Aus der Klinik für Dermatologie, Venerologie und Allergologie
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DISSERTATION

Generation of mast cells from human induced pluripotent stem cells: a novel strategy for studying mast cell-driven disorders

Generierung von Mastzellen aus humanen induzierten pluripotenten Stammzellen: eine neue Strategie zur Untersuchung Mastzell-vermittelter Erkrankungen

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MC: mast cell
hsMC: human skin mast cell
PSCMC: peripheral hematopoietic stem cell-derived mast cell
hiPSC: human induced pluripotent stem cell
hiPSC-MC: human induced pluripotent stem cells derived mast cell
MC_{TC}: mast cell containing tryptase and chymase
MC_{T}: mast cell containing tryptase only
MC_{C}: mast cell containing chymase only
DPBS: Dulbecco's phosphate-buffered saline
MGG: May-Grünwald Giemsa
HSC: hematopoietic stem cell
MPP: multipotent progenitor
CMP: common myeloid progenitor
GMP: granulocyte/monocyte progenitor
MCp: Mast cell progenitor
CRISPR: clustered regularly interspaced short palindromic repeats
ESC: embryonic stem cell
MRGPRX2: Mas-related G protein-coupled receptor X2
IgE: immunoglobulin E
PBMC: peripheral blood mononuclear
SM: systemic mastocytosis
SCF: stem cell factor
OD: optical density
LDL: low-density lipoprotein
FBS: fetal Bovine serum
MACS: magnetic activated cell sorting
SFEM: serum-free expansion medium
EB: embryoid body
TBS: Tris-buffered Saline
MGG: May-Grünwald-Giemsa

DAPI: 4’-6-diamidino-2-phenylindole, dihydrochloride

RT: room temperature

MRGPRX2: Mas-related G protein-coupled receptor X2

Sub.P: Substance P

Abstract

Mast cells (MCs) are well known for their contribution to the pathogenesis of a multitude of inflammatory diseases, such as urticaria and mastocytosis. Growing evidence also suggests that MCs exert important surveillance and host-protective functions by orchestrating immune responses. In principle, *in vitro* studies of MC functions are performed with human MCs isolated from tissues or differentiated from hematopoietic progenitors. However, the recent *in vitro* research has taken on some challenges including donor-dependent heterogeneity, poor proliferation capacities of primary MCs, and the lack of a continuous cell source.

To address this, we developed a novel strategy for the rapid differentiation of MCs from human induced pluripotent stem cells (hiPSC-MCs). hiPSC-MCs exhibit phenotypic and functional characteristics of human skin MCs (hsMCs) and peripheral hematopoietic stem cell-derived MCs (PSCMCs). hiPSC-MCs are able to express the MC-associated receptors such as CD45, CD117, FcεRIα, CD200R, Siglec-8 and MRGPRX2, and degranulate in response to IgE/anti-IgE and substance P.

hiPSCs are immortal cells and able to develop into any type of human body cells, thus, for *in vitro* MCs research, hiPSC lines provide a continuous and stable MC source and overcome the obstacles caused by tissue samples and donor variabilities. In addition, hiPSCs allow for the development of cell models by generating hiPSC-MCs from patients.

Zusammenfassung

Mastzellen (MCs) sind bekannt für ihren Beitrag zur Pathogenese einer Vielzahl von entzündlichen Erkrankungen wie Urtikaria und Mastozytose. Immer mehr Hinweise deuten darauf hin, dass MCs wichtige Überwachungs- und Schutzfunktionen ausüben, indem sie Immunantworten modulieren. Um ihre biologischen Funktionen *in vitro* zu untersuchen, werden humane MCs entweder als reife Zellen aus humanen Geweben wie Haut, Lunge und Darm isoliert oder aus hämatopoetischen Vorläuferzellen aus peripherem oder Nabelschnurblut differenziert. Diese Techniken bergen jedoch mehrere Nachteile und Herausforderungen, darunter eine geringe Proliferationskapazität, eine Spender-abhängige Heterogenität und das Fehlen einer kontinuierlichen Zellquelle.

Ziel dieses Projekts war die Entwickelung einer neuartigen Strategie zur schnellen und effizienten Differenzierung von MCs aus humanen induzierten pluripotenten Stammzellen (hiPSC). Diese aus hiPSCs generierten MCs (hiPSC-MCs) weisen phänotypische und funktionelle Eigenschaften von primären humanen Hautmastzellen (hsMC) und Mastzellen, die aus peripheren hämatopoetischen Stammzellen generiert wurden (PSCMCs), auf. hiPSC-MCs zeigen eine stabile Expression typischer MC-assoziierter Rezeptoren wie CD45, CD117, FcεRIα, CD200R, Siglec-8 und MRGPRX2, und degranulieren als Reaktion auf eine Stimulation mit IgE/Anti-IgE und Substanz. P.

hiPSCs, reprogrammiert aus menschlichen Körperzellen, bieten aufgrund ihrer Selbsterneuerungseigenschaft eine kontinuierliche und nachhaltige Zellquelle für die Forschung. hiPSC-MCs stellen so einen bedeutenden Fortschritt in Richtung einer schnellen und hochproduktiven Generierung phänotypisch ausgereifter und funktioneller MCs dar und erlauben zudem die Entwicklung krankheitsspezifische Zellmodelle durch die Möglichkeit der hiPSC-MC-Generierung von Patienten.

1 Introduction

1.1 Mast cell as drivers of diseases

Mast cells (MCs) are multifunctional effector cells of the immune system and well known for antigen-dependent activation with binding of immunoglobulin E (IgE) to the high affinity IgE receptors (FcεRI).[1-2] However, depending on the circumstances, MCs have two faces: the activation of MCs can indeed confer either great benefit or harm to our body. On the one hand, MCs are able to recognize various stimuli and respond to such stimuli by releasing a panel of inflammatory compounds.[3] This enables MCs to play a key role in orchestrating inflammation, which can be utilized in the host defense against infectious agents. On the other hand, the deregulated activation of MCs contributes to the pathogenesis of allergic conditions, such as asthma and urticaria, as well as to other diseases, like mastocytosis, fibrosis and cancer. The significant clinical impact of MCs leads to the development of several strategies, such as inhibition of MC activation, reduction of MC proliferation, blockade of MC surface receptors and inhibition of MC mediators.[4-5]

Classically, human MCs have been categorized into three MC phenotypes, depending on their tissue distribution and granule content: MC produces tryptase only (MCT), MC expresses chymase only (MCC), and MC makes both tryptase and chymase (MCTC). MCT predominates in the lungs and intestinal mucosa and MCC are found at multiple tissue sites, predominantly in the stomach and lung. MCTC is located in the skin, intestine, stomach, myocardium, conjunctiva, synovium and many other connective tissues.[6-9]

MCs express a wide range of receptors, e.g. for IgE (FcεRI), IgG (FcγR), stem cell factor (SCF) (KIT receptor or CD117), complement and cytokines. The final consequence of such ligand receptor–based activation of MCs is the release of a broad array of mediators. The mature MCs contain abundant preformed cytoplasmic granules. While some mediators are preformed and remain stored in granules such as heparin, histamine, β-hexosaminidase and enzymes mainly chymase and tryptase, others are de novo synthesized only after activation including LTB4, LTD4, PDG2, and PAF, and the cytokines IL-10, IL-8, IL-5, IL-3, IL-1, GM-CSF, TGF-β, VEGF, and TNF-α.[10] Depending on the stimulus, MCs can calibrate their pattern of mediator release, induce the recruitment of other immune cells to modulate the amplification of allergic inflammation.

