**Name of journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 5718**

**Columns: TOPIC HIGHLIGHT**

WJG 20th Anniversary Special Issues (5): Colorectal cancer

**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

**Received:** September 23, 2013 **Revised:** January 24, 2014

**Accepted:** April 21, 2014

**Published online:**

**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.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

**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.

Marech I, Ammendola M, Gadaleta C, Zizzo N, Oakley C, Gadaleta CD, Ranieri G.

Possible biological and translational significance of mast cells density in colorectal cancer. *World J Gastroenterol* 2014;

**Available from:** URL: http://www.wjgnet.com/esps/

**DOI:** DOI:10.3748/wjg.v20.i0.0000

**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.

**REFERENCES**

1 **Nettleship T**, Tay W. Rare forms of urticaria. *Brit Med J* 1869; **2**: 323-330

2 **Ehrlich P**. Beiträge zur Kenntniss der granulirten Bindegewebszellen und der eosinophilen Leukocythen. *Arch Anat Physiol* (Leipzig) 1879; **3**: 166-169

3 **Ellis JM**. Urticaria pigmentosa; a report of a case with autopsy. *Arch Pathol (Chic)* 1949; **48**: 426-435 [PMID: 18149230]

4 **Marone G**, Spadaro G, Genovese A. Biology, diagnosis and therapy of mastocytosis. *Chem Immunol* 1995; **62**: 1-21 [PMID: 7546277 DOI: 10.1159/000319293]

5 **Horny HP**, Sotlar K, Valent P. Mastocytosis: state of the art. *Pathobiology* 2007; **74**: 121-132 [PMID: 17587883 DOI: 10.1159/000101711]

6 **Orfao A**, Garcia-Montero AC, Sanchez L, Escribano L. Recent advances in the understanding of mastocytosis: the role of KIT mutations. *Br J Haematol* 2007; **138**: 12-30 [PMID: 17555444 DOI: 10.1111/j.1365-2141.2007.06619.x]

7 **Shea-Donohue T**, Stiltz J, Zhao A, Notari L. Mast cells. *Curr Gastroenterol Rep* 2010; **12**: 349-357 [PMID: 20711694 DOI: 10.1007/s11894-010-0132-1]

8 **Irani AM**, Schwartz LB. Human mast cell heterogeneity. *Allergy Proc* 1994; **15**: 303-308 [PMID: 7721078 DOI: 10.2500/108854194778816472]

9 **Irani AA**, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci U S A* 1986; **83**: 4464-4468 [PMID: 3520574 DOI: 10.1073/pnas.83.12.4464]

10 **Marshall JS**. Mast-cell responses to pathogens. *Nat Rev Immunol* 2004; **4**: 787-799 [PMID: 15459670 DOI: 10.1038/nri1460]

11 **Mangia A**, Malfettone A, Rossi R, Paradiso A, Ranieri G, Simone G, Resta L. Tissue remodelling in breast cancer: human mast cell tryptase as an initiator of myofibroblast differentiation. *Histopathology* 2011; **58**: 1096-1106 [PMID: 21707711 DOI: 10.1111/j.1365-2559.2011.03842.x]

12 **Ranieri G**, Ammendola M, Patruno R, Celano G, Zito FA, Montemurro S, Rella A, Di Lecce V, Gadaleta CD, Battista De Sarro G, Ribatti D. Tryptase-positive mast cells correlate with angiogenesis in early breast cancer patients. *Int J Oncol* 2009; **35**: 115-120 [PMID: 19513558 DOI: 10.3892/ijo\_00000319]

13 **Ranieri G**, Labriola A, Achille G, Florio G, Zito AF, Grammatica L, Paradiso A. Microvessel density, mast cell density and thymidine phosphorylase expression in oral squamous carcinoma. *Int J Oncol* 2002; **21**: 1317-1323 [PMID: 12429983]

14 **Ranieri G**, Roccaro AM, Vacca A, Ribatti D. Thymidine phosphorylase (platelet-derived endothelial cell growth factor) as a target for capecitabine: from biology to the bedside. *Recent Pat Anticancer Drug Discov* 2006; **1**: 171-183 [PMID: 18221035 DOI: 10.2174/157489206777442241]

15 **Passantino L**, Patruno R, Valerio P, Penna A, Mazzone F, Zito AF, Catalano V, Pellecchia A, Jirillo E, Ranieri G. Thymidine phosphorylase profiles in nonmalignant and malignant pancreatic tissue. Potential therapeutic role of capecitabine on tumoral and endothelial cells and tumor-infiltrating macrophages. *Immunopharmacol Immunotoxicol* 2005; **27**: 95-107 [PMID: 15803863 DOI: 10.1081/IPH-51753]

16 **Raica M**, Cimpean AM, Ceausu R, Ribatti D, Gaje P. Interplay between mast cells and lymphatic vessels in different molecular types of breast cancer. *Anticancer Res* 2013; **33**: 957-963 [PMID: 23482767]

