Review

New insight into Intestinal Mast Cells revealed by Single-Cell RNAseq

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Review

New Insight into Intestinal Mast Cells Revealed by Single-Cell RNAseq

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Abstract: Mast cells are tissue-resident immune cells distributed in all tissues and strategically located close to blood and lymphatic vessels and nerves. Thanks to the expression of a wide array of receptors, mast cells act as tissue sentinels, able to detect the presence of bacteria and parasites and to respond to different environmental stimuli. Mast cells originate from bone marrow progenitors that enter the circulation and mature in peripheral organs under the influence of microenvironment factors, thus differentiating in heterogeneous tissue-specific subsets. Even though mast cell activation has been traditionally linked to IgE-mediated allergic reactions, a role for these cells in other pathological conditions including tumor progression has recently emerged. However, several aspects of mast cell biology remain to be clarified. The advent of single-cell RNA sequencing platforms has provided the opportunity to understand mast cell origin and differentiation as well as the phenotype and functions within different tissues, including the gut. This review recapitulates how single cell transcriptomic studies provided insight into mast cell development as well as into the functional role of intestinal MC subsets in health and disease.

Keywords: intestinal mast cells; gut inflammation; colorectal cancer

1. Introduction

Mast cells (MCs) arise from bone marrow (BM) progenitors that enter the circulation and mature in peripheral tissues under the influence of microenvironment factors [1–3].

Mature MCs are tissue-resident innate immune cells that are present in all organs, particularly in skin, in lung and intestinal mucosa and are distributed close to blood and lymphatic vessels and nerves. Thanks to their strategical localization and to the expression of a wide array of receptors, mature MCs act as tissue sentinels, able to firstly detect the presence of bacteria and parasites and to respond to different microenvironmental stimuli [4–7].

Their functions are mediated by the secretion of a vast array of biologically active molecules, including histamine and proteases that are stored in secretory granules and immediately released upon activation [8,9]. A plethora of newly synthesized lipid inflammatory mediators are secreted within hours [9]. Moreover, by releasing various cytokines and chemokines, MCs orchestrate the recruitment and activation of immune cells to the site of infection and regulate innate and adaptive immunity [10].

Among the main surface receptors, mature MCs are characterized by the expression of c-Kit (CD117) that upon interaction with its ligand (stem cell factor, SCF) regulates MC migration and activation [11], and the high affinity receptor for immunoglobulin E (FcεRI) that orchestrates the IgE-mediated allergic reactions [12]. Indeed, cross-linking of FcεRI-bound IgE by multivalent antigens results in the release of granule-stored mediators such as histamine, accompanied by the generation of newly synthetized soluble mediators [12–14] and high quantities of extracellular vesicles, emerging as important players in intercellular communication [15,16].

Similarly, activation by anaphylatoxins or neuropeptides including substance P, results in degranulation of preformed mediators and de-novo synthesis of chemokines/cytokines [17,18].

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However, MCs also express a wide range of receptors that are pivotal in the host's defense against pathogens, such as Toll-like receptors [7,19]. More recently, a selective expression of human mas-related G protein-coupled receptor X2 (MRGPRX2) and its mouse homologue, Mrgprb2, have been also reported [20]. This receptor can promote IgE-independent pseudo-allergic reactions by binding an array of host and microbial peptides, often generated from proteolytic cleavage of inactive precursors solely in inflamed tissue [20].

Thus, MC activation has been linked not only to allergy but also to other inflammatory conditions within different tissues, including the gut where a cross-talk between MCs and nerves can also provide a neuroimmune network necessary to control local responses [21,22].

Of note, the presence of MCs has also been reported in several solid cancers accompanied by MC ability to shape tumor microenvironment [23,24]. However, MCs can both orchestrate anti-tumoral responses, promoting the recruitment of other immune cells, or tumor progression favoring angiogenesis, lymphoangiogenesis, fibrosis and metastasis [23–25].

More recently, several aspects of MC biology have been solved thanks to the development of single-cell transcriptomic profiling technologies [26–28], as depicted in Figure 1.

This novel approach was able to differentiate MCs from other immune cells, including basophils and eosinophils, and to reveal a unique mouse and human MC identity [26–28]. Moreover, the presence of distinct MC subsets into different connective tissues has been elucidated [29,30], revealing a high degree of MC heterogeneity [31,32].

However, MC phenotype and functions between and within different organs remain to be clarified. Moreover, how MC plasticity is shaped in different physiological and pathological conditions is largely unexplored.

This review recapitulates data obtained from recent single-cell-based studies mainly focusing on intestinal MC subsets and their roles in health and disease.