The most common physiological pathway is IgE (FcεRI)-mediated activation of MCs. Immunoglobulin E (IgE) is produced by the immune system and mostly found in the skin, connective tissue, respiratory and gastrointestinal tract. IgE binds to the high-affinity IgE receptors FcεRI expressed on the surface of MCs, and their crosslinking leads to degranulation and release of mediators. Strategies to inhibit or pre-emptively curb activation of mast cells are currently being explored for therapeutic purposes.[11-13]

1.2 The origin and development of mast cell

MCs are long-lived tissue-resident cells of hematopoietic origin that normally reside in mucosal and connective tissues.[2] Unlike most cells that originate from the myeloid lineage, MCs leave the bone marrow as multipotent hematopoietic stem cells (HSCs) rather than as circulating end-stage cells. This causes a big challenge for collecting and isolating patients-derived MCs from peripheral blood and hinders studying MC-driven diseases.

Until now, the origin of MCs is not yet completely understood. Classically, mature tissue-resident MCs develop from circulating MC progenitors (MCps), which enter the blood by transendothelial migration. MCps are only detected in a very small amount in the peripheral blood, and their origin and development still are controversially discussed. MCps are considered as a progeny of HSC via multipotent progenitors (MPP), common myeloid progenitors (CMPs) and granulocyte/monocyte progenitors (GMPs).[14] To what lineage MCs finally belong to is still debated. Recently, most data suggested that committed MCps originate from progenitors with both mast cell and basophil capacity within the GMPs population (Figure 1).[15-17]

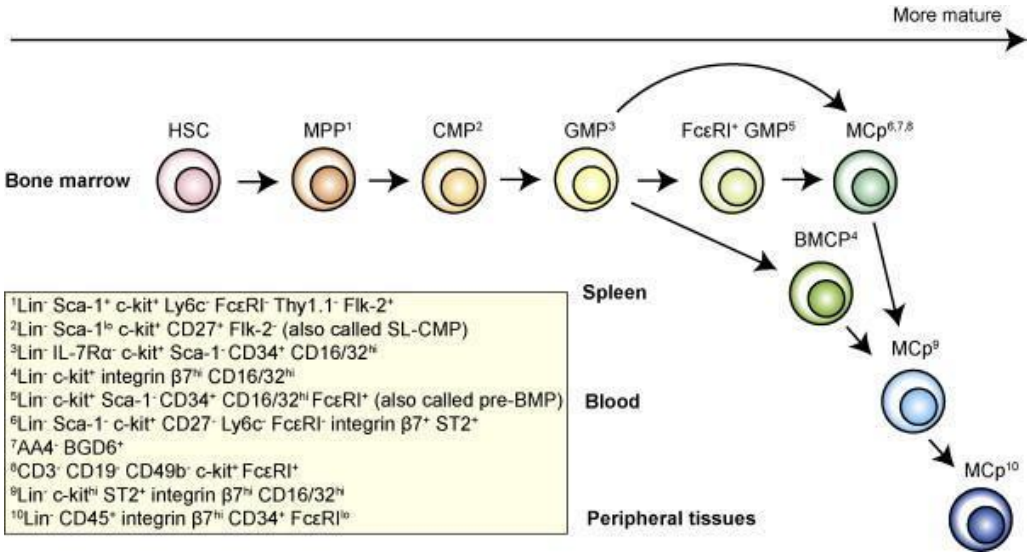

Figure 1. Proposed model for the development of MCps.

MCs originate from bone marrow HSC. MC progenitors (MCps) identified by Lin-CD45+ integrin β7hi CD34+ FcεRI to give rise to mast cells. MCps migrate to peripheral tissues where they further maturate. (From Dahlin JS *et al*., 2015). [15]

1.3 The bottleneck of studying mast cell-driven diseases

To study human MC functions *in vitro*, common MC models include the use of primary MCs isolated from tissues (such as the skin, intestine or lungs), and peripheral hematopoietic stem cell-derived mast cells (PSCMCs), generated from either peripheral or cord blood.[18-19] However, low proliferation capacity of MCs, donor-dependent heterogeneity and the scarce continuous tissue source present big challenges for *in vitro* MC research. To date, three MC lines (i.e. HMC1, LAD2 and LUVA cells) are available. LUVA cells were derived from a patient with aspirin-exacerbated respiratory disease, while HMC1 and LAD2 cells were derived from patients with mast cell leukemia and mast cell sarcoma, respectively. They can be used for studying MC functions, because of their MC phenotype, such as expression of intracytoplasmic histamine, tryptase and chymase, and release β-hexosaminidase in response to FcεRI crosslinking. However, they also exhibit various abnormal phenotypic and functional alterations.[20] Therefore, they can neither replace primary MCs or PSCMCs nor meet the needs for studying MC-driven diseases. Unlike normal MCs, LUVA cells are an immortalized cell line, and grow up fast without stem cell factor. LAD2 cells, on the other hand, are poorly suited for high-throughput screening assays that require large amounts of cells due to their very low proliferation rate with a doubling time of approximately 2 weeks and their poor cytokine

production compared to primary human MCs.[21] HMC1 is also an immortalized neoplastic KIT mutant cell line, it is considered unsuitable for degranulation assays, because of their immature phenotype and the lack of the expression of functional IgE-receptor.[20]

The prevalence of allergy is rising, which is an increasing global public health issue. Since MCs are considered the main effector cells in patients with allergic reactions. MC activation assays could serve as robust *in vitro* tool for exploring MC reactivity in allergy.[22] Allergen-specific IgE levels are commonly assessed to diagnose allergy *in vitro*. To detect biologically active allergen specific IgE in serum samples, MC activation test (MAT) that makes use of *in vitro* generated MCs, sensitized overnight with patients' serum and activated by allergens. However, the heterogeneity of MCs often causes poor reproducibility and instability of such *in vitro* allergy testing.[23] Though, MC lines provide a stable source for MCs with a high homogeneity, their use in the passive IgE sensitization assay is limited. To overcome these problems, alternative sources, such as cultured cell lines expressing the human FcεRI transfected into a rat basophilic leukemia line RBL-2H3. However, because of their rat origin, RBL-2H3 cells are vulnerable to high concentrations of human sera.[24]

Mastocytosis is a clonal MC disorder accompanied with systemic or local increases in MC numbers. A gain-of-function KIT mutation D816V is detectable in about 90% of patients with systemic mastocytosis (SM).[25] The KIT D816V mutation is considered the major driver in the pathogenesis in SM. The induced resistance of the KIT D816V mutant to the tyrosine kinase inhibitor Imatinib has led to large research efforts on novel selective tyrosine kinase inhibitors specialty targeting KIT D816V mutated KIT. A bottleneck in mastocytosis research and drug development is the lack of reliable *in vitro* models. There is only one KIT D816V human cell model available, which is the cell line HMC1.2. Unfortunately, it suffers from disadvantages that limit its utility in certain studies: it is considered an immature MC; it lacks a wild-type control and crucially lacks expression of the high-affinity IgE receptor. Recently, Rosine Saleh *et al.* have created a human transfected KIT D816V MC line, named ROSA KIT D816V. However, unlike normal MCs, it cannot be activated upon stimulation with IgE/antigen.[26] On the other hand, the biggest challenge for cellular research on mastocytosis is the limited availability of primary cell source. As of now, no successful isolation and culture of primary KIT D816V^{+} MCs has been reported.

