cl.19A), promoting their proliferation.

Yoshii *et al*[96] investigated the distribution of MCD (positive to tryptase) in 30 human CC, showing the prevalence of MCD in the invasive front rather than in either the central tumor part or the normal tissue. In addition, the Authors showed a higher density of PAR-2 in the tumor tissue compared to the normal tissue[96].

Interestingly, two Authors explored the tryptase/PAR-2 axis in one human colon carcinoma cell line (DLD-1)[96,97]. Specifically, the proliferation signal induced by tryptase on DLD-1 cells is mediated by PAR-2, that in turn leads to the increase of calcium[98] and transient phosphorylation of mitogen-activated protein kinase/extracellular signal–related kinase (MEKK) and the mitogen-activated protein kinase (MAPK) pathway[96]. In addition, the increase of calcium PAR-2/Phospholipase C-mediated led to the activation of CycloOXygenase-2 (COX-2) and prostaglandin E2 (PGE2) synthesis, suggesting that the MEKK and MAPK pathway activation and PGE2 synthesis were together essential for DLD-1 proliferation[96] (Figure 2).

Sodium-hydrogen antiporter 3 regulator 1 (NHERF-1) is a cytoplasmic adaptor protein present in various cellular types (including intestinal cells). NHERF-1 regulates several transmembrane receptors, transporters and other proteins localized near the plasma membrane, and *via* the Ezrin/protein kinase-A- mediated network seems to lead to CRC progression[99,100].

Interestingly, Malfettone *et al*[90], having confirmed the close interplay between MCT and PAR-2 in tumour progression and invasiveness, showed that the PAR-2(+)/cytoplasmic NHERF-1(+) expression immunophenotype is an unfavourable prognostic factor in CRC patients, as it is associated with the presence of lymph nodal and distant metastasis, poor differentiation grade and lymphovascular invasion. If further studies conducted in stage II CRC patients should confirm the role of the PAR-2(+)/cytoplasmic NHERF-1(+) expression immunophenotype as a negative prognostic biomarker, it will become a prerequisite to the treatment of patients with adjuvant chemotherapy.

Finally, if future studies demonstrate that high MCD positive to tryptase is an independent unfavourable prognostic factor[30,49,50,88,89] related to a significant and increased risk of tumor progression, this parameter could be considered in the decision to give chemotherapy associated with tryptase inhibitors (gabexate and nafamostat mesylate).

Clearly, before being able to use MC targeted agents, a more in-depth knowledge of MC-mediated angiogenic mechanisms and the complex hierarchical relationships between the various angiogenesis signaling pathways will be necessary[101-104].

In this regard, tryptase may induce angiogenesis mainly by the increase of VEGF expression mediated *via* PAR-2, which is expressed also on endothelial cells as well as intestinal cells[12,27,45,54]. Moreover, VEGF and its receptors are widely expressed in intestinal carcinoma cells, and VEGF stimulates VEGFR-2-positive tumor, mast and endothelial cells directly, leading to tumor growth and angiogenesis by paracrine and autocrine stimulation signals[26,105,106].

Considering the central role of MCs in the activation of gastrointestinal and endothelial cells which contribute to tumor angiogenesis and progression, c-KitR could also be a potential therapeutic target for inhibiting their pro-angiogenic cytokine degranulation (VEGF, PDGF, FGF, tryptase) and activation[6,38,67,107]. In fact, MC c-KitR activation potentiates the cross-talk between MCs and endothelial cells (Figure 2), leading to the strengthening of pro-angiogenic signaling. Therefore, MCs could represent a possible therapeutic target through tryptase inhibitors (gabexate and nafamostat mesylate) and c-KitR inhibitors (imatinib, masitinib) to arrest angiogenesis-mediated tumor growth in gastrointestinal cancer[108-110].

**CONCLUSION**

Although the role of MCs was well defined in hypersensitivity reactions, the discovery of their regulatory function in innate and adaptive immunity has allowed us to understand their complex interplay between inflammatory and tumor cells. In fact, much evidence obtained from *in vitro* and *in vivo* studies has demonstrated that common MCs phenotypes, if adequately stimulated by various factors (histamine, heparin, tryptase, chymase, VEGF, FGF-2, PDGF-β, EGF), are able to interfere with tumor cells and the tumor microenvironment inducing tumor angiogenesis and progression[10,12].

Although the majority of studies have reported that several malignancies are associated with an increase of MC infiltration, controversial data about the relationship between MCD and prognosis in CRC have been reported. Considering these studies, conflicting conclusions[48-50], may in part depend on considerable *bias* related to CRC disease (radical surgical treatment with relative lymph node collection, type of resection, histology or stage tumor, colon plus rectal cancer, small sample size)[83,85,86,88], and different methods of MC evaluation (histochemistry with Toluidine blue, Giemsa stain, primary antibody anti-tryptase or anti-chymase for immunohistochemistry, standardization of MC counts with reference to magnification, MC location, microscopic field of evaluation)[76,84,87,90]. Despite these *biases*, the majority of the published studies suggest that high MCD in tumors may play a role as an unfavourable prognostic marker. Should this prognostic marker be validated in expected future studies it would be intriguing to conduct clinical trials employing chemotherapy plus tryptase inhibitors or TK inhibiting MCs c-KitR.