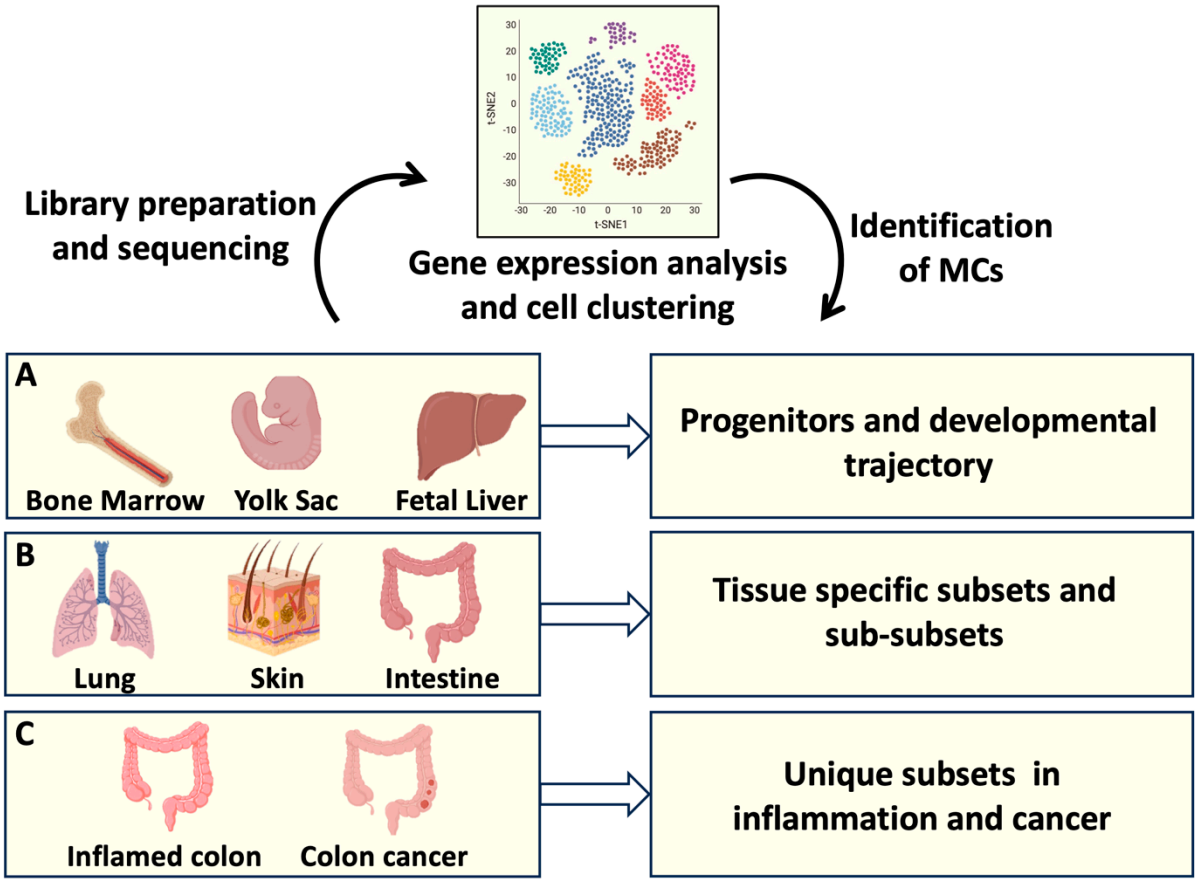

Figure 1. MC origin and tissue heterogeneity analysed by Single cell RNAseq. scRNAseq offers the possibility to identify the transcriptomic profiles of several cells from a tissue of interest. Transcripts

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associated to individual cells are sequenced and analysed, resulting in cell clustering based on gene expression. **A**: Identification of MC progenitors in bone marrow, fetal liver and Yolk sac has defined MC developmental trajectories; **B**: Gene expression profiles of MCs resident in different organs has clarified MC tissue heterogeneity; **C**: Transcript analysis of intestinal MCs has provided information on MC phenotypical and functional plasticity in health and disease. Created using BioRender.com.

2. Transcriptomic Analysis and MC Development

A first transcriptomic study on MC differentiation by Saito and coauthors has been performed using progenitors derived from human umbilical cord blood and adult peripheral blood and has revealed a series of MC-specific genes, including *TPSAB1/2* (tryptase α1 and β1), *HDC* (L-histidine decarboxylase), and *CPA3* (carboxypeptidase A) [33].

