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CHARTING MAST CELL DEVELOPMENT IN HEALTH AND SYSTEMIC MASTOCYTOSIS

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By

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‘There is only one heroism in the world: to see the world as it is, and to love it.’

- Romain Rolland

ABSTRACT

Hematopoietic stem and progenitor cells give rise to various blood and immune cells, including mast cells. In adults, bone marrow hematopoietic stem cells give rise to mast cell progenitors. These mast cell progenitors circulate in the peripheral blood and mature in peripheral tissues. Mast cells are activated by the cross-linking of immunoglobulin E (IgE) immune complexes bound to the high-affinity receptors, FcεRI. This event causes the release of a series of bioactive substances involved in various physiological and pathological processes.

Mast cells play a key role in immunological homeostasis as essential immune cells. By contrast, mast cell dysfunction causes mast cell-related diseases. Systemic mastocytosis is a rare systemic disorder caused by abnormal accumulation of aberrant mast cells. However, in-depth studies of mast cell development in healthy individuals and in patients with mastocytosis are scarce. In the past decade, single-cell RNA sequencing technology has revolutionized the hematopoietic models, making it possible to analyze differentiation trajectories at single cell resolution. Leveraging single-cell RNA-sequencing and cell culture assays, this thesis aims to systematically explore mast cell development in healthy individuals and patients with systemic mastocytosis.

In study I, we examined the cell-forming capacity of FcεRI$^+$ progenitors in the bone marrow. In this study we found that CD203c distinguishes the erythroid and mast cell/basophil differentiation trajectories within the FcεRI$^+$ progenitors.

In study II, we analyzed the hematopoietic landscape in peripheral blood using single-cell RNA sequencing (scRNA-seq). Transcriptome analysis indicated that the genes encoding FcεRI were expressed at the hematopoietic progenitor cell stage and increased in cells that showed a mast cell gene expression signature. *In vitro* culture revealed the ability of the FcεRI$^+$ progenitor population to rapidly differentiate into mature mast cells. Together, the transcriptome and cell culture assays demonstrated that CD34$^+$ c-Kit$^+$ FcεRI$^+$ progenitors constitute mast cell progenitors (MCPs) in peripheral blood. Subsequent screening of cell surface receptors on the gene expression level and *in vitro* culture experiments identified novel regulators of MCPs. IL-3 and IL-5 promoted MCP survival. In addition, IL-3 showed a pro-proliferative effect on MCPs, whereas IL-5 did not. Interestingly, IL-33 significantly downregulated the expression of FcεRI on MCP, suggesting that FcεRI expression can be influenced by the extracellular environment.

In study III, we aimed to explore the hematopoietic landscape in the bone marrow of patients with systemic mastocytosis (SM). In this study, we utilized single-cell RNA-sequencing to analyze the hematopoietic landscape of c-Kit$^+$ hematopoietic progenitors isolated from bone marrow, with a focus on mast cells. Our results provide a comprehensive and in-depth analytical resource for research related to hematopoiesis in mastocytosis. Furthermore, using integrated single-cell transcriptome and immunophenotype data, we identified two distinct mast cell subpopulations with distinct expression of CD25, which is as diagnostic marker.

Further comparison of $CD25^+$ aberrant mast cells with $CD25^-$ mast cells identified a panel of disease-associated markers and revealed new potential pathogenic factors.

Altogether, this thesis provides a comprehensive map of mast cell development in healthy individuals and systemic mastocytosis, laying the foundation for further studies on mast cell development in physiological and pathological states.

LIST OF SCIENTIFIC PAPERS

I. Grootens J, Ungerstedt JS, **Wu Chenyan**, Hamberg Levedahl K, Nilsson G, Dahlin JS.
CD203c distinguishes the erythroid and mast cell-basophil differentiation trajectories among human FcεRI+ bone marrow progenitors.
Allergy. 2020 Jan;75(1):211-214.

II. **Wu Chenyan***, Boey D*, Bril O, Grootens J, Vijayabaskar MS, Sorini C, Ekoff M, Wilson NK, Ungerstedt JS, Nilsson G, Dahlin JS.
Single-cell transcriptomics reveals the identity and regulators of human mast cell progenitors.
Blood Advances. 2022 Aug 9;6(15):4439-4449.
* These authors contributed equally to this study.

III. Boey D*, **Wu Chenyan***, Mo J, Papavasileiou S, Ungerstedt JS, Nilsson G, Dahlin JS.
Single-cell transcriptional analyses uncovers the hematopoietic landscape in systemic mastocytosis.
Manuscript.
* These authors contributed equally to this study.

CONTENTS

LIST OF ABBREVIATIONS

7-AAD	7-aminoactinomycin D
Adv-SM	Advanced systemic mastocytosis
AGM	Aorta-gonado-mesonephros
AML	Acute myeloid leukemia
ASM	Aggressive systemic mastocytosis
BMM	Bone marrow mastocytosis
C5aR	Complement component 5a receptor 1
CITE-seq	Cellular indexing of transcriptomes and epitopes by sequencing
CM	Cutaneous mastocytosis
CMP	Common myeloid progenitor
CPA3	Carboxypeptidase A3
CTMCs	Connective tissue-type mast cells
DAPI	4',6-diamidino-2-phenylindole
DC	Dendritic cell
DCM	Diffuse cutaneous mastocytosis
EMH	Extramedullary hematopoiesis
EMPs	Erythro-myeloid progenitors
EPO	Erythropoietin
FACS	Fluorescent-activated cell sorting
FCS	Fetal calf serum
FcεRI	Fc epsilon receptor I
Flt3L	FMS-like tyrosine kinase 3 ligand
FMO	Fluorescence Minus One
GEMs	Gel Bead-In Emulsions
GIST	Gastrointestinal stromal tumor
GM-CSF	Granulocyte-macrophage colony stimulating factor
GMP	Granulocyte and macrophage progenitor
HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem and progenitor cell

IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
ILC	Innate lymphoid cell
ISM	Indolent systemic mastocytosis
LMPP	Lympho-myeloid primed progenitors
MACS	Magnetic-activated cell sorting
MCL	Mast cell leukemia
MCP	Mast cell progenitor
MEMP	Megakaryocyte-erythroid-mast cell progenitor
MGG	May-Grünwald Giemsa
MMCs	Mucosal-type mast cells
MPCM	Maculopapular cutaneous mastocytosis
MPP	Multipotent progenitor
NK cell	Natual killer cell
PBS	Phosphate-buffered saline
PCW	Postconceptional weeks
SCF	Stem cell factor
scRNA-seq	Single-cell RNA-sequencing
SM	Systemic mastocytosis
SM-AHN	systemic mastocytosis - associated hematologic neoplasm
SSM	Smoldering systemic mastocytosis
TPO	Thrombopoietin
TK	Tyrosine kinase
UMAP	Uniform Manifold Approximation and Projection
UP	Urticaria pigmentosa
WHO	World Health Organization

1 INTRODUCTION

The focus of this thesis is on the formation of mast cells under normal conditions and in systemic mastocytosis (SM). The introduction summarizes the current knowledge of mast cells, mast cell progenitors, and systemic mastocytosis. This provides the context for how the three constituent manuscripts of the thesis advance the research field. My research focuses on human subjects. Therefore, the introduction primarily focuses on research performed using human samples, which is complemented with the description of results from other species.

1.1 HEMATOPOIESIS

Hematopoiesis, the production of blood cells, begins at the embryonic stage and continues throughout the adult life. It is a complex biological process where uncommitted hematopoietic stem cells give rise to various lineage-committed progenitors that form mature blood cells. The continuous production and renewal of blood cells maintain normal physiological activities in everyday life. The production of blood cells is delicately regulated and can for example adapt to respond to specific stressful situations such as injury or inflammation.

During early human development, the embryonic hematopoietic process is divided into three stages, depending on the anatomical site: yolk sac, liver, and bone marrow. It begins in the yolk sac, and as the embryo and fetus develop, the fetal liver becomes the major hematopoietic organ, with the placenta and spleen also involved in hematopoiesis. During late fetal development and after birth, the bone marrow progressively takes over and becomes the main site in which blood cells form(1). By tracing the development of blood cell lineages across time and space, different developmental waves have been described (2). At four postconceptional weeks (PCW), studies characterizing the yolk sac cell population revealed the presence of hematopoietic stem cells (HSCs)-like progenitors, innate immune cell precursors such as macrophages, mast cells, natural killer (NK) cells, and innate lymphoid cells (ILCs), as well as megakaryocytes and erythrocyte progenitors (1, 3). Subsequently, monocyte and dendritic cell (DC) precursors and lymphocyte precursors appear successively in the fetal liver. Definitive hematopoietic stem cells in the fetal liver can form erythroid, megakaryocytic, myeloid and lymphoid lineages (3, 4). Granulocytes such as neutrophils appear with bone marrow hematopoiesis (1, 4, 5).

In postnatal hematopoiesis, hematopoietic stem cells (HSCs), which are mainly distributed in the bone marrow, differentiate into blood cells of various lineages. Rare hematopoietic stem and progenitor cells (HSPC) are also present outside the bone marrow, for example in the spleen, liver and peripheral blood (6, 7). Differentiation of HSPCs outside the bone marrow is called extramedullary hematopoiesis (EMH), mainly associated with hematopoietic stress such as anemia (8), pregnancy (9, 10), and chronic inflammation (11).

A tree-like model is traditionally used to describe the bifurcations of differentiating HSCs. The branching into myeloid and lymphoid progenitors constitute the first bifurcation. The myeloid progenitors branch into erythroid-megakaryocyte progenitors and granulocytes-monocyte progenitors. The lymphocyte lineages branch into the various lymphocytes such as T cells, B

cells and NK cells (Figure 1A). This traditional tree-like model was revised following the discovery of lymphocyte progenitors that also have the capacity to produce some myeloid cells such as monocytes and dendritic cells (12). In this revised tree-like model, lymphocytes, along with neutrophils, monocytes and DCs, originate from lympho-myeloid primed progenitors (LMPP), whereas erythrocytes, megakaryocytes and other myeloid cells such as eosinophils and basophils originate from common erythro-myeloid progenitors (EMP) (13, 14). This revised LMPP-EMP model suggests a shared progenitor of eosinophils and basophils, separate from neutrophil and the erythroid and megakaryocyte lineages (Figure 1B). Notably, mast cells are absent from the classic and the revised tree-like models.