17 **Ribatti D**, Nico B, Finato N, Crivellato E. Tryptase-positive mast cells and CD8-positive T cells in human endometrial cancer. *Pathol Int* 2011; **61**: 442-444 [PMID: 21707849]

18 **Nagata M**, Shijubo N, Walls AF, Ichimiya S, Abe S, Sato N. Chymase-positive mast cells in small sized adenocarcinoma of the lung. *Virchows Arch* 2003; **443**: 565-573 [PMID: 12827514 DOI: 10.1007/s00428-003-0842-y]

19 **Horny HP**, Greschniok A, Jordan JH, Menke DM, Valent P. Chymase expressing bone marrow mast cells in mastocytosis and myelodysplastic syndromes: an immunohistochemical and morphometric study. *J Clin Pathol* 2003; **56**: 103-106 [PMID: 12560387 DOI: 10.1136/jcp.56.2.103]

20 **Galli SJ**, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005; **23**: 749-786 [PMID: 15771585 DOI: 10.1146/annurev.immunol.21.120601.141025]

21 **Oda K**, Kitano H. A comprehensive map of the toll-like receptor signaling network. *Mol Syst Biol* 2006; **2**: 2006.0015 [PMID: 16738560]

22 **Coussens LM**, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999; **13**: 1382-1397 [PMID: 10364156 DOI: 10.1101/gad.13.11.1382]

23 **Nakayama T**, Yao L, Tosato G. Mast cell-derived angiopoietin-1 plays a critical role in the growth of plasma cell tumors. *J Clin Invest* 2004; **114**: 1317-1325 [PMID: 15520864 DOI: 10.1172/JCI22089]

24 **Theoharides TC**, Conti P. Mast cells: the Jekyll and Hyde of tumor growth. *Trends Immunol* 2004; **25**: 235-241 [PMID: 15099563 DOI: 10.1016/j.it.2004.02.013]

25 **Ribatti D**, Crivellato E. Mast cells, angiogenesis and cancer. *Adv Exp Med Biol* 2011; **716**: 270-288 [PMID: 21713661 DOI: 10.1007/978-1-4419-9533-9\_14]

26 **Ribatti D**, Guidolin D, Marzullo A, Nico B, Annese T, Benagiano V, Crivellato E. Mast cells and angiogenesis in gastric carcinoma. *Int J Exp Pathol* 2010; **91**: 350-356 [PMID: 20412338 DOI: 10.1111/j.1365-2613.2010.00714.x]

27 **Ribatti D**, Finato N, Crivellato E, Marzullo A, Mangieri D, Nico B, Vacca A, Beltrami CA. Neovascularization and mast cells with tryptase activity increase simultaneously with pathologic progression in human endometrial cancer. *Am J Obstet Gynecol* 2005; **193**: 1961-1965 [PMID: 16325597 DOI: 10.1016/j.ajog.2005.04.055]

28 **Ranieri G**. Hot topic: targeting tumor angiogenesis: an update. *Curr Med Chem* 2012; **19**: 937 [PMID: 22214460 DOI: 10.2174/092986712799320718]

29 **Saponaro C**, Malfettone A, Ranieri G, Danza K, Simone G, Paradiso A, Mangia A. VEGF, HIF-1α expression and MVD as an angiogenic network in familial breast cancer. *PLoS One* 2013; **8**: e53070 [PMID: 23326384 DOI: 10.1371/journal.pone.0053070]

30 **Ammendola M**, Zuccalà V, Patruno R, Russo E, Luposella M, Amorosi A, Vescio G, Sammarco G, Montemurro S, De Sarro G, Sacco R, Ranieri G. Tryptase-positive mast cells and angiogenesis in keloids: a new possible post-surgical target for prevention. *Updates Surg* 2013; **65**: 53-57 [PMID: 23117746 DOI: 10.1007/s13304-012-0183-y]

31 **Ranieri G**, Coviello M, Chiriatti A, Stea B, Montemurro S, Quaranta M, Dittadi R, Paradiso A. Vascular endothelial growth factor assessment in different blood fractions of gastrointestinal cancer patients and healthy controls. *Oncol Rep* 2004; **11**: 435-439 [PMID: 14719080]

32 **Ranieri G**, Coviello M, Patruno R, Valerio P, Martino D, Milella P, Catalano V, Scotto F, De Ceglie A, Quaranta M, Ribatti D, Pellecchia A. Vascular endothelial growth factor concentrations in the plasma-activated platelets rich (P-APR) of healthy controls and colorectal cancer patients. *Oncol Rep* 2004; **12**: 817-820 [PMID: 15375505]

33 **Mangia A**, Chiriatti A, Ranieri G, Abbate I, Coviello M, Simone G, Zito FA, Montemurro S, Rucci A, Di Leo A, Tommasi S, Berloco P, Xu JM, Paradiso A. H pylori status and angiogenesis factors in human gastric carcinoma. *World J Gastroenterol* 2006; **12**: 5465-5472 [PMID: 17006982]