More recently, a single-cell transcriptomic analysis revealed the identity of human MC progenitors showing a temporal association between the appearance of FcεRI and the MC signature in hematopoietic progenitors isolated from adult peripheral blood [34].

Regarding the existence of a common progenitor between basophils and MCs, a first study integrating flow cytometric and transcriptomic data has been performed on primary BM-derived hematopoietic stem cells showing the presence of a cluster of cells expressing a set of common signature genes between basophils, eosinophils and MCs [35]. Similarly, a single-cell RNA sequencing of progenitors from human cord blood identified an intermediate stage progenitor that co-expresses gene modules of basophil, eosinophil and MC lineages [36].

Notably, erythro-myeloid progenitors were found also in yolk sac, suggesting that, as happens in mice, human MC arise from multiple compartments during and after embryogenesis [37].

More recently, by analysing single-cell dataset of human BM, Hamey and coauthors have provided a road map of MC and basophil development supporting the existence of a common progenitor until a bifurcation into the two specific cell lines [38].

However, a transcriptome analysis of mature human skin MCs in relation to other mature cell lineages, demonstrated a unique MC transcriptional landscape delineating a limited relation between MCs and basophils [39]. Moreover, the low mRNA levels in mature human basophils make it difficult to characterize the basophils' transcriptional profile in-depth.

Thus, although human basophils and MCs express common marker genes (e.g., HDC and FcεRI encoding genes), further studies are needed to explore in-depth the transcriptional differences between them in order to better discriminate their developmental trajectories.

In regard to similarities between distinct lineages, recent human and murine studies have suggested the existence of a hematopoietic progenitor with MC-erythrocyte potential [36,37,40,41]. However, the contributions of these progenitors to the resolution of infection-induced inflammation remain only poorly defined [42], as further discussed in paragraph 4.

3. Insights into Intestinal MC Origin and Phenotype through scRNA-Seq

A tissue compartment in which MCs are particularly abundant is the gut. Intestinal MCs are involved in the maintenance of tissue homeostasis and at the same time act as sentinels of the host defense against different pathogens, orchestrating inflammation [21,43].

In the mouse, the small intestine represents a large reservoir of MC-committed progenitors (MCps) that are recruited by a mechanism that involves α4β7 integrin and the CXC chemokine receptor-2 (CXCR2) [44]. As in all organs, critical signals for homing and recruitment of MCps are also provided by SCF binding to c-Kit [3]. Thanks to the role of c-Kit in MC maturation, migration and proliferation [11], murine models with spontaneous mutations in White spotting locus coding for c-Kit, have been used to identify and understand the contribution of MCs in several biological processes [45].

The study performed by Hamey and coauthors [38], offers valuable insights into the intricate process of MC differentiation, shedding light on the nuances of gene regulation during maturation. Focusing on peritoneal MC, they observed that MC differentiation/maturation is characterized by the downregulation of β7 integrin, as well as the protease genes *Mcpt8* and *Gzmb* (Granzyme B). Of

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note, they also reported the upregulation of MC specific protease genes including *Cpa3, Cma1, Mcpt4, Tpsb2 and Tpsab1* also revealing that their induction occurs in distinct temporal stages (*Cpa3* first, followed by *Tpsb2*, and finally *Tpsab1*) [38].

Mature MCs in the intestine are heterogeneous and comprise two main subsets that differ for localization and protease content [46]. In rodents, MCs are divided in mucosal MCs (MMC) present into the intestinal lamina propria close to the epithelium and positive for Mctp1 and Mcpt2 proteases, and connective-tissue MCs (CTMCs) that reside in gut submucosa and are characterized by the expression of proteases Mcpt4-7 and carboxypeptidase A3 (Cpa3) as well as a higher amount of histamine and heparin compared to MMCs [47].

In humans, mucosal MCs present in lamina propria contain only tryptase in their granules (MC_T), while MCs that predominate in the intestinal submucosa contain tryptase, chymase, the metallopeptidase CPA3, and cathepsin G (MC_{TC}) [48,49]. MCs that exclusively express chymase have also been identified as a rare population that resides in both lamina propria and submucosa [50]. Similarly, an intraepithelial MMC subpopulation has also been described in mice [51]. However, the role of these rare MC populations is still unclear.

Recent advancements in RNAseq profiling technologies and fate mapping revealed different developmental origins between the two main MC populations in mice: MMCs originate from fetal hematopoietic stem cells and depend on adult stem cells for their replacement, while CTMCs originate from yolk sac and can self-maintain independently from BM derived stem cells [41]. A similar conclusion came from the study by Gentek and coauthors revealing that CTMC are maintained independently of adult hematopoietic stem cells [52].