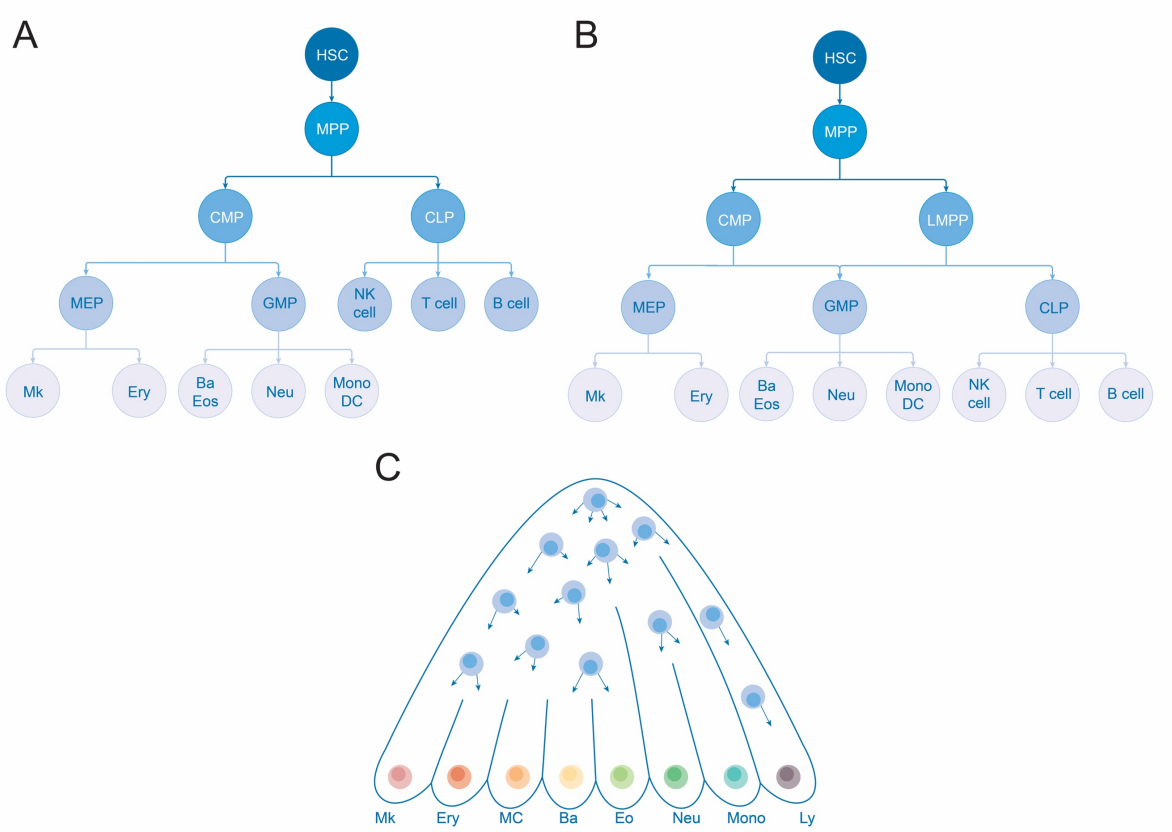

Figure 1. Three models describing hematopoiesis. A) traditional tree-like model, B) revised tree-like model, and C) landscape model proposed in recent years. HSC, hematopoietic stem cell; MPP, multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte-erythroid progenitor; GMP, granulocyte-monocyte progenitor; Mk, megakaryocyte; Ery, erythrocyte; Ba, basophil; Eos, eosinophil; Neu, neutrophil; Mono, monocyte; DC, dendritic cell; LMPP, lymphoid-primed multipotent progenitor; Ly, lymphocyte.

The accuracy of tree-like models has been challenged in recent years along with the development of single cell RNA-sequencing technologies. Several studies found that the development and cell fate determination of hematopoietic cells is a continuous process and a new landscape model of hematopoiesis has been introduced (15-20). In contrast to the traditional tree-like models that highly compartmentalize hematopoiesis into a hierarchical structure, the different progenitors in the continuous hematopoietic model are transient states containing heterogeneous populations of cells distributed according to certain pathways with a high degree of flexibility. In this model, hematopoietic progenitors differentiate through a

continuous landscape of states and progressively lose their potential to form multiple cell types (21). Notably, this model accommodates the mast cell differentiation trajectory (Figure 1C).

1.2 MAST CELLS AND THEIR DEVELOPMENT

1.2.1 An overview of mast cells

Mast cells are tissue-resident multifunctional cells involved in various physiological and pathological processes. They were first discovered in the connective tissue by Paul Ehrlich in 1877 based on their special metachromatically staining characteristics (22, 23). Mast cells are the major effector cells for type I hypersensitivity reactions, such as allergic rhinitis (24) and allergic asthma (25). Focal or systemic accumulation of mast cells with functional abnormalities can be caused by the primary clonal mast cell disease mastocytosis (26). In addition, mast cells are involved in a wide range of physiological roles, including host defense (27, 28), degradation of snake venom (29), wound healing (30), etc. Mature mast cells contain many cytoplasmic granules including biologically active substances, such as histamine, tryptase, and chymotrypsin (24, 31). Mast cells express a wide range of receptors, for example the IgE receptor FcεRI, IgG receptor (FcγRI and FcγRII), stem cell factor receptor (c-Kit), G protein-coupled receptor (MRGPRX2) and various cytokine receptors, which trigger relevant signaling pathways upon activation (32). Activation though receptor-ligand binding can cause mast cells to rapidly release preformed granule-associated substances and/or to start producing mediators that can be involved in various inflammatory responses (33, 34). One of the most well-described processes is IgE-dependent mast cell activation, which can cause local or systemic allergic reactions. In this process, allergens and allergen-specific IgE cross-link the high-affinity IgE receptor, FcεRI, on the cell surface, resulting in the release of various bioactive substances, e.g. histamine, pro-inflammatory cytokines and leukotrienes, leading to an inflammatory response (24, 35-37).

Mast cells constitute a functionally heterogeneous population. The cells are distributed in various tissues and organs of the body. Two distinct types of mast cells were first described in rats (38): connective tissue-type mast cells (CTMCs) and mucosal-type mast cells (MMCs). Each subtype has its own histological staining characteristics and protease expression profile. The presence of MMCs and CTMCs was later confirmed in mice (39, 40). MMCs were found mainly in the lung mucosa and small intestine mucosa. They are involved in adaptive inflammatory responses and are usually short-lived. CTMCs, on the other hand, are considered to be constitutive or innate long-lived cells located in connective tissue (41, 42).

In humans, the heterogeneity of mast cells is often classified according to the expression of proteases. There are mainly two distinct types. MC_{TC} located in the skin and intestinal submucosa contain both tryptase and chymase. MC_T found in the lung and small intestine mucosa only contain tryptase (43-45). The expression of surface receptors can also classify mast cells into different types, for example, complement component 5a receptor 1 (C5aR) is highly expressed in skin MC_{TC} but absent in lung MC_T (46). The mechanisms leading to mast cell heterogeneity have yet to be clarified.

Previous studies have found that culturing $CD34^+$ progenitors from bone marrow and cord blood can produce a homogeneous population of mast cells containing a full range of neutral granule proteases, including tryptase, chymase, carboxypeptidase A3 (CPA3), and cathepsin G (47, 48). It has been suggested that all types of mast cells derive from a common precursor in human, and that the peripheral tissue microenvironment may determine the mast cells phenotype (49). Studies of mouse embryos found that different subtypes of mast cells originate from temporally distinct waves of hematopoiesis. CTMCs are mainly derived from late erythro-myeloid progenitors (EMPs), and MMCs are mainly generated from HSCs (50). Whether the human MC_T and MC_{TC} population have distinct origins remains to be determined.

Recent single-cell transcriptome-based analyses have demonstrated the presence of heterogeneous mast cells within nasal polyp tissue. Unlike the traditional discrete subdivision into two mast cell subsets, the transcriptional profiles of mast cells are continuous, thus dividing them into polarized and transitional subpopulations (51). In agreement with this, another study using high-throughput flow cytometry analysis reported that the human lung mast cell population likely constitute a continuum of cells (52).

1.2.2 Mast cell development in embryonic hematopoiesis

Techniques such as fate mapping have allowed exploration of hematopoiesis and mast cell development in mouse. Due to ethical constrains, only limited tools are available to study human prenatal hematopoiesis. Available techniques include single-cell immunophenotyping and single-cell transcriptomics of aborted fetuses.

In mice, progenitors with mast cell forming potential first appear in the primitive hematopoiesis in the yolk sac. In an early study, Sonoda et al used a limiting dilution and clonal expansion-based method to identify mast cell precursors in the yolk sac of mouse embryos on embryonic day 9 (E9) (53). In a more recent study, a fate mapping-based method confirmed that mast cells can originate from early erythroid–myeloid progenitors (EMPs) in the yolk sac (54). However, late EMPs from yolk sac and hematopoietic stem cells generated in the aorta-gonad-mesonephros (AGM) migrate to the fetal liver and orchestrate transient definitive hematopoiesis (55, 56). The fetal liver becomes the main source of mast cells at this stage (53). Definitive hematopoiesis is initiated in late embryonic development, and hematopoietic stem cells in the fetal bone marrow become the main source of hematopoiesis and persist throughout life (56). Several studies using different mouse models have shown that early EMP-derived mast cells from the yolk sac are replaced by HSC-derived mast cells that originated in definitive hematopoiesis, while late EMP-derived mast cells become the main source of CTMCs, a long-lived cell that can sustained into adulthood (50, 54, 56).