1.4 A novel strategy for studying mast cell-driven diseases

Human induced pluripotent stem cells (hiPSCs) were pioneered by Shinya Yamanaka's lab in Japan in 2006.[27] Like embryonic stem cells (ESCs), hiPSCs retain unlimited expansion ability and enable to differentiate into any body cell; hiPSCs are reverted from somatic cells, such as fibroblast, peripheral blood mononuclear cells and virtually any other cell type could serve source for hiPSC reprogramming.[28-29] Therefore, hiPSCs are enabled to be reprogrammed from patients' somatic cells and can serve as an unlimited cell source for research purposes. In recent years, increasing commercialization of hiPSC reprogramming kits provides an easy and efficient method for generating hiPSC lines from patient's samples. The rapidly growing hiPSC banks around the globe have made the generation and use of hiPSCs much more easily accessible.[30-31]

To study allergic diseases, the generation of MCs from a healthy hiPSC line would provide an unlimited homologous cell source. For the diagnosis of allergic diseases, like urticaria, Akira Igarashi *et al.* have established a novel determining system for IgE and allergens, based on detection of hiPSC derived MCs biologically active and with functional IgE-allergen interaction and is expected to be established as the new allergy testing in clinical diagnostics and research settings in the future.[24]

To study MC-driven diseases, like mastocytosis, patient-derived hiPSCs would gain the ability to resemble the clinical situation more closely than immortalized cell lines derived from cancer patients (e.g. HMC1.2). The group of Martin Zenke reported on the first hiPSC-based model in association with systemic mastocytosis and envisions an expansion of our library of SM-derived hiPSCs carrying the KIT D816V mutation and other mutations. The use of such new research tools has already led to the identification of novel drug targets. Thus, nintedanib has been shown to exhibit KIT D816V inhibitory effects, due to its capacity to reduce the viability of hiPSC-derived KIT D816V hematopoietic progenitor cells and MCs in the nanomolar range.[32]

Another, very powerful application of hiPSC derived MCs would be the availability and generation of an unlimited homologous MC source and the development of *in vitro* models for MC-driven disease *in vitro* models. Despite the rapid development and promising application of hiPSC technology, it has encountered many challenges, especially in the differentiation of the target cells. In principle, hiPSCs can differentiate into any cell type of the human body, however, only a few protocols are yet able for

differentiation of mast cells. The major challenges included time consuming (>70d) or low efficiency procedures.[24, 33-34]

1.5 Aim of this study

MC research suffers from the lack of continuous and sustainable tissue and cell supply. To overcome this obstacle, we were seeking to develop a novel strategy for providing a homogeneous source for the generation of phenotypically mature and functional MCs. This thesis aimed to establish a novel approach for generating MC derived from hiPSCs. The overall aim of this project was divided into the following three objectives:

- Generation of MCs derived from three healthy hiPSC lines.
- Characterization of phenotypic features of hiPSC-MCs as compared to human skin and peripheral stem cell derived MCs.
- Investigation of functional properties of hiPSC-MCs assessed by activation and release assays.

2 Methods

2.1 Generation of mast cells

Human skin MCs (hsMCs) were isolated from breast skin or eyelids from plastic reduction surgeries at the Charité-Universitätsmedizin Berlin. The skin tissues were digested in 2.4 U/mL dispase type II (Corning) overnight at 4°C. After removing the epidermis, the tissue was minced and digested for 1 hour in Dulbecco's phosphate-buffered saline (DPBS) containing Ca2+ and Mg2+ supplemented (ThermoFisher) with 1% Pen/Strep, 5% FBS, 2.5 µg/mL amphotericin B (Corning), 5 mM MgSO4, 10 µg/mL DNaseI (Roche), 0.75 mg/mL hyaluronidase and 1.5 mg/mL collagenase type II (Cellsystems), at 37 °C with shaking. The cell suspension was filtered via 300 µm and 40 µm sieves, then the cells were spinned down at 300 ×g for 15 minutes at 4°C. That digestion cycle was repeated once and then cells were washed in Dulbecco's phosphate-buffered saline without Ca2+ and Mg2+. Mast cells were isolated using CD117 positive MACS enrichment Kit (Miltenyi Biotec) and cultured in basal Iscove's medium (ThermoFisher) supplemented with 1% Pen/Strep, 10% FBS, 1% non-essential amino acids (ThermoFisher) and 226 µM α-monothioglycerol. [35] After 24 hours, hsMCs were cultured in Basal Iscove's Medium (Biochrom) containing 100 ng/ml rhSCF and 20 ng/ml IL-4 (both from Peprotech).

Peripheral hematopoietic stem cell-derived mast cells (PSCMCs) were generated from CD34+ progenitors. Peripheral CD34+ progenitors were isolated and purified from buffy coats using EasySep™ Human CD34 Positive Selection Kit II (STEMCELL Technologies Inc.). In the 1st week, those human peripheral hematopoietic CD34+ stem cells were cultured with StemSpan™ CD34+ Expansion Supplement (STEMCELL Technologies). From 2nd to 4th week, cells were cultured in SFEM II medium (STEMCELL Technologies) supplemented with 50 ng/ml rhSCF, 20 ng/ml IL-3 and 20 µm/ml LDL. On day 28, PSCMCs were purified by positive cell separation using anti-human CD117 magnetic beads (Miltenyi-Biotec). Purified PSCMCs were cultured in SFEM II supplemented with 20 µg/ml LDL, 125 ng/ml rhSCF and 50 ng/ml IL-6.[35]

A four-step protocol has been designed for the generation of hiPSC derived MCs (hiPSC-MCs), based on the use of three hiPSC-lines (Figure 2). Firstly, hiPSCs were differentiated into hematopoietic stem cells (HSCs) using the STEMdiff™ Hematopoietic Kit (STEMCELL Technologies) according to the manufacturer's instructions. After a 10-day differentiation period, the CD34+ HSCs were harvested and purified with EasySep™

Human CD34 Positive Selection Kit II (STEMCELL Technologies). Like the method of PSCMC differentiation, hiPSC-CD34+ progenitors were differentiated into MCs, according to the method previously described. Finally, the cells developed into the mature hiPSC-MCs by at least 2-week culturing SFEM II medium containing LDL, IL-6 and rhSCF (all from PeproTech).[35]

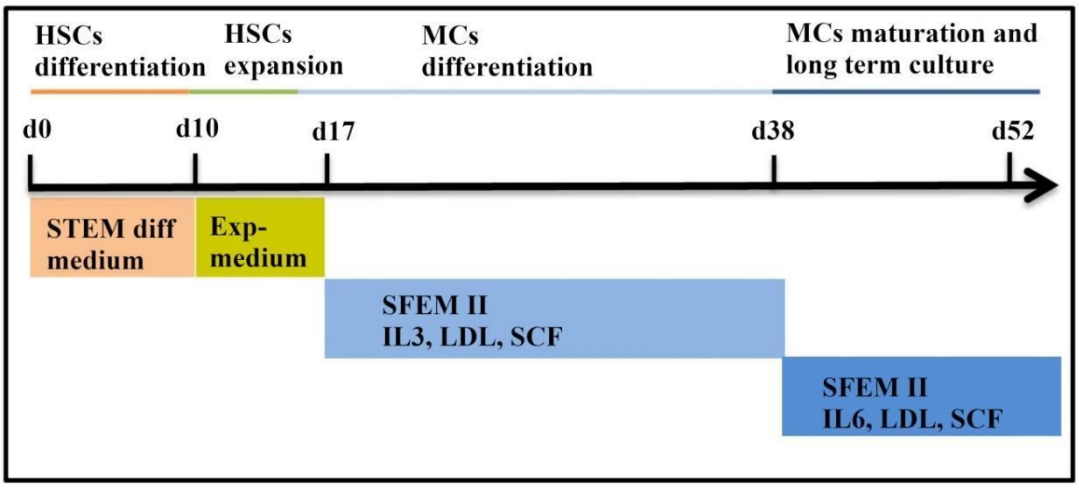

Figure 2. “Four-step protocol for differentiation of mast cells from hiPSCs.