Regarding human MCs, transcriptomic profile obtained by scRNAseq analysis revealed that MCs are characterized by different transcriptomic signatures in diverse organs [53]. In gut, MCs express specific transcripts such as Vascular Endothelial Growth Factor A (*VEGFA*), the cytoskeleton component utrophin (*UTRN*), the chemokine receptor *CXCR4*, the aryl hydrocarbon receptor (*AHR*), and the interleukin 1 receptor associated kinase 3 (*IRAK3*). However, this signature is not a unique characteristic of human intestinal MCs but is shared by MCs resident in bladder, lymph nodes, skeletal muscle, trachea and tongue [53].

Furthermore, by integrating different datasets from Mouse cell Atlas derived from different tissues, Tauber and co-author demonstrated that CTMCs and MMCs are characterized by diverse gene signature across organs. In gastrointestinal tract MMCs, besides mucosal *Mcpt1* and *Mcpt2* protease genes, are characterized by a high expression of genes encoding adhesion molecules (*Itgae, Itga2a, Ly6e*) and the chemokine receptor *Cxcr1*, while CTMCs are enriched in *Cma1, Mcpt4, Tpsb2* and *Cpa3* protease genes, *Ccl2* chemokine gene, lipid metabolism genes (*Apoe*) together with the expression of *Mgbrb2* gene [53]. These results are in line with previous studies showing that Mrgprb2 and the human ortholog MRGPRX2 are exclusively expressed on connective tissue-like MCs [47,54].

The origin of the two subsets was further explored comparing mice at different ages. CTMCs positive for Mrgprb2 were found in both neonatal pups and adults, while $Mrgprb2^-$ $Mcpt1^+$ MMCs were exclusively detected in adult mice, suggesting that $Mrgprb2^+$ CTMCs originate embryonically, whereas $Mrgprb2^-$ MMCs after birth. Moreover, the use of BM chimeras confirmed that the $Mrgprb2^-$ MMCs are continuously renewed from BM progenitors, while the $Mrgprb2^+$ CTMC population appears to be independent of BM-derived cells for turnover not only in the gut but also in the skin and peritoneal cavity [53]. Of note, CTMCs isolated by different organs showed a high degree of heterogeneity [26], definitively demonstrating a microenvironment-dependent MC differentiation and suggesting that tissue-specific MC subsets exist beyond the traditional MMC/CTMC classification.

4. Deciphering Intestinal MC Function in Homeostasis and Inflammatory Conditions

Intestinal MCs contribute to homeostasis controlling physiological processes such as mucosal integrity and epithelial barrier activity [43,55]. Indeed, mice deficient in MCs or Mcpt4 protease have

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a reduced small intestinal permeability and altered epithelial cell migration as well as intestinal morphology and tight junctions [55].

The crucial role of MCs in epithelial integrity is confirmed by their involvement in intestinal inflammatory conditions but also in food allergy and nematode infections (Figure 2).

During parasite infections, including *Trichinella spiralis* and *Trichuris muris*, MMCs are the main subset that increases in number due to a shift from a connective tissue-like phenotype to a mucosal phenotype characterized by the expression of the proteases Mcpt1 and Mcpt2.