Human embryonic hematopoiesis show similarities to that of mice, with several waves of hematopoiesis starting from the extra-embryonic yolk sac, followed by the transfer of AGM-generated HSCs to the fetal liver. Beginning in late embryonic development, the bone marrow becomes the primary site of hematopoiesis and continues throughout the life span (57). A series of recent comprehensive studies have used single-cell RNA sequencing techniques to map the

developmental landscape of blood and immune cells across the development of multiple organs, including the yolk sac, fetal liver, bone marrow, and peripheral tissues such as lymph nodes (3, 58-61). Evidence suggests that mast cells first appear during primitive hematopoiesis detected in the yolk sac, similar to the appearance of mast cells in mice. It was followed by the appearance of a shared megakaryocyte-erythroid-mast cell progenitor (MEMP) during the fetal liver-dominated hematopoietic stage (3, 58). However, whether the heterogeneity of mast cell phenotypes in adulthood is caused by different hematopoietic waves of mast cells, similar to the mouse fetus, requires further investigation.

1.2.3 Mast cell development in adult hematopoiesis

Kitamura Y et al observed that donor bone marrow gives rise to mast cells in irradiated mice following transplantation, providing the first evidence that hematopoietic stem and progenitor cells in bone marrow constitute a source of mast cells (62). Studies from the 1980s validated that bone marrow hematopoietic cells give rise to mast cells in mice using clonal assays (63, 64). The first identification of mast cell progenitors (MCPs) was done by Rodewald et al (65). This study isolated lineage-committed MCPs in mouse fetal blood (65). Subsequent studies reported committed MCPs in adult bone marrow (66, 67), peripheral blood (68), and peritoneal cavity (69). A consensus phenotype of Lin^- c-Kit^+ integrin $\beta7^+$ $ST2^+$ $CD16/32^+$ progenitors, with or without FcεRI expression, describes these committed MCPs.

Deeper insights into the mast cell trajectory relative to other cell lineages was provided in a landmark article by Arinobu et al, in which the authors identified a population of progenitors in the spleen that showed potential to form both mast cells and basophils. Even individual progenitors exhibited capacity to form colonies of basophils and mast cells, but no other cell type. These progenitors were therefore named bipotent basophil–mast cell progenitors (BMCPs) (70). Subsequent studies further identified bipotent basophils and mast cells progenitors in the bone marrow (71, 72) and peritoneal cavity (73). Interestingly, in a study of parasite-infected mice, individual progenitors that exhibited potential to form mast cells and erythrocytes was identified. This result suggests that the mast cell lineage is highly associated with the erythrocyte lineage development under inflammatory conditions (74). Another significant study examined mouse bone marrow hematopoiesis using single-cell transcriptome and cell fate analysis and discovered that mast cell-basophils were tightly related to the erythroid lineage (75). Notably, parallel existence of bipotent BMCPs and erythrocyte-mast cell progenitors is compatible with the landscape model of hematopoiesis, but not the tree-like models of hematopoiesis.

In human postnatal hematopoiesis, bone marrow is the main source of various blood cell lineages, including mast cells. However, the developmental trajectory of mast cells in the bone marrow and the existence of a lineage-committed mast cell progenitors has remained unclear. Early studies revealed the existence of bone marrow hematopoietic stem and progenitor cells that exhibited mast cell-forming capacity, first reported following *in vitro* culture analysis $CD34^+$ progenitors from bone marrow (76). The hematopoietic stem and progenitor cells' capacity to form mast cells was subsequently confirmed in a case of bone marrow

transplantation (77). The discovery of the CD203c surface marker has improved the accuracy of isolating $CD34^+$ progenitors primed for the mast cell lineage in bone marrow. Bühring et al found that $CD34^+$ $CD203c^+$ progenitors isolated from bone marrow are able to give rise to different lineages including both basophils and mast cells (78). Schernthaner et al tracked the expression of cell surface markers on mast cell precursors cultured *in vitro* (79). They found that CD203c was highly expressed in early stages of development and subsequently decreased. In addition, Kirshenbaum et al reported that $CD34^+$ c-Kit^+ $CD13^+$ progenitors from the bone marrow give rise to mast cell and/or monocyte colonies (80). In a recent study, bone marrow derived Lin^- $CD34^{hi}$ c-Kit^+ $Fc\varepsilon RI^+$ progenitors gave rise to multiple cell lineages including mast cell-like cells following *in vitro* culture, indicating that mast cell progenitors are potentially found in this cell compartment (81).

Human umbilical cord blood provides an accessible non-invasive source of cells for studying neonatal mast cell development. Both mononuclear cells and $CD34^+$ progenitors isolated from umbilical cord blood are commonly used as starting material to produce mast cells *in vitro* (82, 83). Detailed analysis using clonal assay analysis has showed that mast cell precursors are enriched in $CD34^+$ $CD38^+$ $HLA\text{-}DR^-$ progenitors from cord blood (84). A recent study in which single-cell transcriptomic analysis of cord blood progenitors was performed, found that Basophil/Eosinophil/Mast cell progenitors share a similar gene expression profile compared with early erythroid progenitor (19, 85). The results may indicate that mast cell and erythrocytes share a common progenitor in fetal hematopoiesis (3), suggesting that the basophil, eosinophil and mast cell developmental trajectories are closely related to the erythroid in early human development.

Circulating mast cell precursors migrate to the peripheral tissues where they mature. Mature mast cells are virtually absent in circulation in healthy state, whereas progenitors with mast cell-forming capacity are detectable in peripheral blood (86, 87). Valent et al demonstrated that peripheral blood mononuclear cells give rise to mast cells following *in vitro* culture with SCF (87). A subsequent study showed that mast cells are derived from Lin^- $CD34^+$ c-Kit^+ progenitors in peripheral blood (88). Sorting of the specific $CD13^+$ cell subset of $CD34^+$ c-Kit^+ progenitors further enriches for mast cell-forming potential (80). Rottem et al showed that $CD34^+$ $Fc\varepsilon RI^-$ progenitors in peripheral blood in patients with systemic mastocytosis produce mast cells (89). FcεRI is generally considered to be absent from mast cell precursors, and the upregulation of FcεRI is often considered a marker of terminal maturation (23, 90, 91). The absence of FcεRI expression on mast cell progenitors has been challenged by a study suggesting that a rare progenitor population characterized as Lin^- $CD34^+$ $CD117^{int/hi}$ $Fc\varepsilon RI^+$ cells constitutes mast cell progenitors in blood (92). This study demonstrates that Lin^- $CD34^+$ $CD117^{int/hi}$ $Fc\varepsilon RI^+$ progenitors in adult peripheral blood exhibit potential to form $CD117^+$ $Fc\varepsilon RI^+$ granulated mast cell-like cells (92).

In recent years, transcriptome analysis has contributed to an increased understanding of mast cell development. Saito et al performed transcriptome analysis of mast cells derived from human umbilical cord blood and peripheral blood, revealing a series of mast cell-specific genes,

e.g. *TPSAB1* (tryptase α1 and β1), *HDC* (*L*-histidine decarboxylase), *CTSG* (cathepsin G), and *CPA3* (carboxypeptidase A) (93). Motakis et al reported a comprehensive view of the transcriptome of mature skin mast cells in relation to other mature cell lineages, indicating limited relation between mast cell and other cell lineages including basophils (94). Another study reported that basophils and eosinophils originate from a common erythromyeloid progenitor, characterized as $CD34^+$ $CD133^{low/-}$, which is distinct from granulocyte and macrophage progenitors (GMPs) (95). However, the mast cell lineage was not analyzed in this study.

Transcriptome analysis at the single-cell level has potential to provide deeper insights into the differentiation process of hematopoietic progenitors. Pellin et al profiled the transcriptome of $linage^-$ $CD34^+$ hematopoietic progenitors from adult bone marrow in a single-cell level, obtaining a snapshot of the hematopoietic landscape revealing that basophil differentiation is more closely related to megakaryo–erythroid lineage than to the granulocyte and macrophage lineages (96). However, no mast cell differentiation trajectory was observed. Another study performed single-cell transcriptome analysis of primary bone marrow hematopoietic stem and progenitor cells to delineate the development path of multiple blood cell lineages, including one cluster expressing *CLC* and *HDC*, which are signature genes of basophil and/or mast cell lineages (97). However, whether this cell cluster exhibited mast cell-forming potential was not investigated. Drissen et al utilized single-cell transcriptome analysis and demonstrated that differential expression of CD131 and CD114 distinguishes the Basophil/Eosinophil/Mast cell lineage from the Neutrophil/Monocyte lineage within common myeloid progenitors and that Basophil/Eosinophil/Mast cell precursors and Erythroid/Megakarocyte progenitors coexist within the $CD131^+$ CMP population (98). However, the association between the mast cell and erythroid and megakaryocyte lineages is still unclear.

Studies performing transcriptional analysis collectively indicate an association between basophils, eosinophils, and mast cells, perhaps suggesting the existence of a common developmental precursor. Another interpretation is that lack of reliable signature genes for basophil and mast cell annotation makes the developmental trajectories indistinguishable. Functioning as the main granulocytes involved in type 2 inflammation, basophils and mast cells show similar transcriptome profiles by expressing common marker genes, e.g. *HDC*, FcεRI encoding genes, and *ENPP3*. The low mRNA levels in mature human basophils make it difficult to characterize the basophils' transcriptional profile in-depth (99). A comprehensive study analyzing transcriptional profile of basophils and mast cells in mouse, revealed their distinct transcriptional features (100). Similar studies are needed to explore in depth into the transcriptional differences between human basophils and mast cells, to better annotate and discriminate them.

1.2.4 Fc epsilon receptor I (FcεRI) in mast cell development

Fc epsilon receptor I, which is the high-affinity receptor of IgE, is expressed on human mast cells and basophils as a complex consisting of an α-chain, a β-chain and two γ-chains. Another type of FcεRI composed of an α-chain and two γ-chains can be found on other cells in human,

for example, monocytes, dendritic cells, eosinophils, neutrophils, and megakaryocyte precursors (101-105). Allergen, binding to allergen specific IgE, can cause cross-linking of FcεRI, which in turn leads to mast cell activation. This event results in allergic reactions and inflammation (24).