34 **Ranieri G**, Patruno R, Ruggieri E, Montemurro S, Valerio P, Ribatti D. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. *Curr Med Chem* 2006; **13**: 1845-1857 [PMID: 16842197 DOI: 10.2174/092986706777585059]

35 **Miyazaki T**, Okada N, Ishibashi K, Ogata K, Ohsawa T, Ishiguro T, Nakada H, Yokoyama M, Matsuki M, Kato H, Kuwano H, Ishida H. Clinical significance of plasma level of vascular endothelial growth factor-C in patients with colorectal cancer. *Jpn J Clin Oncol* 2008; **38**: 839-843 [PMID: 18923001 DOI: 10.1093/jjco/hyn106]

36 **Werther K**, Christensen IJ, Nielsen HJ. The association between preoperative concentration of soluble vascular endothelial growth factor, perioperative blood transfusion, and survival in patients with primary colorectal cancer. *Eur J Surg* 2001; **167**: 287-292 [PMID: 11354321 DOI: 10.1080/110241501300091480]

37 **Ranieri G**, Ruggieri E, Falco G, Zizzo N, Mattioli E, Zito AF, Patruno R, Gasparini G. Drug targets to pro-angiogenetic factors with special reference to primary peritoneal mesothelioma. *Endocr Metab Immune Disord Drug Targets* 2006; **6**: 271-277 [PMID: 17017978 DOI: 10.2174/187153006778250028]

38 **Hassan S**, Kinoshita Y, Kawanami C, Kishi K, Matsushima Y, Ohashi A, Funasaka Y, Okada A, Maekawa T, He-Yao W, Chiba T. Expression of protooncogene c-kit and its ligand stem cell factor (SCF) in gastric carcinoma cell lines. *Dig Dis Sci* 1998; **43**: 8-14 [PMID: 9508539 DOI: 10.1023/A: 1018851415704]

39 **Ribatti D**, Ranieri G, Basile A, Azzariti A, Paradiso A, Vacca A. Tumor endothelial markers as a target in cancer. *Expert Opin Ther Targets* 2012; **16**: 1215-1225 [PMID: 22978444 DOI: 10.1517/14728222.2012.725047]

40 **Macfarlane SR**, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptors. *Pharmacol Rev* 2001; **53**: 245-282 [PMID: 11356985]

41 **Liu Y**, Mueller BM. Protease-activated receptor-2 regulates vascular endothelial growth factor expression in MDA-MB-231 cells via MAPK pathways. *Biochem Biophys Res Commun* 2006; **344**: 1263-1270 [PMID: 16650817 DOI: 10.1016/j.bbrc.2006.04.005]

42 **Taipale J**, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J Biol Chem* 1995; **270**: 4689-4696 [PMID: 7876240 DOI: 10.1074/jbc.270.9.4689]

43 **Gruber BL**, Marchese MJ, Suzuki K, Schwartz LB, Okada Y, Nagase H, Ramamurthy NS. Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. *J Clin Invest* 1989; **84**: 1657-1662 [PMID: 2553780 DOI: 10.1172/JCI114344]

44 **Stack MS**, Johnson DA. Human mast cell tryptase activates single-chain urinary-type plasminogen activator (pro-urokinase). *J Biol Chem* 1994; **269**: 9416-9419 [PMID: 8144524]

45 **Blair RJ**, Meng H, Marchese MJ, Ren S, Schwartz LB, Tonnesen MG, Gruber BL. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. *J Clin Invest* 1997; **99**: 2691-2700 [PMID: 9169499 DOI: 10.1172/JCI119458]

46 **Ribatti D**, Ranieri G, Nico B, Benagiano V, Crivellato E. Tryptase and chymase are angiogenic in vivo in the chorioallantoic membrane assay. *Int J Dev Biol* 2011; **55**: 99-102 [PMID: 21425085 DOI: 10.1387/ijdb.103138dr]

47 **Tomita M**, Matsuzaki Y, Edagawa M, Shimizu T, Hara M, Sekiya R, Onitsuka T. Association of mast cells with tumor angiogenesis in esophageal squamous cell carcinoma. *Dis Esophagus* 2001; **14**: 135-138 [PMID: 11553224 DOI: 10.1046/j.1442-2050.2001.00171.x]

48 **Ammendola M**, Sacco R, Donato G, Zuccalà V, Russo E, Luposella M, Vescio G, Rizzuto A, Patruno R, De Sarro G, Montemurro S, Sammarco G, Ranieri G. Mast cell positivity to tryptase correlates with metastatic lymph nodes in gastrointestinal cancer patients treated surgically. *Oncology* 2013; **85**: 111-116 [PMID: 23887206 DOI: 10.1159/000351145]

49 **Acikalin MF**, Oner U, Topçu I, Yaşar B, Kiper H, Colak E. Tumour angiogenesis and mast cell density in the prognostic assessment of colorectal carcinomas. *Dig Liver Dis* 2005; **37**: 162-169 [PMID: 15888280 DOI: 10.1016/j.dld.2004.09.028]