1. Differentiation: hematopoietic progenitor cells (HSCs) were differentiated from hiPSCs in 10 days using the STEMdiff™ Hematopoietic Kit. 2. Expansion: CD34+ HSCs were purified and then expanded with StemSpan™ CD34+ expansion supplement. 3. MC differentiation: cells were cultured in differentiation medium (i.e. SFEM II medium containing 50ng/ml rhSCF, 20 ng/ml IL-3 and 20 ug/ml LDL) for three weeks. 4. MC maturation and culture: cells gave rise to fully mature and functional mast cells in MC medium (i.e. SFEM II supplemented with 20 ug/ml LDL, 125 ng/ml rhSCF and 50 ng/ml IL-6) at least for 14 days. The hiPSC-MCs were cultured in the MC medium at least up to 3 months”. (From Luo Y *et al*., 2022)[35]

2.2 Characterization of hiPSC-derived mast cells

2.2.1 Cytospin preparations

The phenotypic characteristics and activation of hiPSC-MC was analyzed by comparison with hsMC and PSCMCs. Cytospin preparations were made from an aliquot of 20,000 cells, centrifuged onto each slide by a cytospin (700 rpm, 5 minutes) and dried overnight. The air-dried slides were fixed in acetone for 10 minutes at room temperature and then

stored at -20 °C. After removal from the freezer and reaching room temperature, the slides were hydrated in TBS cytospins for staining.

2.2.2 Mast cell staining

Toluidine blue staining was performed according to the protocols by Mathilde Leclere *et al*. In brief, the toluidine working solution was prepared by 0.5 ml of toluidine blue stock solution (0.5 g toluidine blue O in 50 ml of 70% EtOH) in 4.5 ml of 1% sodium chloride (0.5 g NaCl in 50 ml double-distilled H_2O). Cytospins were stained in toluidine blue working solution for 60 minutes, washed in PBS three times and dehydrated quickly in 100% EtOH.

For May-Grunwald Giemsa (MGG) staining, the cytospins were placed in May-Grunwald solution for 4 minutes, washed in distilled water, then stained with Giemsa solution (Merck) for 4 minutes. After washing, slides were passed through increasing grades of alcohol for 2 minutes each, cleared in xylene and mountd.[36]

The cytospin slides were also stained with chymase and tryptase. Firstly, cytospins were incubated with Serum-free Protein Block (Dako) for 10 minutes at RT, and washed 3 times in TBS, incubated in primary antibodies: 1: 50 mouse-anti-chymase (Abcam) for 12 hours at 4 °C or 1:500 mouse anti-tryptase (Abcam) for 1 hours at RT. After washing, cells were then conjugated with 1:400 goat anti-mouse IgG antibody Alexa Fluor® 594 (Jackson ImmunoResearch Laboratories, Inc) in TBS + 2%GNS for 30 minutes at RT and again washed three times. The slides were mounted with a DAPI mount solution. Images were captured with a BZ-X800 upright fluorescence microscope.

2.2.3 Immunophenotyping

Immunophenotyping was investigated by flow cytometry. For cell surface staining, MCs were blocked for 15 minutes at 4 °C with human AB-serum (Biotest, Dreieich, Germany) and incubated with specific antibodies for 15 minutes at 4 °C. The antibodies were as follows: anti-CD34-allophycocyanin (APC) (BD Biosciences, Heidelberg, Germany), CD45-fluorescein isothiocyanate (FITC) (BD Biosciences), CD117-PE-Vio 615 (Miltenyi-Biotec), and FcεRIa- phycoerythrin (PE) (BioLegend), Mas-related G protein–coupled receptor X2 (MRGPRX2)-PE (Biolegend), CD200R-PE (BioLegend), and siglec-8-PE(R&D Systems, Minneapolis). Dead cells were excluded by 4’-6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Invitrogen, Karlsruhe, Germany) staining. The

stained cells were measured on the Facscalibur (BD Biosciences, San Jose, CA, USA) and analyzed with the FlowJo analysis software (FlowJo LLC, Ashland, OR, USA).

2.3 Mast cell activation assays

2.3.1 IgE-dependent activation

Cells were fed one day before the experiment. The cells were washed and resuspended in Tyrode's buffer, then aliquots of 100 µl were seeded into 96-well-plates (2×10^4 per well) and sensitized with 3.5 µg/ml human IgE (Merck, Darmstadt, Germany) at 37 °C for 1 hour. Then supernatant was removed by centrifuge (300 g, 3 minutes, RT). The cells were stimulated by 5 µg/ml anti-human IgE (Bio Legend) and 20 mg/ml ionomycin calcium salt (Sigma-Aldrich,St Louis, Mo) at 37 °C for 1 hour. The supernatant was collected by centrifugation at 300 g, RT for 3 minutes, and the pellet MCs rapidly froze with 100 µl H_2O at -80 °C for 1 hour. After thawing, aliquots of 50 µl supernatant and lysate separately were added 3.2 µl OPT reaction (containing 9 µl NaOH and 2.3 µl o-phthalaldehyde) and incubated for 4 minutes at room temperature. The assay is stopped with 3 µl HCl per 50 ul sample. Fluorescence intensity of histamine was measured using a 360 nm excitation filter and a 450 nm emission filter.[35]

To stimulate MCs, cells were incubated in Tyrode's buffer containing 3.5 µg/ml human IgE (Merck) for 1 hour and stimulated with 5 µg/ml anti-human IgE (Bio Legend) or 20 ug/ml calcium ionophore (Sigma), or 0.31 µM, 1.25 µM, 5 µM Sub.P (R&Dsystems) for 1 hour.

2.3.2 Non IgE-mediated activation

Cells were fed and inoculated into a 96-well plate at 2×10^4 per well one day before stimulation. To stimulate the MCs, cells were washed with Tyrode’s buffer once, and then stimulated with different concentrations of Sub.P (0, 0.31, 1.25 or 5 µM) (R&Dsystems) for 1 hour at 37 °C. As a positive control, cells were incubated in Tyrode's buffer containing 3.5 µg/ml human IgE for 1 hour and stimulated with 5 µg/ml anti-human IgE. For the β-hexosaminidase assay, the supernatant of each cell culture was withdrawn, and cells were lysed with distilled water of the same volume at -80 °C for 1 hour. Thawed lysates and the supernatant were transferred to the black 96 well plate. The same volume of 1:10000 diluted 4-Mug (4-Methylumbelliferyl-β-D-Glucuronidhydrat) working (Millipore Sigma) solution was added, incubated for 1h at 37 °C. Then the reaction was stopped by

addition of 100 µl of Na-carbonate buffer pH 10.7. Optical density (OD) was measured in the supernatants by Victor X5 2030 Multilabel Reader. β-hexosaminidase was measured at excitation 355 nm and emission 460 nm. For detecting MC activation markers, cells were stimulated with 0 or 5 µM Sub.P stimulation. Cell samples were stained by anti-CD63-PE antibody (Abcam). CD63 expression was assessed by flow cytometry.