Even though FcεRI expression is a signature marker for mast cells, the stage during mast cell development that FcεRI is expressed remains controversial. Rodewald et al reported a population of committed mast cells progenitors in fetal mouse blood that lacks FcεRI (65). Later, Chen et al identified committed MCPs in adult mice as Lin^- c-Kit^+ $Sca\text{-}1^-$ $FcεRI^-$ $CD27^-$ $b7^+$ $T1/ST2^+$ in mice (66). Absence of FcεRI on committed mast cell progenitors was also reported in another study published the same year (106). Thus, $FcεRI^-$ hematopoietic progenitors may indeed include committed mast cell progenitors. However, mast cell progenitors that express FcεRI have been reported in mouse blood (107) and intestine (108). In addition, BMCPs – that is cells preceding the committed mast cell progenitor stage – can express FcεRI (71, 73).

In human, Rottem et al first discovered that mast cells can derive from $CD34^+$ $FcεRI^-$ progenitors isolated from peripheral blood. This study involved both healthy donors and mastocytosis patients (89). It is generally accepted that FcεRI expression is absent in mast cell precursors but intermediate to high upon maturation (80, 87, 91, 109). However, Dahlin et al isolated a rare cell population characterized as Lin^- $CD34^+$ $CD117^{int/hi}$ $FcεRI^+$ cells in peripheral blood, which give rise to $CD117^+$ $FcεRI^+$ mast cell-like cells after culture (92). Thus, there are conflicting reports on when during FcεRI appears during mast cell differentiation (Figure 2), both in human and mouse.

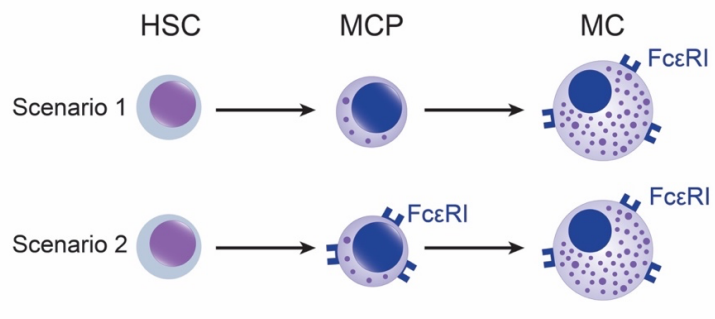

Figure 2. Schematic plot showing two scenarios of mast cell differentiation. Scenario 1 demonstrates the widely accepted view that FcεRI is absent on the MCP and expressed at terminal differentiation. Scenario 2 exhibits the new perspective that FcεRI arises at the MCP stage.

1.3 CYTOKINES REGULATION IN MAST CELL DEVELOPMENT

Mast cells express multiple cytokine receptors, and several cytokines can influence the cells' developmental processes by targeting the corresponding cytokine receptor. The discovery of human stem cell factor (SCF), the ligand of KIT, is a milestone for mast cell research. SCF stimulates all stages of mast cell differentiation, including the maturation. The production of recombinant SCF has therefore enabled *in vitro* generation of mast cells in large numbers (87, 110-113). The powerful effects of SCF on mast cell differentiation made the research field

conclude that SCF is an indispensable factor for *in vitro* culture of mast cells (114, 115). However, the absolute requirement of SCF for promoting all stages of mast cell development has recently been challenged. Dahlin et al demonstrated that IL-3 and IL-6 are sufficient to promote mast cell progenitor proliferation and the formation of granulated pre-mast cells (116).

In mice, *in vitro* culture of bone marrow hematopoietic stem and progenitor cells revealed that multiple interleukins (ILs) including IL-3, IL-9, and IL-10 promote the expansion and maturation of mast cells (117-120). Many studies have also shown that SCF can promote the proliferation and differentiation of progenitor cells, with a key effect on mast cell development in particular (121-123).

Various cytokines are known to influence the human mast cell differentiation process. Studies have shown that IL-3 promotes proliferation and differentiation of early mast cell precursors yet is not sufficient to support full cell maturation (80, 83, 109, 124, 125). IL-6 has been found to have the function of promoting mast cell differentiation (109, 113).

There are many other growth factors mediating mast cell survival, differentiation and maturation. T helper cell type 2 cytokines such as IL-3, IL-5, IL-6, IL-9, and GM-CSF each promotes mast cell progenitor proliferation in the presence of SCF (91). However, their effect on primary MCPs has yet to be determined. Furthermore, some cytokines have divergent effects on mast cells. It is reported that IL-10 and IL- 4 can cooperate with IL-3 or SCF to better stimulate mast cell proliferation (91, 119, 126). IL-4 has also been reported to play a key role in the expression of chymase and FcεRI during mast cell development (127-131). However, long-term exposure of bone marrow-derived mast cell to IL-4 and IL-10 results in down-regulation of mast cell effector molecules such as c-Kit and FcεRI and cause cell apoptosis (127, 131, 132).

1.4 SYSTEMIC MASTOCYTOSIS (SM)

1.4.1 Disease overview

Systemic mastocytosis (SM) is a hematologic disease caused by the clonal proliferation and accumulation of aberrant mast cells, affecting the skin and/or various organs (133). SM is more common in adults than in children (134). SM often manifests as systemic symptoms, such as headache, abdominal pain, diarrhea, flushing and anaphylaxis caused by systemic multi-organ involvement of aberrant mast cells and release of mast cell mediators (135). According to reports, the prevalence of mastocytosis in Europe is about 0.5-1 per 10,000 people (136, 137).

1.4.2 Diagnosis and classification

The World Health Organization updated the classification of mastocytosis in 2016. According to this classification (Table 1), the main criterion for the diagnosis of SM is the detection of multifocal and dense mast cell infiltration (defined as ≥ 15) in at least one organ outside the skin. One or more minor criteria are also required for SM diagnosis including abnormally shaped mast cells infiltrating the bone marrow or visceral organs, detection of the KIT D816V

somatic mutation, aberrant marker CD2/CD25 expression on mast cells and increased levels of tryptase in serum. SM can be divided into subtypes according to the burden of mast cells and organ damage. The 5 major subtypes include: indolent SM (ISM), smoldering SM (SSM), SM-associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and mast cell leukemia (MCL). SM-AHN, ASM and MCL are typically grouped together and referred to as advanced SM (Adv-SM) (138, 139). Recently, the 5th WHO classification of myeloid hematologic neoplasms added a new subtype of SM, bone marrow mastocytosis (BMM), to the existing classification of SM (140). Different subtypes of mastocytosis have different prognosis. The life expectancy of ISM patients is the same as that of healthy people, whereas MCL patients often only have a few months to live after diagnosis (141).

Table 1. WHO diagnostic standards of diagnosing systemic mastocytosis (138, 140). The diagnosis of SM can be made if at least A) 1 major and 1 minor, or B) 3 minor criteria are satisfied.

Criterion	Description
Major	Mast cell multifocal dense infiltration (≥ 15 mast cells in aggregates) in bone marrow biopsies and/or other extracutaneous organ sections
Minor	> 25% of mast cells exhibit spindle shape or other atypical morphology in bone marrow smears or visceral organ sections
	Detection of KIT codon 816 point mutation or in other critical regions of *KIT** in the bone marrow or another extracutaneous organ
	One or more expression of CD2, CD25, and CD30* in the mast cells
	Elevated baseline serum-tryptase levels of >20ng/mL

* Updated in the 2022 WHO diagnostic criteria for SM.

1.4.3 Mast cell immunophenotyping in SM

Aberrant mast cells in SM typically have an atypical immunophenotype. A bone marrow biopsy is used for clinical diagnosis. Immunohistochemical staining of tryptase and c-Kit are used to quantify mast cells in bone marrow biopsy sections, but neither marker distinguish between normal and aberrant mast cells (142-144). Neoplastic mast cells can be distinguished based on characteristic phenotypes such as CD2 and CD25 expression. Abnormal expression of CD25 and/or CD2 is a minor WHO criterion for the diagnosis of SM (138, 145). Studies have shown that CD25 is stably expressed in various subtypes of abnormal mast cells and has high sensitivity and specificity for disease, so it is considered to be a reliable marker for identifying pathological mast cells (146, 147). In addition, neoplastic mast cells may express atypical expression of cell surface antigens, including CD30, CD64, CD45RA, and CD123, but the prognostic implications are unclear (138, 148-152).

Mature mast cells are virtually undetectable in peripheral blood of healthy subjects (86). However, circulating aberrant mast cells exist in a subset of patients with SM. This was demonstrated in a study in which a population of $CD117^+$ $Fc\varepsilon RI^+$ tryptase-expressing mast cells were isolated (86).

1.4.4 KIT mutations

The human proto-oncogene *KIT*, which encodes a transmembrane tyrosine kinase receptor, can activate cell mitosis when stimulated (153). The KIT receptor belongs to the type III tyrosine kinase family, together with macrophage colony-stimulating factor 1 receptor, the FMS-like tyrosine kinase 3, and the platelet-derived growth factor receptor. As the receptor of stem cell factor, KIT is widely involved in the development of various cells in the body, especially blood cells. KIT is widely involved in hematopoiesis and the receptor is expressed on the surface of a variety of progenitor cells of different lineages. During cell differentiation, most progenitors lose KIT expression, but not cells of the mast cell lineage. The KIT receptor is critical for mast cell survival, differentiation, and migration (154-156).

Abnormal tyrosine kinase activity caused by a KIT mutation is found in many diseases, such as malignant melanoma, acute myeloid leukemia (AML), gastrointestinal stromal tumor (GIST) and SM (157). Excess mast cell proliferation and accumulation caused by *KIT* activating mutations are considered an important pathogenic mechanism of SM. The most common mutation in SM is the aspartic acid (D) amino acid at position 816 that is replaced by valine (V) in exon 17 of the *KIT* gene. The KIT D816V mutation can be detected in more than 80% of the patients with SM, irrespective of the subtype (158, 159). The D816V mutation is an activating *KIT* mutation, which alters downstream signaling pathways and makes ligand binding dispensable for activation, leading to pathological proliferation, accumulation and invasion of mast cells (160-162). Other mutations such as the D816Y, D816F, D816H and D816I mutations in KIT are occasionally detected in SM (163).

1.4.5 Associated mutations

Oncogenic tyrosine kinases (TK) found in SM usually function as a weak promotor of cell differentiation and maturation, but not proliferation (164). Presence of the KIT D816V does not fully explain the onset and diverse pathological features of SM, reflected in the fact that the mutation is a minor disease criterion.