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**Figure 1 The close relationship between mast cells and angiogenesis-mediated tumor progression.** FGF-2: Fibroblast growth factor-2; VEGF: Vascular endothelial growth factor; PDGF-β: Platelet-derived growth factor-β; EGF: Epidermal growth factor; IL: Interleukin; GM-CSF: Granulocyte/macrophage colony stimulating factor; TNF-α: Tumor necrosis factor-α; ECM: Extracellular matrix; MMP: Matrix metalloproteinase.



**Figure 2 In both intestinal and endothelial cells, the tryptase/proteinase-activated receptor-2 and vascular endothelial growth factor/vascular endothelial growth factor receptor axes, induced by mast cells, lead to tumor angiogenesis and intestinal cell growth.** Note that targeting mast cells with molecular agents (c-KitR tyrosine kinase and tryptase inhibitors) could prevent angiogenesis-mediated colorectal cancer progression.c-KitR: c-Kit receptor; PAR-2: Proteinase-activated receptor-2; VEGFR: Vascular endothelial growth factor receptor; SCF: Stem cell factor: VEGF: Vascular endothelial growth factor; NHERF-1: Na+/H+ exchanger regulatory factor-1; MEKK-1: Mitogen-activated protein kinase/ extracellular signal–related kinase-1; MEKK-4: Mitogen-activated protein kinase/ extracellular signal–related kinase-4; JNK: c-Jun N-terminal kinase; c-Jun: Jun proto-oncogene; SAPK: Mitogen-activated protein kinase-9; GEF: Rho/rac guanine nucleotide exchange factor; Rho: Rhodopsin transcription termination factor; SOS: Son of sevenless protein; Grb2: Growth factor receptor-bound protein 2; Shc: Shc transforming protein kinase; Ras: Ras protein kinase; Raf: Raf protein kinase; Mitogen-activated protein kinase/extracellular signal–related kinase-1/2; Erk: Elk-related tyrosine kinase; DAG: Diacylglycerol; IP-3: Inositol triphosphate; PK-C: Protein kinase-C; COX-2: Cyclooxygenase-2; PGE2: Prostaglandin E2; PGES-1: Prostaglandin E synthase-1; PK-A: Protein kinase-A.

**Table 1 Principal studies correlating mast cell density with survival/stage in colorectal cancer patients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Author, year** | **Disease stage/main stages** | **Neoadjuvant therapy** | **Patients (*n*)/site** | **Methods of MCs identification** | **Correlation with overall survival/stage** | ***P* value** |
| Xia *et al*[83], 2011 | All TNM stages(mainly II-III) | No | 155CC | Immunohistochemistryprimay anti-tryptase and anti-chymase abs | No with OS | NS |
| Xia *et al*[84], 2011 | Stage IIIB | No | 93CC | Immunohistochemistryprimay anti-tryptase ab | No with OS | NS |
| Nielsen *et al*[85], 1999 | All Dukes’ stage(mainly B-C) | No | 584CRC | Immunohistochemistryprimay anti-tryptase ab | Yes, high MCD with high OS | 0.02 |
| Tan *et al*[86], 2005 | All TNM stages | NR | 60CRC | Immunohistochemistryprimay anti-tryptase and anti-chymase abs | Yes, high MCD with high OS |  < 0.01 |
| Fisher *et al*[88], 1989 | All Dukes’ stage(mainly B-C) | No | 331RC | Giemsa method | Yes, high MCD with low OS | NE |
| Yodavudh *et al*[89], 2008 | All TNM stages(mainly II-III) | No | 130CRC | Immunohistochemistryprimay anti-tryptase ab | Yes, high MCD with low OS | < 0.0001 |
| Elezoglu *et al*[87], 2012 | All TNM stages(mainly II-III) | NR | 204CRC | Toluidine blue histochemistry | Yes, high MCD with high OS | 0.035 |
| Acikalin *et al*[49], 2005 | All TNM stages(mainly II-III) | No | 60CRC | Giemsa method | Yes, high MCD with low OS | 0.0013 |
| Gulubova *et al*[50], 2009 | All TNM stages(mainly II) | No | 106CRC | Immunohistochemistryprimay anti-tryptase ab; Toluidine blue histochemistry | Yes, high MCD with low OS | 0.038 |

CC: Colon cancer; ab/s: Antibody/ies; OS: Overall survival: NS: Not significant; CRC: Colorectal cancer; MCD: Mast cell density; NR: Not reported; RC: Rectal cancer; NE: Not evaluated.