2.4 Statistics

All data are presented as mean ± SEM of 3 donors or 3 independent experiments. One-way ANOVA with Tukey multiple comparisons test and unpaired Student t test were used. Data analysis was performed by GraphPad Prism (GraphPad Software).

2.5 Ethical Statement

Four healthy hiPSC lines (BIHi005-A, BIHi001-A, BIHi001-B) were obtained from the Berlin Institute of Health. hiPSCs were derived previously from fibroblasts commercially available at ATCC: CRL-2429 (BIHi001) and approved by Stanford University (BIHi005-A, approval no. 350–panel:3). The Ethics Committee of Charité – Universitätsmedizin Berlin has approved this work (EA2/150/19). The generation and use of primary human cells has been previously approved by the Ethics Committee of Charité – Universitätsmedizin Berlin (EA1/141/12).[35]

3 Results

3.1 Generation of hiPSC-derived mast cells

In the 1st stage (day 0 - 10), hiPSCs were differentiated into CD34+/CD45+ hematopoietic stem cells (HSCs). Starting from 40±7 hiPSC colonies with an average diameter of about 150 µm (Figure 3Ai), the first appearance of round non-adherent cells was observed at day 5 (Figure 3Aii). On day 10 (Figure 3Aiv), 2,200,000 ± 253,000 non-adherent cells were collected. Those cells were round with an average diameter of 8 µm. At this timepoint, 56.1 - 94.5% of cells were positive for CD34. The number of CD34/CD45 double-positive cells varied among the three hiPSC lines 19.3% (BIHi001-A), 5.3% (BIHi001-B) and 25.5% (BIHi005-A), respectively and was lower when compared to HSCs isolated from the peripheral blood, as assessed by flow cytometric analyses (Figure 3B).

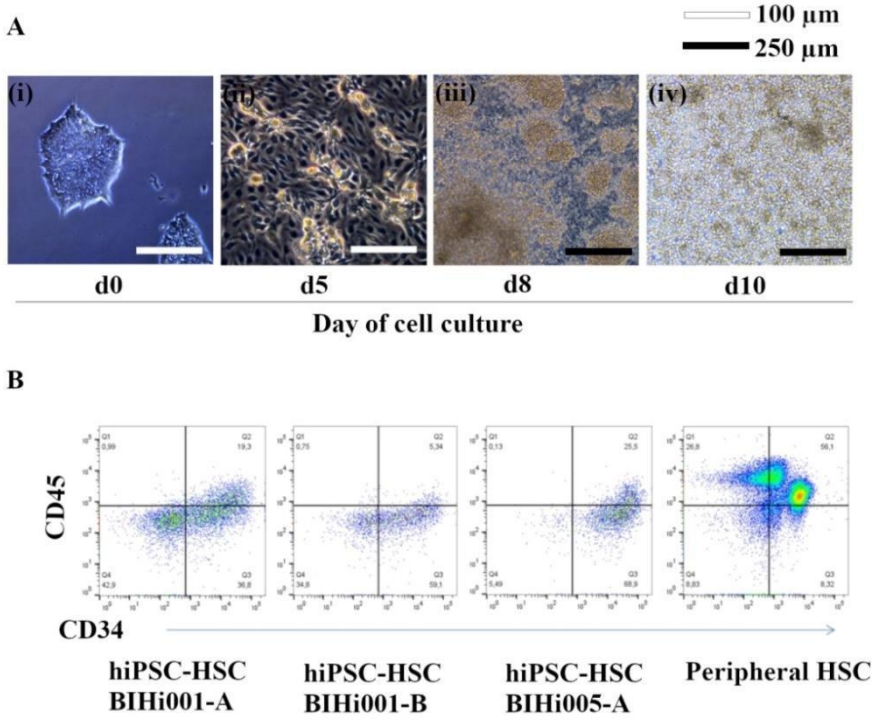

Figure 3. “Differentiation of hematopoietic stem cells from human hiPSC (hiPSC-HSC). (A) The microscopic morphology of hiPSCs and HSCs: on day 0, the differentiation started with hiPSC colonies about 100-250 µm in diameter; from day 5, first non-adherent cells appeared; on day 8, clusters of spherical cells appeared; on day 10, large numbers of non-adherent cells were present. (B) Flow cytometric analysis of hiPSC-HSCs on day 10 and freshly isolated CD34+ peripheral stem cells.” (From Luo Y *et al*., 2022)[35]

In the 2nd stage (day 10 - 17), HSCs were cultured and expanded with StemSpan™ CD34+ Expansion Supplement for one week.

During the 3rd stage (day 18 - 38), HSCs differentiated into hiPSC-MCs in the SFEM medium containing IL3, LDL and SCF. The cultures exhibited an increasing expression of the maturation markers CD45, CD117 and the high-affinity IgE receptor FcεRIα, whereas expression of the stem cell marker CD34 decreased (Figure 4B). At the end of this stage, few adherent cells were seen in the culture, but many cells exhibited the heterogeneous phenotypes (Figure 4Aiii).

In the 4th stage (day 38 - 52), the cells developed further into the mature MCs in the SFEM medium containing IL6, LDL and SCF. At the end of this stage, no more adherent cells were seen. They presented as a homogenous population (Figure 4Aiv). Under the microscope, all of the suspended cells were round with a relatively high nuclear-to-cytoplasm ratio and a diameter of about 20 µm. The level of MC surface markers on hiPSC-MCs was comparable to hsMCs and PSCMCs (Figure 4B-C).