Mutations in genes other than *KIT* are often found in advanced SM subtypes. Associated mutations are most common in SM-AHN, followed by ASM, and are rare in ISM (165, 166). Several of the mutations detected in advanced SM can also be found in other myeloid neoplasms, for example myelodysplastic syndromes, or myeloproliferative neoplasms (167). The most common associated mutations in SM include mutations in *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *N/KRAS* and *IDH2*, genes involved in a variety of different functions including cell signaling, epigenetic regulation and mRNA splicing (168). Most mutations are associated with poor prognosis, and the screening of a panel of mutated genes can therefore be used to assess the prognosis of a given patient (165, 168, 169). For example, the presence of mutations in

SRSF2 / ASXL1 / RUNX1 (S/A/R) are correlated with poor outcome and short overall survival in SM (168, 169). The role of mutated TET2 is unclear in SM. Mutated TET2 did not show a significant effect on overall survival in one study (170), but another study showed that TET2 is a key regulator of mast cell proliferation and differentiation (171). A recent study revealed a new non-gene level molecular change in Adv-SM, that is, the tumor suppressor gene SETD2 loses its function during post-transcriptional translation (172), suggesting an important role of epigenetics in SM pathogenesis. This provides new research directions and targets for the pathogenesis and treatment of SM.

1.4.6 Hematopoiesis in SM

The cellular origin of the aberrant mast cell remains unknown. It has been reported that the KIT D816V mutation can be found in various stem cells or progenitors in the bone marrow of patients with systemic mastocytosis, despite evidence that the overall hematopoietic progenitor composition remains unaffected (152, 173-175). KIT D816V mutation has been detected in various lineages, including $CD34^+$ progenitors (152, 175, 176), erythrocytes (177), granulocyte/monocyte lineages (177, 178), lymphoid lineage (175, 179). A reasonable speculation is that the KIT D816V mutation originates from $CD34^+$ progenitor cells and is distributed in various lineages along with the hematopoietic process. However, the distribution of mutated cells in the hematopoietic landscape and the impact on hematopoiesis of other lineages remain unclear. In one study, the KIT D816V mutation was found in hematopoietic progenitor cells in the majority of the patients with advanced SM and around 1/4 of ISM patients (175). Mutations in undifferentiated progenitors are associated with multilineage involvement and advSM; conversely, mutations arising in committed MC progenitors or mature MC result in relatively mild forms of disease and are commonly found in stable ISM (175, 180).

Limited studies have explored the transcriptomes of hematopoietic progenitors and mast cells in the bone marrow of patients with systemic mastocytosis. D'Ambrosio et al used gene expression microarrays to compare the transcriptional differences between SM patients and healthy donor's bone marrow mononuclear cells (181). Teodosio et al compared the gene expression of MCs isolated from healthy individuals and SM patients and reported a list of genes upregulated in SM patients (182). However, how the hematopoiesis is altered in SM patients at the gene expression level remains unclear.

Above, I have summarized the research related to mast cell development and systemic mastocytosis, and highlighted gaps in the research field’s knowledge. Recent advances in single-cell RNA-sequencing technologies is profoundly changing our understanding of hematopoiesis. Utilization of single-cell RNA-sequencing allows us to chart the mast cell differentiation trajectory at the single cell level in both health and disease.

2 RESEARCH AIMS

The overall aim of this thesis is to provide new insights into the identity and development of mast cell progenitors in the human bone marrow and peripheral blood. The main focus is on hematopoiesis and mast cell differentiation in health and in patients with systemic mastocytosis, to expand our knowledge on the pathogenesis of systemic mastocytosis and mast cell-related diseases.

The specific aims were:

Study I To characterize the FcεRI^{+} progenitor population in human bone marrow

Study II To investigate the identity and regulators of mast cell progenitors in human peripheral blood

Study III To chart the single-cell transcriptional landscape of hematopoiesis and the mast cell population of patients with systemic mastocytosis

3 MATERIALS AND METHODS

3.1 Ethical considerations and human samples

Ethical approvals for the collection of bone marrow and peripheral blood samples from human subjects were obtained from the regional ethical committees in Uppsala and Stockholm, and the Swedish Ethical Review Authority. All participants involved in the studies provided informed consent.

Human bone marrow samples were included in study I. Buffy coats of peripheral blood collected from healthy donors were the main source of primary cells in study II. In addition, we collected 3 peripheral blood samples from patients with indolent mastocytosis. For study III, we collected 5 bone marrow samples from patients diagnosed with or under evaluation for systemic mastocytosis (4 ISM and 1 SM-AHN) and one from a healthy donor.

3.2 Sample preparation

Cells were isolated from bone marrow using a standard protocol depleting mature erythrocytes by PharmLyse (Study I and III). In study II, we isolated mononuclear cells from buffy coats and peripheral blood using Ficoll-Paque PLUS to remove excessive granulocytes.

For study II, a magnetic beads-based enrichment of progenitor cells and mast cells from mononuclear cells was performed using CD117 MicroBead Kit.

3.3 Multi-color flow cytometry

Several multi-color flow cytometry panels were applied for cell analysis (study I-II). We designed the panel based on published studies where cell populations were verified by functional or culture experiments. Lineage markers including CD3, CD14, CD15, and CD19 were typically used to exclude lymphocytes, granulocytes and monocytes from the analyses. Viability dyes 4',6-diamidino-2-phenylindole (DAPI) and 7-aminoactinomycin D (7-AAD) were used to remove dead cells during the analysis. Flow cytometric data was analyzed using FlowJo v10 software.

Fluorescence Minus One (FMO) controls are commonly applied in multicolor flow cytometry experiments to measure the spillover of a given channel, to help decide the correct position of a given gate. Here we combined the FMO control with isotype control staining when analyzing cell culture experiments, to rule out the non-specific binding of antibodies to the limited number of cells following culture (study II).

3.4 Fluorescence-activated cell sorting

We isolated cells for downstream experiments using fluorescence-activated cell sorting on the FACS Aria Fusion platform. In study I, a two-step sorting protocol was used when sorting rare populations from blood or bone marrow. We used a one-step sorting protocol for sorting experiments in study II and III when c-Kit$^+$ cells were pre-enriched by magnetic beads selection. Cells were sorted into sterile filtered FACS buffer (PBS with 2% Fetal Calf Serum). The purity

of sorted cells was checked by recording a limited number of sorted cells after thoroughly cleaning the cell sorter.

3.5 Cell cultures

In studies I and II, progenitors were cultured *in vitro* to evaluate their differentiation potential. Several combinations of cytokine panels were used for analyzing various lineage differentiation. For promoting mast cell differentiation, in study II, we used a standard protocol that includes IL-3 (10 ng/mL), IL-6 (50 ng/mL), and SCF (100 ng/mL). IL-3 was added only for the 1st week and subsequently removed, whereas the concentrations of IL-6 and SCF were maintained for the remaining approximately 3 weeks. However, the high concentration of SCF leads to the internalization of c-Kit, making c-Kit expression unreliable by flow cytometry detection. Therefore, in Study I, we used a reduced SCF concentration (5 ng/mL) in the myeloerythroid-promoting panel, which also contained EPO and GM-CSF. Other cytokines including IL-5, GM-CSF, EPO, TPO, Flt3L, and IL-33 were used to explore their effects on promoting mast cell development in study II.

3.6 Cell culture analysis

The cell fate of primary hematopoietic progenitors was determined using flow cytometry analysis following cell culture (study I and II). We tracked the morphological changes of differentiating progenitors using cytochemical May-Grünwald Giemsa (MGG) staining (study I and II). Enzymatic tryptase staining using Z-Gly-Pro-Arg-4-Methoxy-b-naphthylamide substrate was applied to assess mast cell maturation throughout the culture period (study II) (183).

To investigate the function of mature mast cells after culture, cells were incubated with human IgE overnight, followed by analyzing the activation marker CD63 using flow cytometry after treatment with mouse anti-human IgE antibody.

3.7 Single-cell RNA sequencing

FACS-purified cells were partitioned into droplets containing gel beads tagged with short fragments of oligonucleotide cell barcodes. In brief, individual cells were encapsulated into each droplet together with beads tagged with cell barcodes. In each droplet, single cells were lysed and released mRNA that was captured by the indexed beads. Following the reverse transcription in each droplet, cDNA was collected and PCR amplified. A sequencing library was constructed from the amplified cDNA. Libraries were sequenced on the Illumina sequence platform.

3.8 Cellular indexing of transcriptomes and epitopes by sequencing

Cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) utilizes oligo-conjugated antibodies that target cell surface proteins. The oligo serves as an artificial gene that can be detected in the 10X Genomics analysis pipeline and allows investigation of surface protein expression for each individual cell. Thus, the CITE-seq analysis adds

quantitative protein level data on top of the transcriptome analysis (184). Different ways of tagging antibodies with oligonucleotides exist, of which we picked TotalSeq-B reagents that match the Feature Barcode technology for 3' Single Cell Gene expression Kit v3.1. In study III, for evaluating surface marker expression on mast cells in SM patients, sorted hematopoietic progenitor cells with additional barcoded mast cells were stained with TotalSeq-B anti-human CD117 (c-Kit), FcεRIα, CD2, CD25, and CD71 following the recommended protocol.

3.9 Cell hashing with oligonucleotide-tagged antibody system

Designed for sample barcoding, hashtag reagents consist of oligonucleotide-conjugated antibodies targeting ubiquitous surface proteins, allowing users to combine multiple samples, and be demultiplexed in downstream analysis.

Here, we customized the hashing protocol to allow the analysis of barcode information in the flow cytometry analysis and in the single-cell transcriptomics data analysis. The approach allowed us to accurately quantify the frequency of two pooled cell populations in studies II and III.

In study II, c-Kit$^+$ cells enriched by magnetic-activated cell sorting (MACS) were split into 2 fractions. One fraction was stained with a biotinylated β2-microglobulin antibody targeting all cells, while the other fraction was stained with an unlabeled β2-microglobulin antibody. Subsequently, both fractions of cells were stained with flow cytometry antibodies and oligonucleotide-labeled streptavidin-phycoerythrin (PE). We FACS-isolated Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ cells with the hashtag (PE$^+$) and Lin$^-$ CD34$^+$ c-Kit$^+$ cells without the hashtag (PE$^-$) separately from the corresponding fraction. Cells were pooled and processed using the Chromium platform (10X Genomics).