50 **Gulubova M**, Vlaykova T. Prognostic significance of mast cell number and microvascular density for the survival of patients with primary colorectal cancer. *J Gastroenterol Hepatol* 2009; **24**: 1265-1275 [PMID: 17645466 DOI: 10.1111/j.1440-1746.2007.05009.x]

51 **Peng SH**, Deng H, Yang JF, Xie PP, Li C, Li H, Feng DY. Significance and relationship between infiltrating inflammatory cell and tumor angiogenesis in hepatocellular carcinoma tissues. *World J Gastroenterol* 2005; **11**: 6521-6524 [PMID: 16425427]

52 **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**: 630-636 [PMID: 15166270 DOI: 10.1136/jcp.2003.014498]

53 **Tuna B**, Yorukoglu K, Unlu M, Mungan MU, Kirkali Z. Association of mast cells with microvessel density in renal cell carcinomas. *Eur Urol* 2006; **50**: 530-534 [PMID: 16426730 DOI: 10.1016/j.eururo.2005.12.040]

54 **Ibaraki T**, Muramatsu M, Takai S, Jin D, Maruyama H, Orino T, Katsumata T, Miyazaki M. The relationship of tryptase- and chymase-positive mast cells to angiogenesis in stage I non-small cell lung cancer. *Eur J Cardiothorac Surg* 2005; **28**: 617-621 [PMID: 16125954 DOI: 10.1016/j.ejcts.2005.06.020]

55 **Carlini MJ**, Dalurzo MC, Lastiri JM, Smith DE, Vasallo BC, Puricelli LI, Lauría de Cidre LS. Mast cell phenotypes and microvessels in non-small cell lung cancer and its prognostic significance. *Hum Pathol* 2010; **41**: 697-705 [PMID: 20040391 DOI: 10.1016/j.humpath.2009.04.029]

56 **Ribatti D**, Ennas MG, Vacca A, Ferreli F, Nico B, Orru S, Sirigu P. Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. *Eur J Clin Invest* 2003; **33**: 420-425 [PMID: 12760367 DOI: 10.1046/j.1365-2362.2003.01152.x]

57 **Benítez-Bribiesca L**, Wong A, Utrera D, Castellanos E. The role of mast cell tryptase in neoangiogenesis of premalignant and malignant lesions of the uterine cervix. *J Histochem Cytochem* 2001; **49**: 1061-1062 [PMID: 11457936 DOI: 10.1177/002215540104900816]

58 **Ranieri G**, Patruno R, Lionetti A, Di Summa A, Mattioli E, Bufo P, Pellecchia A, Ribatti D, Zizzo N. Endothelial area and microvascular density in a canine non-Hodgkin's lymphoma: an interspecies model of tumor angiogenesis. *Leuk Lymphoma* 2005; **46**: 1639-1643 [PMID: 16236617 DOI: 10.1080/10428190500205150]

59 **Nico B**, Mangieri D, Crivellato E, Vacca A, Ribatti D. Mast cells contribute to vasculogenic mimicry in multiple myeloma. *Stem Cells Dev* 2008; **17**: 19-22 [PMID: 18205547 DOI: 10.1089/scd.2007.0132]

60 **Ribatti D**, Molica S, Vacca A, Nico B, Crivellato E, Roccaro AM, Dammacco F. Tryptase-positive mast cells correlate positively with bone marrow angiogenesis in B-cell chronic lymphocytic leukemia. *Leukemia* 2003; **17**: 1428-1430 [PMID: 12835741 DOI: 10.1038/sj.leu.2402970]

61 **Ribatti D**, Polimeno G, Vacca A, Marzullo A, Crivellato E, Nico B, Lucarelli G, Dammacco F. Correlation of bone marrow angiogenesis and mast cells with tryptase activity in myelodysplastic syndromes. *Leukemia* 2002; **16**: 1680-1684 [PMID: 12200681 DOI: 10.1038/sj.leu.2402586]

62 **Molica S**, Vacca A, Crivellato E, Cuneo A, Ribatti D. Tryptase-positive mast cells predict clinical outcome of patients with early B-cell chronic lymphocytic leukemia. *Eur J Haematol* 2003; **71**: 137-139 [PMID: 12890156 DOI: 10.1034/j.1600-0609.2003.00110.x]

63 **Tomita M**, Matsuzaki Y, Onitsuka T. Correlation between mast cells and survival rates in patients with pulmonary adenocarcinoma. *Lung Cancer* 1999; **26**: 103-108 [PMID: 10568681 DOI: 10.1016/S0169-5002(99)00076-8]

64 **Norrby K**. Mast cells and angiogenesis. *APMIS* 2002; **110**: 355-371 [PMID: 12076253 DOI: 10.1034/j.1600-0463.2002.100501.x]