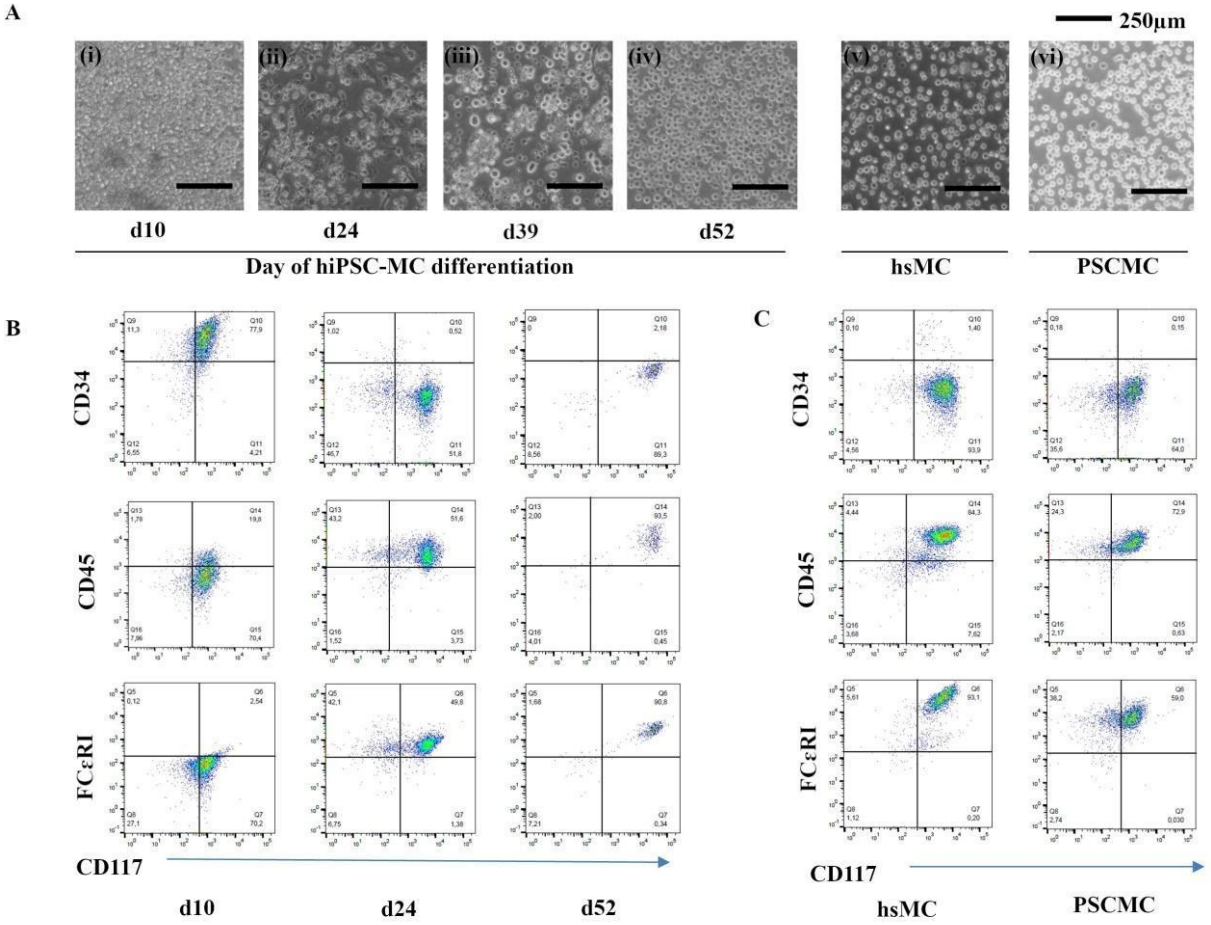

Figure 4. “Changes in cell morphology and surface receptor expression during differentiation of hiPSCs-HSCs into hiPSC-MCs:
(A) Changes in cellular morphology of hiPSC-MCs BIHi00-5A as a representative example during culturing in MC differentiation medium (i)-(iv). Cultures of hsMCs preparation (v) (one day after isolation) and a PSCMC preparation (vi) (one day after CD117 enrichment) were shown for comparison. (B) Flow cytometric analysis of changes in CD34, CD45, FcεRiα and CD117 expression during differentiation from hiPSC-HSCs into hiPSC-MCs at culture day 10 and day 24 as well as hiPSC-MCs at culture day 52. (C) Flow cytometric analysis of hsMCs and PSCMCs cultures was shown for comparison. Data were representative examples from 6 individual experiments (two cultures for each hiPSC line and 2 hsMC and 2 PSCMC preparations)”. (From Luo Y *et al*., 2022)[35]

Taken together, in these experiments we used 40±7 colonies of hiPSCs, which resulted in 692,000 ± 107,000 viable MCs. The yield of MCs can readily be scaled up at

the stage of hiPSCs and/or HSCs depending on the experimental needs. The yield of PSCMCs from peripheral blood and primary MCs from skin tissue has been compared to hiPSC-MCs (Figure 5, Table 1).

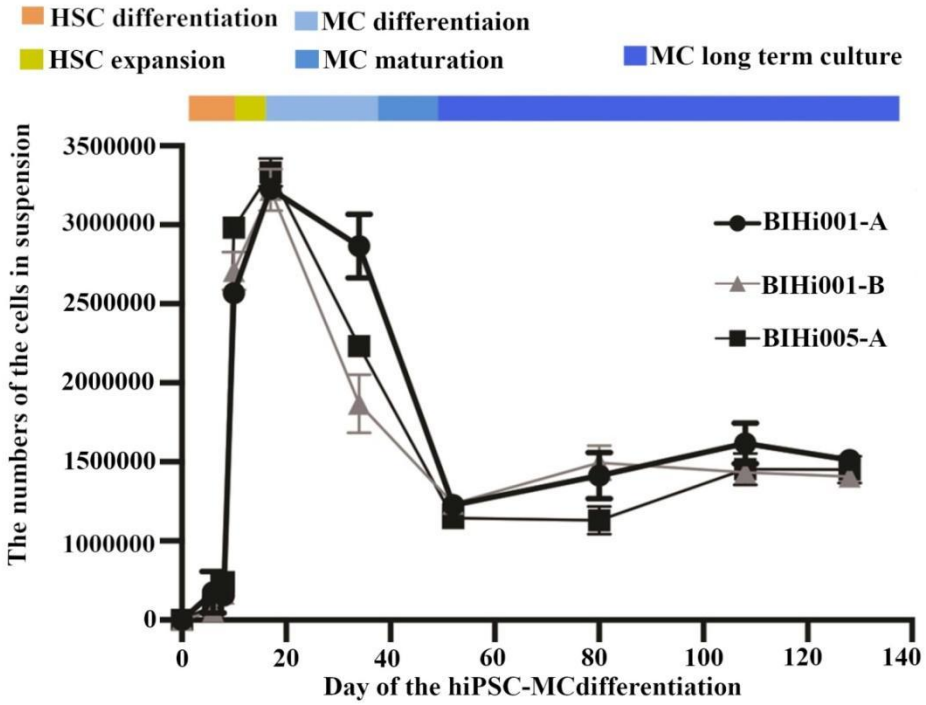

Figure 5. “Proliferation of hiPSC-MC cultures:
HSCs were differentiated from all of the three hiPSC lines when starting from 40 ± 7 hiPSC colonies. During the HSC differentiation (stage 1) and expansion phase (stage 2), the progenitor stem cells proliferate strongly. During the MC differentiation phase (stage 3) the cell numbers decreased but stabilized upon dead cell removal (day 52) and the generated MCs proliferated during long term culture (stage 4). All data were presented as mean ± SEM of 3 independent experiments”. (From Luo Y *et al*., 2022)[35]

Table 1. Generation and frequency of hiPSC-MCs, PSCMC and hsMC.

Experiment	hiPSC lines on day 0 (40 ± 7 colonies)	# of HSCs on day 10	# of MCs on day 52
1	BIHi001-A	2,000,000	800,000
2	BIHi001-A	2,000,000	700,000
3	BIHi001-B	2,200,000	760,000
4	BIHi001-B	2,000,000	740,000
5	BIHi005-A	2,600,000	650,000
6	BIHi005-A	2,400,000	500,000
Mean±SEM		2,200,000 ± 253,000	692,000 ± 107,000
	Skin tissue [per gram]		199,000 ± 50, 000
	Peripheral blood [per 50ml]		2,183,000±1,371,000

Note: At day 0, 40 ± 7 hiPSC colonies were seeded in 2 wells of 12-well plate, the numbers of hiPSC-MCs were counted at day 10 and 52. The numbers of PSCMC were counted on day 35. hsMC was counted one day after isolation. Data were from three donors and results were presented as mean ± SEM. (Modified from Luo Y *et al.*, 2022)[35]

3.2 Phenotypical characterization of hiPSC-MC

The morphology of mature hiPSC-MCs was assessed by MGG and toluidine blue staining. The MGG-stained hiPSC-MCs contained purple cytoplasmic granules. Granular metachromasia as assessed by toluidine blue staining was similar in hiPSC-MCs, PSCMCs, and hsMCs (Figure 6). All three populations showed equally strong expression

of the proteases chymase and tryptase as assessed by immunofluorescence staining (Figure 6). The expression profile of selected functional receptors, i.e., MRGPRX2, CD200R and Siglec-8, was examined by flow cytometry on hiPSC-MCs that were cultured for 84 days (Figure 7A and 8A).