Due to the limited number of mast cells in bone marrow samples, the cell hashing technique allowed us to enrich for and trace spiked-in mast cells in the hematopoietic landscape. In study III, red blood cell lysed bone marrow sample was divided into 2 fractions for hashtag staining. Labeled (PE$^+$) CD45$^+$ c-Kit^{high} mast cells were spiked-in into unlabeled (PE$^-$) Lin$^-$ CD45$^+$ c-Kit$^+$ progenitor cells. The sample pool was processed using the Chromium platform.

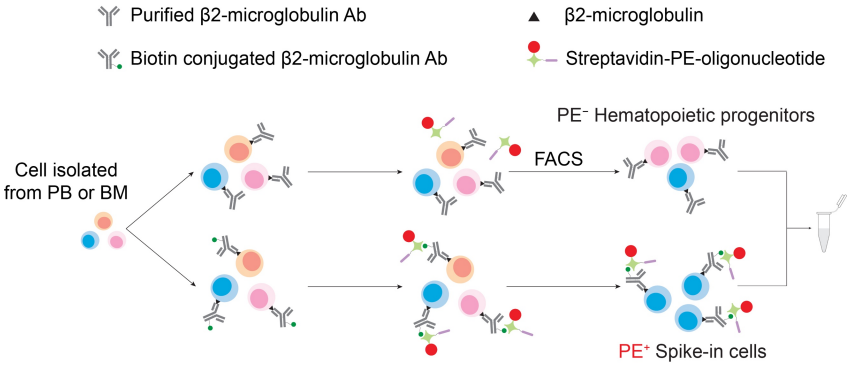

Figure 3. Schematic figure of cell hashtag using PE-oligonucleotide barcode. Adapted from Wu et al, 2022 (185) with permission from ASH. BM, bone marrow; PB, peripheral blood.

4 RESULTS AND DISCUSSION

This chapter briefly outlines the main results of each study and discusses their relevance.

4.1 BONE MARROW MAST CELL AND BASOPHIL PROGENITORS (STUDY I)

Postnatal hematopoiesis in humans occurs primarily in the bone marrow. Bone marrow hematopoietic stem and progenitor cells give rise to blood cells and immune cells, including mast cells and basophils. Unipotent and bipotent mast cell and basophil progenitors have been described in mouse bone marrow, but it is unclear whether similar progenitors exist in human bone marrow.

The high-affinity IgE receptor, FcεRI, is one of the characteristic markers of mature mast cells and basophils. Cross-linking of FcεRI by IgE-antigen complexes results in degranulation of mast cells or basophils and release of inflammatory mediators. A previous study has described $FcεRI^+$ hematopoietic progenitors in bone marrow (152). However, the population has not been fully characterized.

Here, we characterized $FcεRI^+$ progenitor cells in human bone marrow. Specifically, we studied $FcεRI^+$ progenitors that fall within the common myeloid progenitor (CMP) gate. Flow cytometry analysis distinguished three subpopulations, namely $CD203c^+$ cells, $CD203c^-$ Integrin $β7^+$ cells, and $CD203c^-$ Integrin $β7^-$ cells. These three subpopulations were sorted by FACS and cultured *in vitro* under myeloerythroid-promoting conditions to assess their fate potential. The cell fate assays showed that $CD203c^+$ cells only have the potential to differentiate into mast cells and basophils, whereas the two $CD203c^-$ cell populations showed potential to differentiate into erythrocytes in addition to mast cells and basophils.

Our results show that $FcεRI^+$ $CD203c^-$ progenitors exhibited significant erythroid-forming capacity. By contrast, no erythroid cells were observed in the $CD203c^+$ cell population, suggesting that CD203c is associated with the loss of erythroid developmental potential. The results in this study indicated that the mast cell and basophil lineages are closely associated. The data also suggested that the erythroid lineage is closely coupled to the mast cell and basophil lineages. However, our study has the limitation that the sorted progenitors are cultured in bulk, and not at the single-cell level. Therefore, we cannot definitively conclude that the mast cell, basophil, and erythroid lineage are closely related. However, our data is compatible with data from mice, in which mast cells and basophils have a shared bipotent progenitor. Our observation that $FcεRI^+$ $CD203c^-$ progenitors give rise to mast cell, basophil, and erythroid cells is also compatible with data in mouse, in which progenitors with combined erythroid and mast cell/basophil potential exist (18). Further studies are required to investigate the relation between the erythroid, mast cell and basophil lineages in human hematopoiesis.

4.2 MAST CELL PROGENITORS IN PERIPHERAL BLOOD (STUDY II)

Mast cells are activated mainly through IgE cross-linking to release a series of bioactive substances to participate in innate immunity, allergy and other physiological and pathological

activities. Several reports have concluded that the high-affinity IgE receptors (FcεRI) appear at the terminal stage of mast cell development, and that they are absent on mast cell progenitors. Others reports indicate that FcεRI appear already at the mast cell progenitor stage. Here, we specifically addressed the temporal upregulation of FcεRI during mast cell differentiation.

Single-cell transcriptome analysis of FACS-isolated c-Kit$^+$ progenitors yielded a landscape comprising multipotent progenitors (MPPs) and progenitors of distinct cell lineages. Uniform Manifold Approximation and Projection (UMAP) graphs visualized the high-dimension transcriptome data in 2 dimensions. We found a cluster of cells with a gene expression profile characteristic of mast cells, e.g. expression of tryptase and histamine-related genes. Notably, we also found expression of genes encoding the FcεRI subunits. The low expression of *CD34* gene expression indicated that the mast cell-like cluster had characteristics of immature cells. We identified a corresponding population by flow cytometry, Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ cells, which warranted further investigation (Figure 1 in study II).

We successfully sorted Lin$^-$ CD34$^+$ c-Kit$^+$ progenitor cells and added additional antibody-oligonucleotide-labeled (PE$^+$) Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ cells to establish a new single-cell RNA sequencing library. The single-cell transcriptomics data provided a high resolution map of hematopoiesis, with specific focus on the Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ mast cell-like cells. Plotting of mast cell signature genes and FcεRI subunit genes uncovered a cell population with mast cell transcriptome signatures, recapitulating results of the first single-cell dataset. Mapping PE hashtag expression on the landscape highly overlapped with the mast cell-like population, demonstrating that Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ cells in the peripheral blood have the gene expression signature characteristic of mast cells (Figure 2 in study II). In subsequent *in vitro* culture experiments using a standard long-term protocol that promotes the formation of mast cells, Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ progenitors differentiated into mature mast cells, which was confirmed by immunophenotypic, morphologic, and functional analysis of the cultured cells. Notably, the Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ population developed the characteristics of mature mast cells faster than the control group, i.e. cultured FcεRI$^-$hematopoietic progenitors. Taken together, our data shows that the Lin$^-$ CD34$^+$ c-Kit$^+$ FcεRI$^+$ cell population in peripheral blood constitutes mast cell progenitors (Figure 3 in study II).

The single-cell transcriptomics data allowed us to search for potential regulators of mast cell progenitor development. Specifically, we screened for gene expression of cytokine receptor in the single-cell hematopoietic landscape. The results indicated that *IL5RA*, *EPOR* and *IL1RL1* were enriched on mast cell progenitors. To investigate the functions of these and other receptors, the ligands were supplemented to culture of MACS isolated c-Kit$^+$ cells *in vitro*. We found that compared with IL-3, a cytokine that promotes hematopoiesis, IL-5 has the effect of maintaining the survival of mast cell progenitor cells but not promoting their proliferation. Strikingly, IL-33 showed capacity to down-regulate the expression of FcεRI, indicating the expression level of FcεRI may be affected by certain factors in the cellular microenvironment (Figure 4-6 in study II).

As mentioned above, FcεRI, has generally been considered to be a marker of mature mast cells that is absent on progenitor cells (23). A previous study reported that $CD34^+$ $FcεRI^-$ progenitors isolated from peripheral blood differentiate into mast cells *in vitro*. $CD34^+$ $FcεRI^+$ progenitors could not be detected in peripheral blood of patients with mastocytosis, further hinting that mast cell are derived from $FcεRI^-$ precursors (89). This view has been challenged by a recent study, in which a population of $CD34^+$ $c\text{-}Kit^+$ $FcεRI^+$ cells was found in the peripheral blood (92). This cell population showed some characteristics of mast cells following short-term *in vitro* culture (92). Our study confirmed the mast cell-forming potential of $CD34^+$ $c\text{-}Kit^+$ $FcεRI^-$ progenitors, consistent with previous knowledge. Furthermore, we successfully isolated a population of $CD34^+$ $c\text{-}Kit^+$ $FcεRI^+$ cells from the peripheral blood of both healthy donors and systemic mastocytosis patients. This FcεRI-expressing cell population show characteristics of mast cells according to single-cell transcriptomics, and differentiates into phenotypically, morphologically, and functionally mature mast cells after 4 weeks of culture in mast cell-promoting conditions. Given that both our study and that of Rottem et al used human peripheral blood samples, the reason why $FcεRI^+$ mast cell progenitors could be detected in our study, but not in that of Rottem et al is likely due to differences in the progenitor isolation methods. In our study, c-Kit-based MACS magnetic bead isolation was used for progenitor isolation, where $FcεRI^+$ cells express residual levels of CD34. Our observation that mast cell progenitors express low levels of CD34 indicates that the methods used in Rottem et al likely fails to enrich for $FcεRI^+$ mast cell progenitors (89).