65 **Erba F**, Fiorucci L, Pascarella S, Menegatti E, Ascenzi P, Ascoli F. Selective inhibition of human mast cell tryptase by gabexate mesylate, an antiproteinase drug. *Biochem Pharmacol* 2001; **61**: 271-276 [PMID: 11172730 DOI: 10.1016/S0006-2952(00)00550-5]

66 **Mori S**, Itoh Y, Shinohata R, Sendo T, Oishi R, Nishibori M. Nafamostat mesilate is an extremely potent inhibitor of human tryptase. *J Pharmacol Sci* 2003; **92**: 420-423 [PMID: 12939527 DOI: 10.1254/jphs.92.420]

67 **Bai Y**, Bandara G, Ching Chan E, Maric I, Simakova O, Bandara SN, Lu WP, Wise SC, Flynn DL, Metcalfe DD, Gilfillan AM, Wilson TM. Targeting the KIT activating switch control pocket: a novel mechanism to inhibit neoplastic mast cell proliferation and mast cell activation. *Leukemia* 2013; **27**: 278-285 [PMID: 22907049 DOI: 10.1038/leu.2012.218]

68 **Patruno R**, Zizzo N, Zito AF, Catalano V, Valerio P, Pellecchia V, D'Errico E, Mazzone F, Ribatti D, Ranieri G. Microvascular density and endothelial area correlate with Ki-67 proliferative rate in the canine non-Hodgkin's lymphoma spontaneous model. *Leuk Lymphoma* 2006; **47**: 1138-1143 [PMID: 16840207 DOI: 10.1080/10428190600565859]

69 **Bloom HJ**, Richardson WW. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 1957; **11**: 359-377 [PMID: 13499785 DOI: 10.1038/bjc.1957.43]

70 **Crivellato E**, Nico B, Vacca A, Ribatti D. Ultrastructural analysis of mast cell recovery after secretion by piecemeal degranulation in B-cell non-Hodgkin's lymphoma. *Leuk Lymphoma* 2003; **44**: 517-521 [PMID: 12688324 DOI: 10.1080/1042819021000047001]

71 **Crivellato E**, Ribatti D, Mallardi F, Beltrami CA. Granule changes of human and murine endocrine cells in the gastrointestinal epithelia are characteristic of piecemeal degranulation. *Anat Rec* 2002; **268**: 353-359 [PMID: 12420282 DOI: 10.1002/ar.10149]

72 **Ward SM**, McLaren GJ, Sanders KM. Interstitial cells of Cajal in the deep muscular plexus mediate enteric motor neurotransmission in the mouse small intestine. *J Physiol* 2006; **573**: 147-159 [PMID: 16513671 DOI: 10.1113/jphysiol.2006.105189]

73 **Mikkelsen HB**. Interstitial cells of Cajal, macrophages and mast cells in the gut musculature: morphology, distribution, spatial and possible functional interactions. *J Cell Mol Med* 2010; **14**: 818-832 [PMID: 20132411 DOI: 10.1111/j.1582-4934.2010.01025.x]

74 **Hiromatsu Y**, Toda S. Mast cells and angiogenesis. *Microsc Res Tech* 2003; **60**: 64-69 [PMID: 12500262 DOI: 10.1002/jemt.10244]

75 **Gounaris E**, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, Lee DM, Zhang G, Glickman JN, Shin K, Rao VP, Poutahidis T, Weissleder R, McNagny KM, Khazaie K. Mast cells are an essential hematopoietic component for polyp development. *Proc Natl Acad Sci U S A* 2007; **104**: 19977-19982 [PMID: 18077429 DOI: 10.1073/pnas.0704620104]

76 **Heijmans J**, Büller NV, Muncan V, van den Brink GR. Role of mast cells in colorectal cancer development, the jury is still out. *Biochim Biophys Acta* 2012; **1822**: 9-13 [PMID: 21146606 DOI: 10.1016/j.bbadis.2010.12.001]

77 **Chichlowski M**, Westwood GS, Abraham SN, Hale LP. Role of mast cells in inflammatory bowel disease and inflammation-associated colorectal neoplasia in IL-10-deficient mice. *PLoS One* 2010; **5**: e12220 [PMID: 20808919 DOI: 10.1371/journal.pone.0012220]

78 **Eaden JA**, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 2001; **48**: 526-535 [PMID: 11247898 DOI: 10.1136/gut.48.4.526]

79 **Lachter J**, Stein M, Lichtig C, Eidelman S, Munichor M. Mast cells in colorectal neoplasias and premalignant disorders. *Dis Colon Rectum* 1995; **38**: 290-293 [PMID: 7882795 DOI: 10.1007/BF02055605]

80 **Ranieri G**, Ria R, Roccaro AM, Vacca A, Ribatti D. Development of vasculature targeting strategies for the treatment of chronic inflammatory diseases. *Curr Drug Targets Inflamm Allergy* 2005; **4**: 13-22 [PMID: 15720230 DOI: 10.2174/1568010053622966]