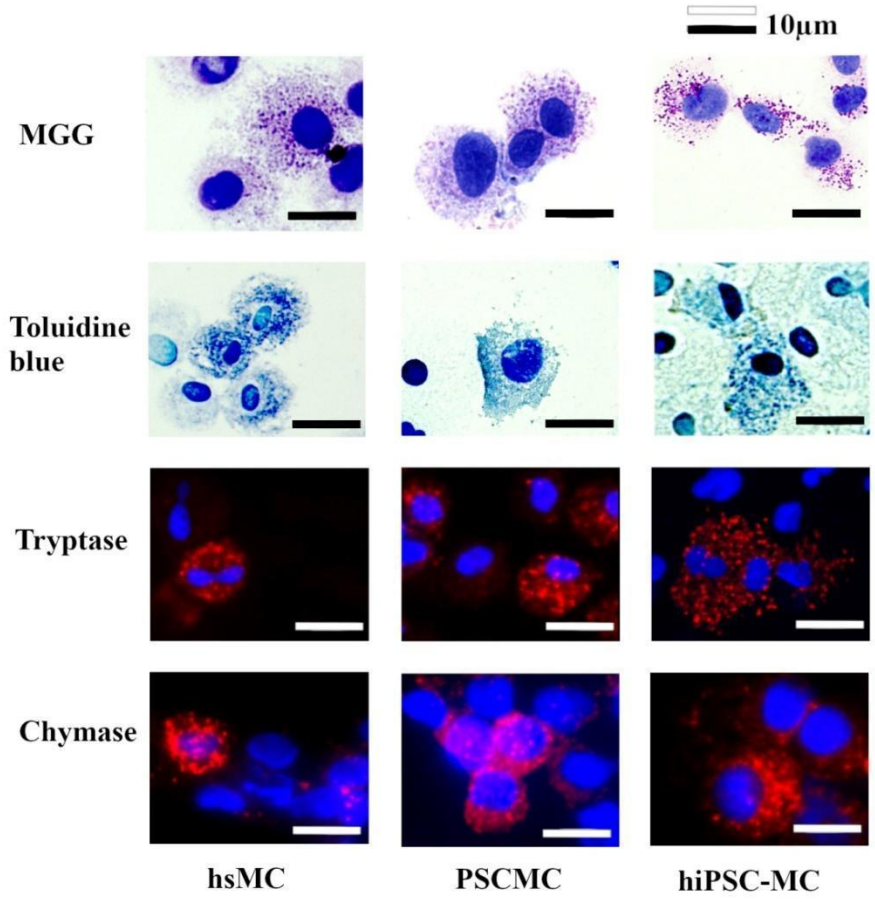

Figure 6. “Morphology and phenotypic characteristics of mature hiPSC-MCs:
MGG and toluidine blue staining on mature hiPSC-MCs, PSCMCs and hsMCs. Moreover, hiPSC-MCs, hsMCs and PSCMCs were stained with anti-tryptase and anti-chymase antibodies. Nuclei were stained with DAPI.” (From Luo Y *et al*., 2022)[35]

3.3 Activation of hiPSC-MC

Functional analyses revealed the activation of hiPSC-MC via IgE/anti-IgE dependent pathway. Anti-IgE induced activation of hiPSC-MCs resulted in the release of histamine (Figure 7B). Furthermore, MRGPRX2 expression and an IgE-independent activation of

hiPSC-MC were also observed (Figure 8A). The results showed the increased expression of MC activation marker CD63 and updose-dependent degranulation upon stimulation with Sub.P alone, a known MRGPRX2 agonist (Figure 8B-C).

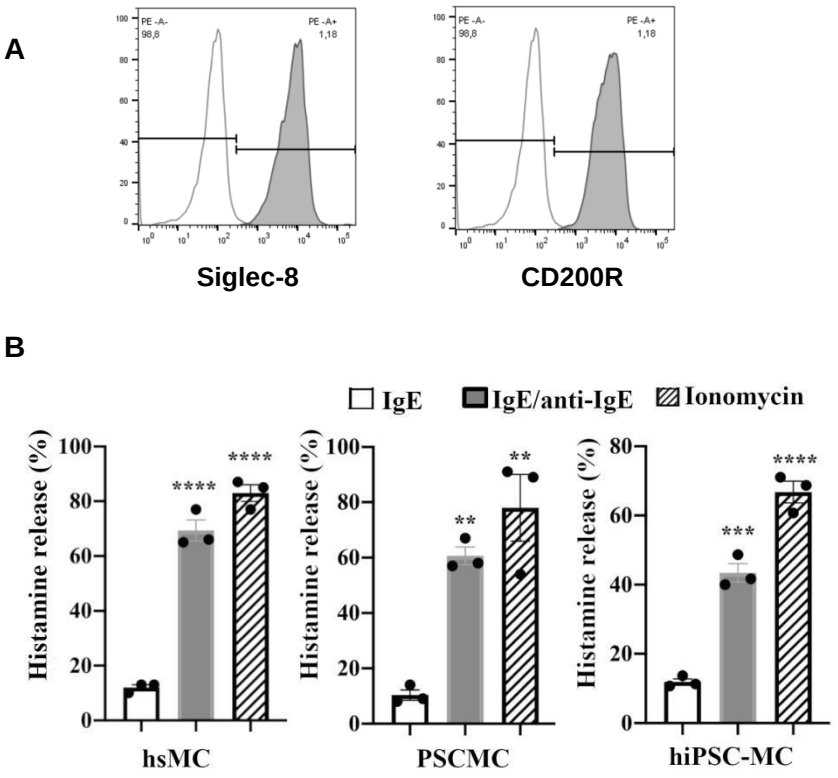

Figure 7. IgE-dependent activation of mast cells:
"(A) Expression of selected functional receptors on hiPSC-MCs (BIHi00-5A; 3 months in culture). Histograms were representative examples from 3 individual experiments. (B) Histamine release stimulated by IgE, IgE/anti-IgE antibody and ionomycin. All data were presented as mean ± SEM of experiments conducted from 3 healthy donors. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05, unpaired Student's t-test." (Modified from Luo Y *et al.*, 2022)[35]