By analyzing the developmental trajectory from multipotent progenitors to mast cell progenitors in peripheral blood, and tracking the dynamic regulation of genes during this process, we found that the emergence of genes encoding the FcεRI subunits was associated with the upregulation of mast cell signature genes. However, the analysis does not necessarily suggest that FcεRI is always linked to mast cell development. Mature mast cells distributed in different anatomical locations show variable levels of FcεRI. For example, mature mast cells with no or low FcεRI expression in the lung parenchyma have been observed (52, 186). A possible explanation to the presence of mast cells without FcεRI expression could be that the mast cells have been exposed to high concentrations of IL-33 during their development. In cultured mast cells, FcεRI was undetectable on both primary umbilical cord blood-derived mast cells and the widely used cell line HMC-1 (187-190). Previous studies have shown that various factors can regulate the expression level of FcεRI on the surface of differentiated mast cells, thereby affecting mast cell function. For instance, IL-4 enhances the expression of FcεRI and acts synergistically with IgE (127, 191, 192). In fact, IL-33 has been reported to significantly downregulate the expression level of FcεRI on the surface of mature mast cells (193). Interestingly, unlike IL-4 on mast cell progenitors, which serves the opposite functions of promoting apoptosis and downregulating FcεRI and c-Kit expression (194), our study shows that IL-33 also inhibits FcεRI on precursor cells expression, but its effect on mast cell development and function remains to be further explored.

We identified different effects of IL-3 and IL-5 in maintaining survival and promoting the proliferation of mast cell progenitors. IL-3 is routinely added to human mast cells in the early stages of *in vitro* culture (76, 124). Even though IL-3 alone is insufficient to promote terminal mast cell differentiation and maturation, it helps to promote the proliferation and development of hematopoietic progenitors (109, 195, 196). Previous studies have compared the effects of IL-3 and IL-5 on differentiated mast cells (197). IL-3 was found to promote mast cell proliferation whereas IL-5 does not (198). The present study shows that at the progenitor cell stage, IL-5 can maintain their survival although it does not promote proliferation.

The receptor complexes for IL-3, IL-5, and GM-CSF share a common beta chain, known as CD131, which is essential for signal transduction (199, 200). One of the most important signaling molecules in the downstream pathway of the GM-CSF receptor is phosphoinositide 3-kinase (PI3K), which is reported to be a concentration dependent binary switches have the function of regulating "survival" or "survival proliferation" (201-203). In the current study, increasing the concentration of IL-5 still did not achieve the same effect on mast cell proliferation as IL-3. This may be due to the limited number of IL-5 receptors on the cell surface. A binary switch mechanism for “survival” or “survival proliferation” may exist in IL-5 signaling, similar to that jn GM-CSF signaling. However, the “survival proliferation” effect of IL-5 can likely not be achieved in mast cell progenitors.

4.3 HEMATOPOIESIS IN SYSTEMIC MASTOCYTOSIS (STUDY III)

In Study III, we turned our attention to bone marrow hematopoiesis. Using multi-omics data, combining hashtag-based single-cell transcriptomes with CITE-seq antibody-based cell-surface molecular phenotypes, we aimed to delineate the hematopoietic landscape of systemic mastocytosis and investigate mast cell abnormalities at the single-cell level. The analysis was focused on bone marrow aspirates from patients with systemic mastocytosis, in which we profiled hematopoietic progenitor differentiation and the mast cell population.

We FACS isolated the entire $Lin^{-/low}$ $c\text{-}Kit^{+}$ cell population, which includes hematopoietic progenitors and mast cells, from three samples. These bone marrow samples were from one healthy donor and two patients with systemic mastocytosis. The cells were subjected to single-cell RNA sequencing, followed by visualizing the hematopoietic landscape using UMAP. One cell cluster exhibited mast cell characteristics, expressing genes encoding c-Kit (*KIT*), tryptase (*TPSB2*) and Carboxypeptidase A3 (*CPA3*). Further analysis revealed that this mast cell-like cluster was mainly derived from one of the patients with systemic mastocytosis. Therefore, the study of the mast cell lineage requires another approach to capture sufficient numbers of mast cells in any patient with systemic mastocytosis (Figure 1 in study III).

We used the method described in study II to hashtag and spike-in mast cells, overcoming the issue with capturing too few mast cells. We also leveraged the single-cell RNA-seq data with the CITE-seq technology to quantify cell surface proteins on each cell. Three bone marrow samples were collected from patients with systemic mastocytosis with this novel method. Specifically, unlabeled hematopoietic stem and progenitor cells, gated as PE^{-} Lin^{-} $CD45^{+}$ c-

Kit^{+} cells, and spiked-in oligo hashtag-labeled PE^{+} Lin^{-} CD45^{+} c-Kit^{high} mast cells were FACS sorted and pooled. The pooled cells were labeled with CITE-seq antibodies and subjected to the single-cell sequencing protocol. A novel single-cell landscape of patient bone marrow covering progenitors of various blood cell lineages and mast cells was depicted. Demultiplexing hashtag markers help us identify the mast cell population. In addition, plotting cell surface signature markers (c-Kit and FcεRI) using CITE-seq data and the corresponding gene expression (*KIT* and *FCER1A*) further confirm the cells' identity (Figure 2 in study III). By plotting the expression of the aberrant mast cell markers CD2 and CD25, which are widely used in clinic, we found that these 2 markers were highly expressed on the mast cell population at both the protein level captured by CITE-seq antibodies and the gene expression level. Notably, the presence of CD25^{-} mast cells suggests heterogeneity within the mast cell population in the patient's bone marrow. Comparing the molecular profiles of CD25^{+} versus CD25^{-} mast cells, we discovered a set of genes that were significantly upregulated, highlighting factors that possibly could be pathogenic in systemic mastocytosis (Figure 3 in study III).

In the present study, the single-cell transcriptomics combined with immunophenotyping were used to map the hematopoietic stem and progenitor cells plus mast cells in the bone marrow of patients with systemic mastocytosis. Previous studies have reported altered immune cell composition in the peripheral blood of patients, such as skewed lymphocyte subgroups, increased group 2 innate lymphoid cells, and decreased monocytes (204-206). However, previous studies have shown little or no effect on the proportion of hematopoietic stem and progenitor cells across lineages (152). Despite no or little skewing in the composition of the hematopoietic stem and progenitor cell frequencies (152, 176, 207, 208), pathological mast cells are accumulated in the bone marrow.

Here, we obtained a mast cell-enriched bone marrow hematopoietic landscape. Notably, we distinguished 2 distinct subpopulations of mast cells with different expression levels of the clinically relevant marker CD25. The analysis of up-regulated gene expression in CD25^{+} mast cells compared to CD25^{-} mast cells allowed us to obtain more confident results by using a within-subject control without batch and individual variants. Few studies have been performed that explore the transcriptome of mast cells from bone marrow of patients with systemic mastocytosis. In an early study, microarray analysis was used to compare the differential expression of bone marrow mononuclear cells from patients and healthy individuals. Among them, α-tryptase related genes were found to be highly expressed in patients (181), but it is impossible to distinguish whether this up-regulation is due to the up-regulation of the gene in pathological mast cells or whether the upregulation is a consequence of mast cell accumulation. A study by Teodosio et al compared the gene expression profiles of mast cells isolated from the bone marrow of patients and healthy individuals and reported that a series of genes were upregulated in patients with mastocytosis (182). Notably, our study transcriptionally compared CD25^{+} and CD25^{-} mast cells in the same subjects for the first time and found that *CCL23*, *IFI27*, and *CLU* were among the top ten genes up-regulated in CD25^{+} mast cells. These genes

match the list of genes that are higher in systemic mastocytosis compared with healthy control (182). The mechanisms that lead to the deregulated gene expression warrants further investigation.

5 CONCLUSIONS AND OUTLOOK

This thesis has systematically analyzed the generation of mast cells from different anatomical sites, including bone marrow and peripheral blood. It describes the hematopoietic process in healthy subjects and in patients with systemic mastocytosis, a rare mast cell-related hematological disease. However, there are still a number of outstanding questions related to mast cell development in steady state conditions and in systemic mastocytosis.

The main findings of this thesis include: 1) The identification of cellular markers that distinguish developing mast cell and basophil progenitors from erythrocyte/basophil/mast cells progenitors in adult bone marrow. Our results hint that the mast cell and basophil differentiation trajectories are closely related in human hematopoiesis. 2) Peripheral blood mast cell progenitor cells express FcεRI, and the expression of FcεRI can be regulated by the microenvironment. These observations show that FcεRI expression is not necessarily associated with mast cell progenitor differentiation. 3) The generation of a single-cell transcriptional plus immunoprofiling resource of systemic mastocytosis, revealing the expression profile of $CD25^+$ and $CD25^-$ mast cells.

Is there a bipotent basophil-mast cell progenitor in human hematopoiesis similar to mouse? Arinobu et al isolated bipotent progenitor cells from murine spleen and verified their ability to differentiate into both mast cells and basophils by *in vitro* culture at the single cell level (70). Analyzing the composition of the colony derived from a single progenitor cell is a valid approach to address the fate potential of hematopoietic progenitors. However, it is challenging to assess the mast cell forming potential by single cell culture assay due to the extremely low proliferation rate of mast cell progenitors (92). Therefore, an improved culture system is warranted to increase the yield of mast cells in *in vitro* culture, which can assess their differentiation potential in mixed populations.

A recent study in mouse introduced unique DNA barcodes in individual hematopoietic progenitors (209), which are transcribed and propagated into the progeny of each cell. Combining single-cell transcriptomics and analysis of the unique cell barcode therefore allows investigation of clonal relationships between progenitors cultured in myeloerythroid-promoting conditions. This approach may serve as an effective tool to track the origin and early fate decisions of mast cells and basophils, where analysis of single colonies may prove challenging.

In study II, we identified $FcεRI^+$ MCPs in peripheral blood. These MCPs exhibited a pure source of cells that form mature and functional mast cells, without showing the ability to form basophils. This reflects the belief that basophils develop and mature in the bone marrow, and their progenitors are supposedly lacking in the peripheral blood (210). However, we cannot completely rule out that basophil-forming potential is lacking in the MCP population. In the study, we mainly used a mast cell-promoting culture system, in which IL-3 was present only during the first week of culture. The consensus is that IL-3 is a key factor promoting the growth and differentiation of basophils and basophil differentiation might therefore not be fully

supported (124, 211, 212). Other factors known to promote basophil differentiation *in vitro*, including e.g. IL-5 (213) or GM-CSF (214), were not present in the mast cell-promoting culture system. However, we note that c-Kit^{-} FcεRI^{+} basophil-like cells were virtually undetectable following culture of FcεRI^{+} progenitors with IL-3 or IL-5 for 5 days. However, more detailed studies are required to fully assess whether the mast cell progenitor population has any basophil-forming potential.