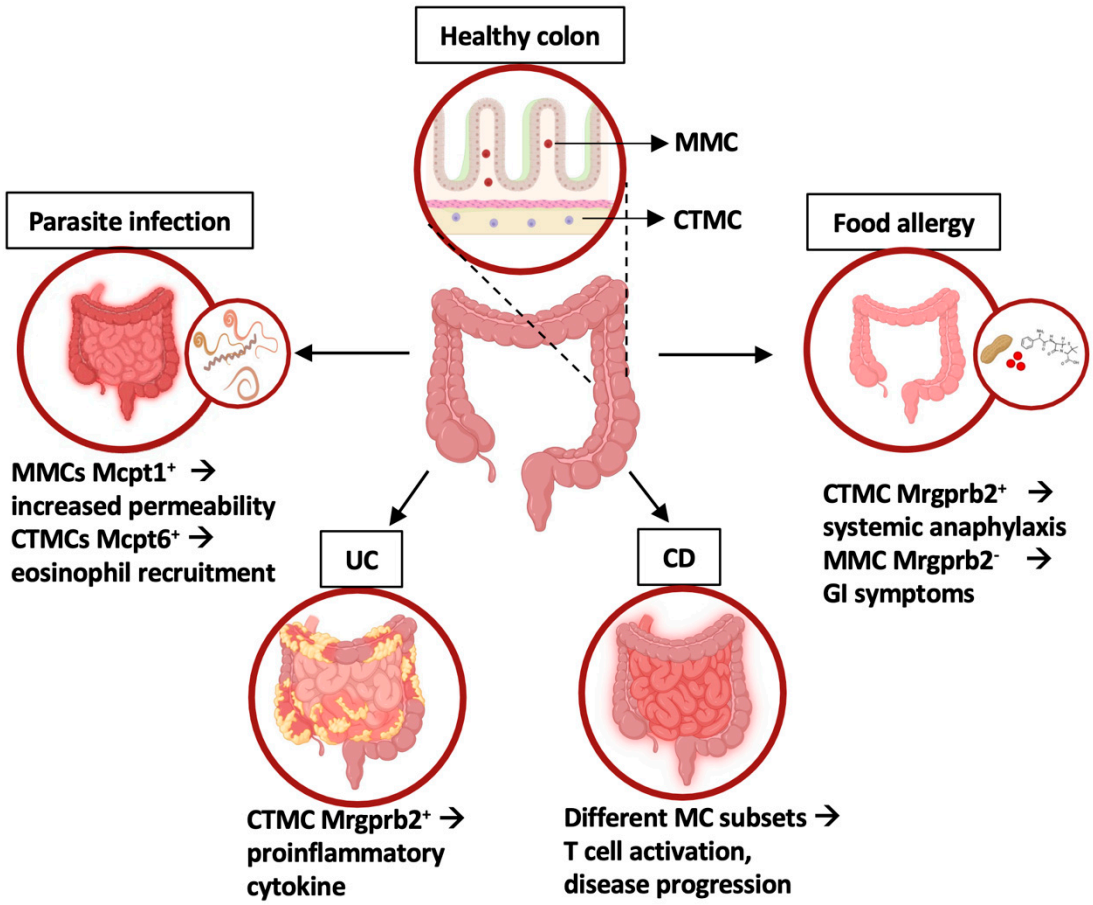

Figure 2. Intestinal mast cell phenotype and functions in homeostasis and inflammatory conditions. In healthy colon, two MC subsets have been identified: mucosal MC (MMC) and connective tissue-like MC (CTMC). In disease states, distinct MC subsets with unique gene expression profiles contribute to the intestinal inflammation, as highlighted by different transcriptomic approaches. UC: Ulcerative Colitis; CD: celiac disease. Created using BioRender.com.

In particular, Mcpt1 appeared to be responsible for the degradation of occludin, thus increasing intestinal permeability and facilitating worm expulsion [56–58]. On the other hand, the connective tissue MC-specific tryptase Mcpt6 was shown to be required for eosinophil recruitment and the eradication of *T. spiralis* [59].

Notably, by single cell RNA-sequencing Inclan-Rico and coauthors demonstrated that infection by *T. spiralis* induces the recruitment into the intestine of a hematopoietic progenitor with dual MC-erythrocyte potential [42], likely contributing to eradicate the infection and to alleviate blood loss.

Besides parasite infections, intestinal MCs are involved in IgE-mediated response to food antigens contributing to both local and systemic development of food allergy.

An increase in MC number, mainly due to the expansion of intestinal MMCs, has been demonstrated both in humans and mice sensitized by food allergens [60,61], and correlates with the

 doi:10.20944/preprints202404.0537.v1

severity of symptoms. However, using two common models of IgE-mediated food allergy, Benedé and Berin demonstrated that systemic anaphylaxis was uniquely associated with activation of connective tissue-like MCs, while gastrointestinal manifestations of food allergy were associated with an increase of Mcpt1-expressing MCs together with a clear activation of both mucosal and connective tissue-like MCs [62]. More recently, Tauber and co-authors confirmed these findings demonstrating that depletion of $Mrgprb2^{+}$ CTMC subset protects murine models from anaphylactic shock, while $Mrgprb2^{-}$ MMCs in gut is not implicated in anaphylaxis, despite being the first population to encounter the allergen [53].

MC ability to rapidly sense and adapt to specific triggers including neuropeptides can explain the activated MC phenotype described in different human gastrointestinal disorders such as celiac disease, irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [22]. IBD are complex multifactorial diseases of the gastrointestinal tract, including Ulcerative Colitis (UC), triggered by environmental factors in genetically susceptible individuals [22,63]. Current therapies based on the use of monoclonal antibodies directed against cytokines offer amelioration and prolonged period of remission but have important limitations. Indeed, more than 30% of patients do not initially respond to therapy while others lose response over time [64,65]. Thus, new treatment strategies are needed.