81 **Kashiwase Y**, Inamura H, Morioka J, Igarashi Y, Kawai-Kowase K, Kurosawa M. Quantitative analysis of mast cells in benign and malignant colonic lesions: immunohistochemical study on formalin-fixed, paraffin-embedded tissues. *Allergol Immunopathol (Madr)* 2008; **36**: 271-276 [PMID: 19080799 DOI: 10.1016/S0301-0546(08)75222-4]

82 **Taweevisit M**. The association of stromal mast cell response and tumor cell differentiation in colorectal cancer. *J Med Assoc Thai* 2006; **89 Suppl 3**: S69-S73 [PMID: 17718271]

83 **Xia Q**, Ding Y, Wu XJ, Peng RQ, Zhou Q, Zeng J, Hou JH, Zhang X, Zeng YX, Zhang XS, Chen YB. Mast Cells in Adjacent Normal Colon Mucosa rather than Those in Invasive Margin are Related to Progression of Colon Cancer. *Chin J Cancer Res* 2011; **23**: 276-282 [PMID: 23358806 DOI: 10.1007/s11670-011-0276-z]

84 **Xia Q**, Wu XJ, Zhou Q, Jing-Zeng JH, Pan ZZ, Zhang XS. No relationship between the distribution of mast cells and the survival of stage IIIB colon cancer patients. *J Transl Med* 2011; **9**: 88 [PMID: 21651824 DOI: 10.1186/1479-5876-9-88]

85 **Nielsen HJ**, Hansen U, Christensen IJ, Reimert CM, Brünner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. *J Pathol* 1999; **189**: 487-495 [PMID: 10629548 DOI: 3.0.CO; 2-I']

86 **Tan SY**, Fan Y, Luo HS, Shen ZX, Guo Y, Zhao LJ. Prognostic significance of cell infiltrations of immunosurveillance in colorectal cancer. *World J Gastroenterol* 2005; **11**: 1210-1214 [PMID: 15754407]

87 **Elezoğlu B**, Tolunay S. The relationship between the stromal mast cell number, microvessel density, c-erbB-2 staining and survival and prognostic factors in colorectal carcinoma. *Turk Patoloji Derg* 2012; **28**: 110-118 [PMID: 22627628 DOI: 10.5146/tjpath.2012.01109]

88 **Fisher ER**, Paik SM, Rockette H, Jones J, Caplan R, Fisher B. Prognostic significance of eosinophils and mast cells in rectal cancer: findings from the National Surgical Adjuvant Breast and Bowel Project (protocol R-01). *Hum Pathol* 1989; **20**: 159-163 [PMID: 2562788 DOI: 10.1016/0046-8177(89)90180-9]

89 **Yodavudh S**, Tangjitgamol S, Puangsa-art S. Prognostic significance of microvessel density and mast cell density for the survival of Thai patients with primary colorectal cancer. *J Med Assoc Thai* 2008; **91**: 723-732 [PMID: 18672639]

90 **Malfettone A**, Silvestris N, Saponaro C, Ranieri G, Russo A, Caruso S, Popescu O, Simone G, Paradiso A, Mangia A. High density of tryptase-positive mast cells in human colorectal cancer: a poor prognostic factor related to protease-activated receptor 2 expression. *J Cell Mol Med* 2013; **17**: 1025-1037 [PMID: 23991686 DOI: 10.1111/jcmm.12073]

91 **Song YX**, Gao P, Wang ZN, Liang JW, Sun Z, Wang MX, Dong YL, Wang XF, Xu HM. Can the tumor deposits be counted as metastatic lymph nodes in the UICC TNM staging system for colorectal cancer? *PLoS One* 2012; **7**: e34087 [DOI: 10.1371/journal.pone.0034087]

92 **Morikawa T**, Tanaka N, Kuchiba A, Nosho K, Yamauchi M, Hornick JL, Swanson RS, Chan AT, Meyerhardt JA, Huttenhower C, Schrag D, Fuchs CS, Ogino S. Predictors of lymph node count in colorectal cancer resections: data from US nationwide prospective cohort studies. *Arch Surg* 2012; **147**: 715-723 [PMID: 22508672 DOI: 10.1001/archsurg.2012.353]

93 **Liu X**, Cai H, Shi Y, Wang Y. Prognostic factors in patients with node-negative gastric cancer: a single center experience from China. *J Gastrointest Surg* 2012; **16**: 1123-1127 [PMID: 22488657 DOI: 10.1007/s11605-012-1881-y]

94 **Sjo OH**, Merok MA, Svindland A, Nesbakken A. Prognostic impact of lymph node harvest and lymph node ratio in patients with colon cancer. *Dis Colon Rectum* 2012; **55**: 307-315 [PMID: 22469798 DOI: 10.1097/DCR.0b013e3182423f62]