The hematopoietic landscape of peripheral blood with spiked-in FcεRI^{+} cell populations revealed two clusters of MCPs. One can therefore ask: Are MCPs in peripheral blood heterogeneous in phenotype and function? When we compared the transcriptomes of the two main MCP subpopulations, i.e. population A1 and A2, genes including *CPA3*, tryptase-encoding genes, and *SRGN* (215) were higher in population A2. The observation that population A2 exhibited higher expression of several mast cell signature transcripts suggests that this population is more differentiated than population A1. Whether it is possible to isolate these subpopulations have yet to be investigated. Identifying surface markers that distinguishing the subpopulations could potentially be guided by the differential expression analysis. Another possibility is to perform an antibody screen to distinguish the subpopulations. Such analysis could potentially be performed using a CITE-seq-based approach with tens or hundreds of oligo-labeled antibodies simultaneously.

The high-affinity receptor for IgE, FcεRI, is often considered a maturation marker of mast cells. By contrast, our study demonstrates that FcεRI is expressed on circulating mast cell progenitor cells. Does the presence of the high-affinity IgE receptor on mast cell progenitors have a physiological function? Previous studies have found that IgE monomeric binding to mast cells promotes mast cell survival, but without proliferation (216). However, whether IgE promotes the survival, proliferation and differentiation of FcεRI-expressing MCPs requires further cell culture and transcriptome analysis.

In acute allergic reactions, antigen specific IgE is bound to the mast cell surface receptors FcεRI, sensitizing them to release mediators in response to specific antigens that are subsequently encountered (217, 218). It was previously thought that the sensitization process occurs in peripheral tissues (24), consistent with the belief that FcεRI expression is upregulated during the terminal mast cell development in the peripheral tissues (23). Plasma IgE levels are elevated in patients with allergic diseases (217). Whether this suggests that MCPs acquire IgE and are sensitized in the peripheral blood have yet to be determined. A recent study reported that human peripheral blood MCP can be activated by IgE cross-linking *in vitro* (216). However, whether MCPs can be activated *in vivo* is not known and its potential physiological functions need to be further explored.

Little is known about the pathogenesis of mastocytosis. Tracing the distribution of KIT D816V in the hematopoietic landscape is essential to further understand the origin of the somatic mutation. Previous studies proposed several methods for integrating mutational information with single-cell transcriptome data (219, 220). By using these tools, mutations at single cell resolution, including *KIT* and other non-*KIT* mutations, could in theory be detected in the

hematopoietic single-cell transcriptome landscape. Another approach to trace the KIT mutation is based on single-cell DNA mutation analysis. For example, Grootens et al index-sorted individual hematopoietic progenitors and analyzed them with qPCR to trace the KIT D816V mutation in the hematopoietic landscape (152).

We derived a list of genes that are elevated in $CD25^+$ abnormal mast cells in patients with SM. To further explore the role of these genes for disease development, *in vitro* experiments could be performed. One possibility would be to knock out genes of interest in mast cells using a CRISPR-Cas9-based system with appropriate readouts to assess the role of each gene. Due to the limited number of primary mast cells that can be obtained from patients, mast cell lines can be used to analyze the function. Patient-derived induced pluripotent stem cell-derived mast cells, with retained mutational profile could also be a source of mast cells for *in vitro* studies (221).

Collectively, the studies presented in this paper provide novel insights into mast cell development in health and systemic mastocytosis, which is expected to provide a starting point for research to benefit patients with mast cell-related diseases.

6 POPULAR SCIENCE SUMMARY

Blood cells in our bodies perform a variety of functions, including supplying oxygen to tissues, fighting infection, and clotting in the event of an injury. The body must constantly produce new blood cells to maintain these functions. The evolution of multiple types of blood cells from hematopoietic stem cells in the bone marrow, known as hematopoiesis, begins in early embryonic life and continues after birth. The blood cell formation is strictly regulated in order to maintain the number and function of the blood cells. If the regulation is disturbed, one or more types of blood cells will be produced in insufficient or excessive quantities, accompanied by abnormal blood cell function. This may cause hematologic diseases, such as leukemia or anemia. However, the exact manner in which blood cell formation is regulated is not fully understood.

Growing from hematopoietic stem cells to mature blood cells with various functions is analogous to growing from an infant to an adult in all aspects of life. Following a general education as a child, those who received a social sciences education during adolescence are more likely to pursue a career in literature and politics later in life; while students who received a natural science education are more likely to work in agriculture, machinery, and other industries later in life. Similarly, progenitor cells are intermediate stages in the hematopoietic process that have the ability to develop into a large class of blood cells while losing the ability to differentiate into other cell types. Progenitor cells have fewer options for differentiation as they develop, eventually becoming a specific type of mature blood cell. The fate decision of blood cells during development is the focus of current research.

Mast cells play an important role in allergy and are found throughout the body, including the skin, digestive mucosa, and respiratory mucosa. Mast cells can release a variety of factors that cause symptoms such as sneezing, runny nose, and red and itchy skin. Mast cells are primarily derived from bone marrow hematopoietic stem cells in a healthy state, where they develop and mature under the sophisticated regulation of multiple factors. When the regulation is disrupted, the mast cells proliferate and function abnormally, which can result in a variety of local or systemic reactions such as itching, gastrointestinal discomfort, and muscle pain. Systemic mastocytosis is an example of such a mast cell-related hematologic disease.

The relationship between all blood cell lineages remains unclear. In **study I**, we provide new insights into the origin of the mast cells. Specifically, our results suggest that mast cells in human are related to two unexpected types of blood cells (basophils and red blood cells, instead of neutrophils and monocyte).

In **study II**, we identified and characterized immature mast cells – referred to as mast cell progenitors – in blood. We found that the molecule responsible for triggering allergies (FcεRI) appear already at the mast cell progenitor stage. This overturned the previous view that this molecule is only expressed when mast cells are fully mature. Moreover, we showed how the appearance of the allergy-triggering molecule is regulated. This study helps us to better

understand allergies, and provide a basis for the development of new therapies to treat mast cell-related diseases.

In **study III**, we investigated the formation of blood cells and mast cells in the bone marrow of patients with systemic mastocytosis, a rare disease caused by the accumulation of abnormal mast cells. We found two populations of normal-like and abnormal-like mast cells, and studied these cells with molecular precision. This study provides a solid foundation for further research on systemic mastocytosis – specifically on how blood cell formation is dysfunctional in disease.

To summarize, the research in this thesis focuses on the development of mast cells in healthy people and patients with systemic mastocytosis. The study of blood cell formation and abnormal mast cells in patients with systemic mastocytosis provides a reference for future in-depth investigation of the pathogenesis and therapeutic targets.

7 肥大细胞的发育

我们体内的血细胞具有多种功能，例如为组织供氧、抵抗感染、在受伤时凝结。血细胞的产生从胚胎早期开始，出生后源源不断地由骨髓中的造血干细胞演变为多种类型的血细胞并释放入血液循环，这一过程称为造血。为了维持各种血细胞数量的平衡和功能的稳定，造血过程受到严格的调控，一旦调控被扰乱，某一种或多种血细胞将会产生不足或过量，并伴随血细胞功能的异常，进而发展为白血病、贫血等血液系统疾病。然而，造血调控的具体方式尚未完全明了。

从造血干细胞演变为具备各种功能的成熟血细胞，就像从婴儿成长为各行各业的成年人。经过孩童时期的通识教育，青少年时期接受了人文学科教育的人，日后更有可能从事文学、政治等相关事业；而接受自然科学教育的中学生，未来倾向于从事农业、机械等行业的工作。类似地，造血过程中存在中间阶段的前体细胞，称为祖细胞，它们具有向某一大类血细胞发育的能力，同时丧失向其他类型细胞分化的潜能。随着发育的推进，祖细胞的分化路径选择逐渐减少，最终发育成为特定种类的成熟血细胞。血细胞在发育过程中的路径选择是当前研究的重点。

肥大细胞是参与过敏的关键细胞，它们广泛分布于全身，例如皮肤、消化道黏膜和呼吸道黏膜。它们可以释放多种因子引起打喷嚏、流鼻涕、皮肤红肿瘙痒等症状。健康人的肥大细胞主要来源于骨髓造血干细胞，在多种因素精密地调控下发育成熟。但当调控发生异常时，肥大细胞会过度增生并伴随功能异常，从而产生一系列局部或全身的症状，例如瘙痒、胃肠道不适和肌肉疼痛。这是一种罕见的血液系统疾病，称为系统性肥大细胞增多症。

各种血细胞发育路径之间的关系仍不清楚。在本论文的研究 I 中，我们提供了对肥大细胞起源的新见解：人类的肥大细胞与两种意想不到的血细胞类型——嗜碱性粒细胞和红细胞有关，颠覆了先前认为的肥大细胞与嗜中性粒细胞和单核细胞更接近的观点。

在研究 II 中，我们鉴定了血液中未成熟的肥大细胞（称为肥大细胞祖细胞），并描绘了它们的特征。我们发现负责触发过敏的分子（FcεRI）在肥大细胞祖细胞阶段已经出现，这推翻了先前的观点——这种分子只有在肥大细胞完全成熟时才会表达。此外，我们展示了如何调节 FcεRI 的出现。这项研究有助于我们更好地了解过敏，并为开发肥大细胞相关疾病的新疗法提供了理论基础。

在研究 III 中，我们研究了系统性肥大细胞增多症患者骨髓中血细胞尤其是肥大细胞的形成。我们发现了正常和异常两个肥大细胞群，并在分子精度比较了这些细胞。这项研究为进一步研究系统性肥大细胞增多症的发病机制提供了坚实的基础。

综上所述，本论文的研究关注肥大细胞在健康人和系统性肥大细胞增多症患者中的发育过程。研究结果展示了骨髓和外周血中肥大细胞祖细胞的存在和特征，对系统性肥大细胞增多症患者异常造血的研究为今后深入探究发病机制和治疗靶点提供了参考。

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