Several studies have reported MC accumulation in patients affected by celiac disease (CD) and UC, but their contribution in disease progression remained unclear.

In this context, Atlasy and co-authors compared transcriptomic profile of immune infiltrate isolated from small intestine of patients affected by active CD. They found four MC subsets differentially enriched in healthy and affected intestine: MCs more abundant in control patients show a profile associated with 'humoral immune response' and 'positive regulation of B cell activation' biological processes, while MC clusters accumulated in active disease display a transcriptomic profile associated to 'protein to ER process', 'antigen processing and presentation' and 'positive regulation of T cell-mediated cytotoxicity' processes [66], suggesting their active role in disease progression.

Similarly, Smillie and coauthors focused on colonic tissues from UC patients and healthy donors and using single-cell RNA sequencing mapped different cell circuits. They identified 51 cell subsets (epithelial, stromal, and immune cells) revealing an increase of inflammatory-associated genes in UC patients compared with healthy volunteers. Of note, together with cytotoxic and regulatory T cells a selective MC subset expressing the activation marker CD69 increase in inflamed tissues [67]. However, this MC subset was not further characterized in term of protease content.

More recently, Chen and coauthors compared acutely inflamed and uninflamed UC tissue to establish the requirement of MRGPRX2-specific MC activation in inflamed colonic tissues [68]. Using both bulk RNASeq and scRNASeq, they reported a key role for adrenomedullin ADM (ADM) and its proteolytic product, PAMP-12, in perpetuating UC inflammation. Moreover, by single-cell RNA sequencing they were also able to show that both activated fibroblasts and epithelial cells express adrenomedullin and that interferon γ is a key upstream regulator of MC gene expression [68], thus defining a new potential therapeutic target.

5. Exploring Intestinal Mast Cell Role in Tumor Biology: Colon Cancer under RNA Seq Microscope

MC physiologic function in tumor biology has raised particular interest for decades since these cells potentially influence different aspects of tumorigenesis including tumor angiogenesis, invasiveness and immunosuppression [69–71]. However, the MC contributions in cancer initiation and progression remain controversial. Indeed, several studies have demonstrated both positive and negative correlation of MCs in the development of different types of cancers [72], including colorectal cancer (CRC).

CRC is the third most common type of malignancy that affects the colon or rectum [73]. The majority of CRC cases emerge sporadically, 20% present a familial history and 5% are attributed to inherited syndromes such as Familial Adenomatous Polyposis and Lynch syndrome [74,75].

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Moreover, lifestyle as well as chronic inflammation in patients with IBD all represent independent risk factors for CRC development [76].

The tumor microenvironment (TME) and the interactions between cancer cells and surrounding stromal cells, also influences CRC development [77].

The density of tumor-infiltrating cytotoxic and memory T cells, which are associated with a better prognosis [78,79], define the "immunoscore" as an additional parameter to classify CRC. However, the knowledge about innate immune cell infiltration, including MCs, is limited [80–82]. Recent advancements in sequencing investigations have provided crucial opportunities to dissect the heterogeneity and functional role of MCs within the CRC microenvironment and adjacent normal tissue.

By examining the transcriptomic profile among wild-type (WT) mice, MC-deficient mice $Kit^{W\text{-}sh}$), and $Kit^{W\text{-}sh}$ mice engrafted with MCs derived from WT mice, Ko and coauthors identified several genes downregulated in MC-deficient mice but recovered by MC engraftment that were named "mast cell–dependent genes" [83].

These genes were found associated to pathways related to cancer progression including immunosuppression, apoptosis and angiogenesis. Interestingly, these pathways are enriched in lung, breast, and colon cancer compared to normal tissues, supporting a pro-angiogenic and anti-apoptotic role for MCs in tumor microenvironment. Moreover, gene associated to lymphocyte cytotoxicity are upregulated in the absence of MCs, suggesting that these cells promote immunosuppression [83]. These results support *in vitro* and *in vivo* evidence demonstrating a role for tumor-infiltrating MCs in favoring a suppressive microenvironment and in promoting tumor growth [84–89]. In particular, a role for SCF in favoring tumor progression has been envisaged: SCF-activated MCs can release adenosine to directly suppress T cells and NK cells and can contribute to the production of pro-inflammatory cytokines [88,89].