95 **Ducroc R**, Bontemps C, Marazova K, Devaud H, Darmoul D, Laburthe M. Trypsin is produced by and activates protease-activated receptor-2 in human cancer colon cells: evidence for new autocrine loop. *Life Sci* 2002; **70**: 1359-1367 [PMID: 11883712 DOI: 10.1016/S0024-3205(01)01519-3]

96 **Yoshii M**, Jikuhara A, Mori S, Iwagaki H, Takahashi HK, Nishibori M, Tanaka N. Mast cell tryptase stimulates DLD-1 carcinoma through prostaglandin- and MAP kinase-dependent manners. *J Pharmacol Sci* 2005; **98**: 450-458 [PMID: 16093613 DOI: 10.1254/jphs.FPJ05002X]

97 **Jikuhara A**, Yoshii M, Iwagaki H, Mori S, Nishibori M, Tanaka N. MAP kinase-mediated proliferation of DLD-1 carcinoma by the stimulation of protease-activated receptor 2. *Life Sci* 2003; **73**: 2817-2829 [PMID: 14511767 DOI: 10.1016/S0024-3205(03)00702-1]

98 **Sendo T**, Sumimura T, Itoh Y, Goromaru T, Aki K, Yano T, Oike M, Ito Y, Mori S, Nishibori M, Oishi R. Involvement of proteinase-activated receptor-2 in mast cell tryptase-induced barrier dysfunction in bovine aortic endothelial cells. *Cell Signal* 2003; **15**: 773-781 [PMID: 12781870 DOI: 10.1016/S0898-6568(03)00014-7]

99 **Voltz JW**, Weinman EJ, Shenolikar S. Expanding the role of NHERF, a PDZ-domain containing protein adapter, to growth regulation. *Oncogene* 2001; **20**: 6309-6314 [PMID: 11607833 DOI: 10.1038/sj.onc.1204774]

100 **Mangia A**, Saponaro C, Malfettone A, Bisceglie D, Bellizzi A, Asselti M, Popescu O, Reshkin SJ, Paradiso A, Simone G. Involvement of nuclear NHERF1 in colorectal cancer progression. *Oncol Rep* 2012; **28**: 889-894 [PMID: 22766563 DOI: 10.3892/or.2012.1895]

101 **De Luisi A**, Ferrucci A, Coluccia AM, Ria R, Moschetta M, de Luca E, Pieroni L, Maffia M, Urbani A, Di Pietro G, Guarini A, Ranieri G, Ditonno P, Berardi S, Caivano A, Basile A, Cascavilla N, Capalbo S, Quarta G, Dammacco F, Ribatti D, Vacca A. Lenalidomide restrains motility and overangiogenic potential of bone marrow endothelial cells in patients with active multiple myeloma. *Clin Cancer Res* 2011; **17**: 1935-1946 [PMID: 21307145 DOI: 10.1158/1078-0432.CCR-10-2381]

102 **Basile A**, Moschetta M, Ditonno P, Ria R, Marech I, De Luisi A, Berardi S, Frassanito MA, Angelucci E, Derudas D, Specchia G, Curci P, Pavone V, Rossini B, Ribatti D, Bottazzi B, Mantovani A, Presta M, Dammacco F, Vacca A. Pentraxin 3 (PTX3) inhibits plasma cell/stromal cell cross-talk in the bone marrow of multiple myeloma patients. *J Pathol* 2013; **229**: 87-98 [PMID: 22847671 DOI: 10.1002/path.4081]

103 **Ranieri G**, Mammì M, Donato Di Paola E, Russo E, Gallelli L, Citraro R, Gadaleta CD, Marech I, Ammendola M, De Sarro G. Pazopanib a tyrosine kinase inhibitor with strong anti-angiogenetic activity: a new treatment for metastatic soft tissue sarcoma. *Crit Rev Oncol Hematol* 2014; **89**: 322-329 [PMID: 24041629]

104 **Gnoni A**, Marech I, Silvestris N, Vacca A, Lorusso V. Dasatinib: an anti-tumour agent via Src inhibition. *Curr Drug Targets* 2011; **12**: 563-578 [PMID: 21226671 DOI: 10.2174/138945011794751591]

105 **Yang XP**, Li Y, Wang Y, Wang Y, Wang P. beta-Tryptase up-regulates vascular endothelial growth factor expression via proteinase-activated receptor-2 and mitogen-activated protein kinase pathways in bone marrow stromal cells in acute myeloid leukemia. *Leuk Lymphoma* 2010; **51**: 1550-1558 [PMID: 20578818 DOI: 10.3109/10428194.2010.496013]

106 **Zhang H**, Wu J, Meng L, Shou CC. Expression of vascular endothelial growth factor and its receptors KDR and Flt-1 in gastric cancer cells. *World J Gastroenterol* 2002; **8**: 994-998 [PMID: 12439912]

107 **Passantino L**, Passantino G, Cianciotta A, Ribaud MR, Lo Presti G, Ranieri G, Perillo A. Expression of proto-oncogene C-kit and correlation with morphological evaluations in canine cutaneous mast cell tumors. *Immunopharmacol Immunotoxicol* 2008; **30**: 609-621 [PMID: 18608529 DOI: 10.1080/08923970801949265]