By a RNAseq approach, Sakita and co-authors showed that MC role in colon cancer development and progression is multifaceted and context-dependent [88]. Indeed, in a model of spontaneous CRC, MC deficiency promoted tumor development while in colitis-dependent CRC the absence of MCs reduced tumor burden and increased the frequency of tumor-infiltrating $CD8^+$ T cells [88]. Bulk RNAseq analysis of colitis-dependent tumor masses showed that MC deficiency upregulated the cytokine-cytokine receptor interaction pathway further supporting a role for MCs in suppressing immune responses during tumorigenesis [88].

Thus, a characterization of murine MC infiltrating CRC and their role in tumor progression is still unavailable. Moreover, whether different MC subsets may play an anti-tumorigenic or pro-tumorigenic role in different stages of the disease is still unknown.

Using a murine colitis-dependent model of tumorigenesis we have recently demonstrated that tumor masses are enriched of CTMCs with an activated phenotype [89]. However, a single cell RNAseq approach is necessary to better define tumor-infiltrating MC subsets in different murine models of CRC.

Regarding human CRC, several groups have profiled immune and non-immune cells isolated from tumoral lesions [90–102]. MCs were identified based on a unique set of genes including those coding for the receptor c-Kit (*KIT*), the chymase (*CMA1*), the carboxipeptidase (*CPA3*), and tryptase (*TPSAB1, TPSB2*), and compose one of the most represented cell types present in the TME [90–102].

However, there is still inconsistency regarding the frequency of MCs in transformed and not transformed tissue and their pro/anti-tumor activity (Table 1).

Regarding MC frequency, by employing single cell RNAseq approach two different research groups demonstrated a comparable MC enrichment in both tumors and normal mucosa [91,92], while single cell analysis of tumor infiltrating immune cells from different kind of cancers demonstrated accumulation of MCs in several tumors, including CRC, compared to non-tumoral adjacent tissue [95]. Furthermore, a higher number of MCs was reported in advanced CRC stages [94] and in right-sided tumors compared to the left part of the colon [98].

On the other hand, a reduced number of MCs in tumor lesions in respect to matched unaffected tissue was observed by transcriptomic profiling of five non metastatic CRC patients [96] and in the

 doi:10.20944/preprints202404.0537.v1

bioinformatic analysis of three single cell RNAseq datasets of both tumor and tumor-free tissue [101].

Few data are currently available also regarding the role of MCs in human CRC (Table 1).

Indeed, the majority of single-cell RNAseq analysis have been mainly focused on tumor microenvironment (epithelial and stromal cells) or on macrophages and adaptive immune infiltrate.

Sakita and co-workers found a negative correlation in CRC between the number of activated MCs and infiltrating $CD8^+$ T lymphocytes, supporting a pro-tumoral role for MCs [88].

Cheng and coauthors performed a meta-analysis by combined previously published and newly generated sc-RNA-seq data set to compare transcriptomic signature associated to MCs infiltrating different cancer types [95].

Focusing on CRC patients, they found a down-modulation of *TNFA* transcript and an upregulation of *VEGFA* in respect to adjacent healthy tissue and suggest association of this signature with decreased patient survival. However, whether this signature is associated to a selective MC subset (mucosal vs connective) is not clear.

Table 1. MC characterization by scRNAseq analysis of human CRC samples.

Sample type	Cells	Method	MC frequency	MC function	Ref
CRC biopsies and adjacent tissues	Immune cells	10X Genomics Smart-seq2	Comparable frequency	n.d.	92
CRC biopsies (different stages) and adjacent tissues	Immune cells	Smart-seq2 DNBelab C4	Increased frequency in advanced stages	n.d.	94
CRC biopsies and adjacent tissues	Immune cells	10X Genomics Analysis of published datasets	Increased frequency in CRC	Protumoral activity	95
CRC biopsies and liver metastasis	Immune cells	10X Genomics Smart-seq2	Increased in metastasis	Protumoral activity	100
CRC biopsies and adjacent tissues	Immune cells	Analysis of published datasets	n.d.	Protumoral activity	88
CRC biopsies and adjacent tissues	Immune, epithelial and stromal cells	10X Genomics	Comparable frequency	n.d.	91
CRC biopsies and adjacent tissues	Immune, epithelial and stromal cells	10X Genomics	Reduced frequency in CRC	n.d.	96
CRC biopsies and adjacent tissues	Immune, epithelial and stromal cells	Analysis of published datasets	Reduced frequency in CRC	Antitumoral activity	101
CRC biopsies and adjacent tissues	Immune, epithelial and stromal cells	Analysis of published datasets	Increased frequency in CRC	Protumoral activity	102
CRC biopsies (LCC and RCC) and adjacent tissues	Immune, epithelial and stromal cells	10X Genomics	Increased frequency in RCC	n.d.	98
CRC biopsies (LCC and RCC) and adjacent tissues	Immune, epithelial and stromal cells	Analysis of published datasets	n.d.	Antitumoral activity	99

LCC- left-sided colon cancer; RCC-right-sided colon cancer

Focusing on CRC patients, they found a down-modulation of *TNFA* transcript and an upregulation of *VEGFA* in respect to adjacent healthy tissue and suggest association of this signature with decreased patient survival. However, whether this signature is associated to a selective MC subset (mucosal vs connective) is not clear.