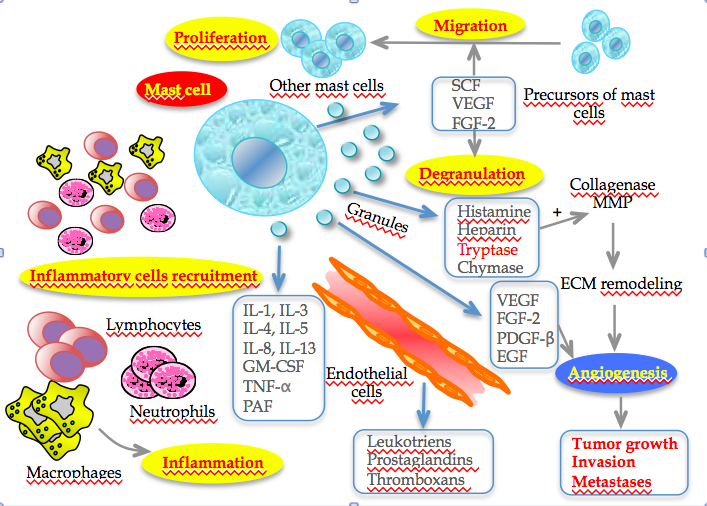
108 **Ranieri G**, Gadaleta CD, Patruno R, Zizzo N, Daidone MG, Hansson MG, Paradiso A, Ribatti D. A model of study for human cancer: Spontaneous occurring tumors in dogs. Biological features and translation for new anticancer therapies. *Crit Rev Oncol Hematol* 2013; **88**: 187-197 [PMID: 23561333 DOI: 10.1016/j.critrevonc.2013.03.005]

109 **Ranieri G**, Passantino L, Patruno R, Passantino G, Jirillo F, Catino A, Mattioli V, Gadaleta C, Ribatti D. The dog mast cell tumour as a model to study the relationship between angiogenesis, mast cell density and tumour malignancy. *Oncol Rep* 2003; **10**: 1189-1193 [PMID: 12883679]

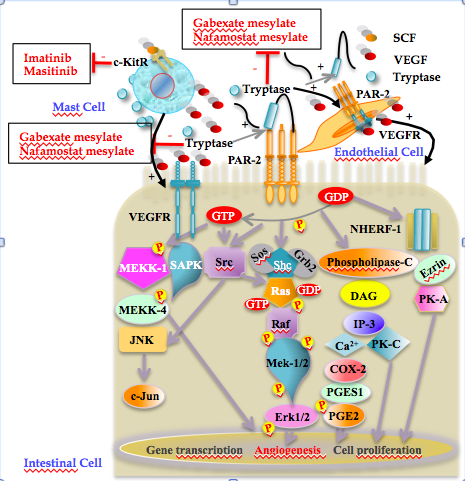
110 **Ranieri G**, Pantaleo M, Piccinno M, Roncetti M, Mutinati M, Marech I, Patruno R, Rizzo A, Sciorsci RL. Tyrosine kinase inhibitors (TKIs) in human and pet tumours with special reference to breast cancer: a comparative review. *Crit Rev Oncol Hematol* 2013; **88**: 293-308 [PMID: 23768779]

**P-Reviewers:** Chen JL, Huang ZH, Stanojevic GZ **S-Editor:** Gou SX

**L-Editor: E-Editor:**

****

**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 | 155  CC | Immunohistochemistry  primay anti-tryptase and anti-chymase abs | No with OS | NS |
| Xia *et al*[84], 2011 | Stage IIIB | No | 93  CC | Immunohistochemistry  primay anti-tryptase ab | No with OS | NS |
| Nielsen *et al*[85], 1999 | All Dukes’ stage  (mainly B-C) | No | 584  CRC | Immunohistochemistry  primay anti-tryptase ab | Yes, high MCD with high OS | 0.02 |
| Tan *et al*[86], 2005 | All TNM stages | NR | 60  CRC | Immunohistochemistry  primay 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 | 331  RC | Giemsa method | Yes, high MCD with low OS | NE |
| Yodavudh *et al*[89], 2008 | All TNM stages  (mainly II-III) | No | 130  CRC | Immunohistochemistry  primay anti-tryptase ab | Yes, high MCD with low OS | < 0.0001 |
| Elezoglu *et al*[87], 2012 | All TNM stages  (mainly II-III) | NR | 204  CRC | Toluidine blue histochemistry | Yes, high MCD with high OS | 0.035 |
| Acikalin *et al*[49], 2005 | All TNM stages  (mainly II-III) | No | 60  CRC | Giemsa method | Yes, high MCD with low OS | 0.0013 |
| Gulubova *et al*[50], 2009 | All TNM stages  (mainly II) | No | 106  CRC | Immunohistochemistry  primay 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.