The integrated analysis of different CRC datasets revealed with more details the potential function of tumor-infiltrating MCs [101].

 doi:10.20944/preprints202404.0537.v1

Based on the expression level of several markers, MCs were clustered into distinct subpopulations and their relative abundance was compared in tumor versus adjacent tissue.

In healthy control tissue, the most representing subset expresses high levels of the protease *CMA1* and upregulates the angiogenesis pathway. In TME, activated MCs express abundant amounts of *KIT* and FcεRI subunits (*FCER1A, FCER1G*, *MS4A2*), high levels of genes for Th2 cytokines, and transcripts for proteases and enzymes involved in histamine and lipid mediator synthesis [101]. However, these results are in apparent contrast with the MC signature reported by Cheng and coauthors [95].

All these discrepant results may depend on the different isolation procedures (whole tissue vs immune infiltrate) or by different protocols used to prepare the sequencing library. Moreover, special considerations should be taken into account when linking genomic data, for instance the heterogeneity of patients in terms of tumor stages. To this regard, a clear CRC stratification of patients in different tumor stages could help to understand whether during the onset of intestinal transformation and in later stages of CRC progression different MC subsets are involved. It could be also possible that in the transcriptomic analysis of whole tissue the expression of epithelial and stromal genes affects the relative abundance of MCs and their related genes.

Considering the very low frequency of MCs in the whole CRC immune infiltrate, single cell RNAseq analysis of sorted MCs could help to better discriminate different clusters and subclusters associated to CRC development and progression.

Regarding a potential interplay between MCs and other cells in TME, Wang and coauthors conducted cell-cell communication analysis mapping the expression of ligand-receptor pairs.

Their finding highlighted a possible MC interaction with B cells, epithelial cells and fibroblasts [102]. Of note, MC co-localization with fibroblasts and endothelial cells was also reported in the stromal region of CRC tissue by spatial transcriptomic analysis [101].

Thus, in future studies the exact localization of MCs within tumor tissue and their interaction with different cell types in CRC could be clarified by integrating single cell with spatial transcriptomic analysis.

6. Conclusions and Future Perspectives

MCs are innate immune cells distributed in all tissue and particularly abundant in the intestine where they play different roles in homeostasis as well as in inflammatory diseases. Moreover, the increase of MCs in different tumors including colonic tumors has been demonstrated in the last years. MCs are characterized by a vast heterogeneity among tissues and their phenotypical and functional plasticity allow them to respond to different environmental stimuli. However, whether distinct MC subsets are involved in intestinal diseases and their functions are poorly understood.

The advent of single-cell RNA sequencing platforms has provided a step forward in the understanding of many biological processes and in the definition of cell functions. Several aspects of MC origin and differentiation into peripheral tissues have been elucidated.

Even though MCs represent an abundant population in healthy intestines, their number appeared increased during inflammation. It could be interesting to clarify whether and how the recruitment of new progenitors contributes to expansion of MCs during inflammation. Moreover, the role of classical MMC and CTMC subsets in different inflammatory states including allergy to food antigens, parasite infections or autoinflammatory diseases is still poorly investigated. It is also largely unknown whether MC populations with unique phenotype and functions arise during inflammation.

In regard to MC role during colonic transformation, it is still unknown how tumor microenvironment shapes MC plasticity in term of phenotype and function and whether unique MC subset(s) differentiate in diverse stages of progression.

Finally, MCs are located near nerves and the bidirectional interaction of MCs with the enteric nervous system plays an important role in gastrointestinal inflammation. It could be interesting to investigate whether these interactions are also involved in tumor progression.

 doi:10.20944/preprints202404.0537.v1

Spatial transcriptomic combined with single cell RNA-seq could help to decipher MC cross-talk with nervous system as well as additional MC interactions in the tumor microenvironment and construct an immune landscape for CRC.

A better characterization of intestinal MCs at various stages of gut inflammation and tumorigenesis would help to define novel potential targets for a therapeutic intervention.

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