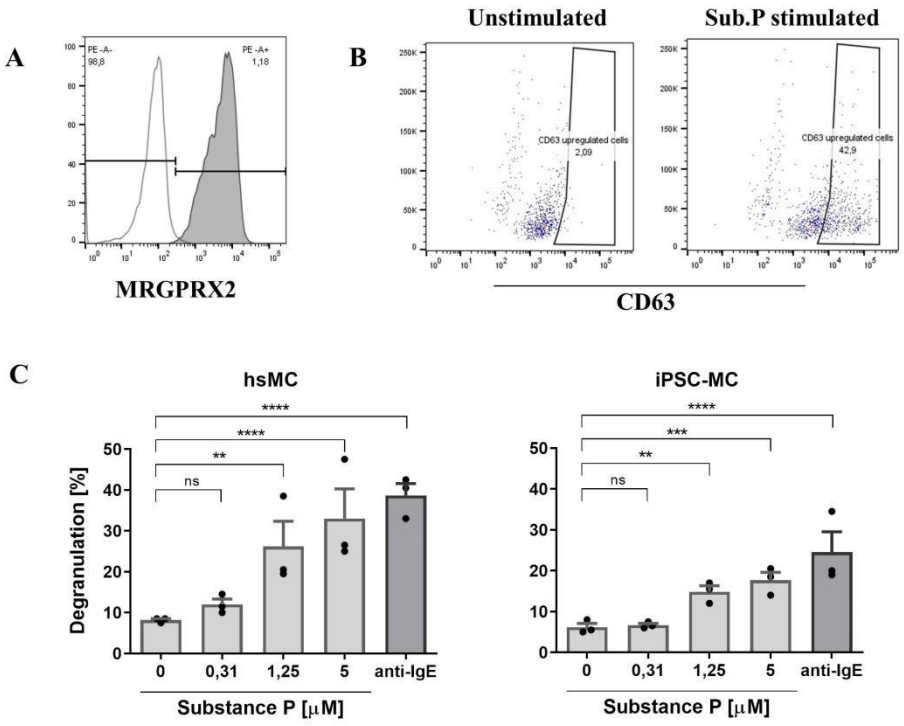

Figure 8. IgE-independent activation of mast cells:

(A) Expression of MRGPRX2 receptors by hiPSC-MCs. (B) The expression of MC activation marker CD63 increased on hiPSC-MCs surface after Sub.P stimulation (5μM). (C) Dose-dependent degranulation of human skin MCs (n = 3 donors) and hiPSC-MCs (n = 3 donors) measured by ß-hexosaminidase release upon stimulation by Sub.P, a known MRPGRX2 agonist (concentration as indicated). Degranulation is defined as the brutto release of ß-hexosaminidase in % of content total, anti-IgE was used as positive control (5 µg/ml). hiPSC-MCs were differentiated from hiPS cell line BIHi00-5A and cultured for 3 months in MC medium. Histograms were representative examples from 3 individual experiments. Bars represent mean±SEM; **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$ (One way-ANOVA with Tukey's multiple comparisons test). (Modified from Luo Y *et al*., 2022)[35]

4 Discussion

4.1 Short summary of results

From the studies conducted for this dissertation, a novel strategy for MC generation has been described. This approach provided a productive generation of functional MCs from human induced pluripotent stem cells (hiPSC-MCs). hiPSC-MCs exhibit tryptase and chymase double positivity, express typical MC-associated receptors such as CD45, CD117, FcεRIα, CD200R, Siglec-8 and MRGPRX2, and degranulate in response to IgE/anti-IgE and SP. Moreover, this study is, to the best of our knowledge, the first to report that: (1) hiPSC-MCs exhibit features that allow for non-IgE-mediated activation; (2) hiPSC-MCs share critical phenotypic and morphologic features comparable to human skin MCs and PSCMCs; (3) by exploring several iPSC lines from three independent healthy donors, iPSC-MCs exhibit a robust reproducibility and homogeneity.

4.2 Interpretation of results

In the past, the comparative potentials of two culture platforms for supporting *in vitro* hematopoietic differentiation of hPSCs have been explored: embryoid body (EB) three-dimensional (3D) formation and via monolayer two-dimensional (2D) culture.[37] Both harbor substantial challenges for the generation and differentiation of human MCs, such as time consuming, low efficiency or poor limited functionality. In this study, we improved a feeder-free 2D method for the generation of MCs derived from iPSCs. At day 52, more than $5x10^5$ functional MCs differentiated from 40±7 colonies of each hiPSC line. The productivity of functional hiPSC-MCs on day 52 in our study equals the expected outcome of monolayer/2D culture protocols reached at day 70 to 90.[24,34] In fact, in our study, the differentiation of mature hiPSC-MCs from HSCs was achieved within only 44 days, excluding an optional 7-day period of HSC expansion, which markedly increased the total amount of MCs.

The shortening of the differentiation period in our study may have resulted in a higher rate of CD34/CD45 double-positive HSCs productivity that was among the three hiPSC lines (5.3-25.5%), as compared to a previously published protocol (0.5%) by Igarashi *et al*.[24] Though, the ontogeny of MCs is still to be traced, it has been previously suggested that tissue-resident MCs may develop from circulating Lin-CD45+ integrin β7hi

CD34+ FcεRI+ MC progenitors. Thus, increasing the yield and purity of CD34/CD45 double-positive hiPSC-HSCs could contribute to a more rapid and more efficient production of hiPSC-MCs. As of yet, the most efficient protocol for iPSC-MCs differentiation has been reported by Kauts *et al*., who made use of a Gata2 transfection strategy, known as important signaling pathway for HSC and MC differentiation. With this approach, hiPSCs started to give rise to MCs within 12 to 16 days via EB formation and by OP9 co-culture method.[33] However, although this method showed good results on murine cells, it turned out to be unfortunately extremely less efficient on human MCs. Nevertheless, these species-specific results led to the suggestion that sorting for HSC with a propensity for MC development could improve the generation of human MCs. So far, there are only two published protocols aiming to the generation of iPSC-MCs via EB/3D formation.[32] It is yet not clear whether hiPSC-MCs that are differentiated via EB/3D formation will exhibit reasonable morphologic and functional features.

MCs can be activated by two main pathways: IgE-dependent and -independent stimuli. To evaluate the activation and function of hiPSC-MCs, we measured surface expression of the high-affinity IgE receptors and assessed the release of histamine in response to FcεRIα/IgE-dependent stimulation, as it has been previously attempted by Igarashi *et al.*[24] In addition to the elaboration on the IgE-dependent activation pathway, our study demonstrated, for the first time, that mature hiPSC-MCs highly express functional Mas-related G protein-coupled receptor X2 (MRGPRX2). When stimulated with SP, a known MRGPRX2 agonist, hiPSC-MCs showed signs of degranulation in a concentration-dependent manner. Thus, hiPSC-MCs seem to be well suited for use in further studies investigating the pathophysiological function of the MRGPRX2 expressed by MCs.

4.3 Embedding the results into the current state of research

4.3.1 hiPSC-MC as a diagnostic tool for IgE-mediated disorders

MCs play a significant role in allergic and inflammatory disorders through IgE-dependent and -independent activation pathways. Previously, research on both MC activation and host response mechanism focused on the FcεRI/IgE-mediated pathway. Cell-based *in vitro* diagnostics are currently limited by various challenges including the lack of a continuous cell source and the donor-dependent heterogeneity of PSCMCs. hiPSC lines

are considered as an unlimited cell source with robust proliferation capacities and a high rate of reproducibility and homogeneity within different cell batches. Igarashi *et al.* reported that human iPSC-MCs exhibit a stable expression of the high-affinity IgE receptors and functionally respond to various allergens when previously sensitized with human sera from allergic patients containing allergen-specific IgE. These iPSC-MCs were applied in a passive IgE sensitization system, named iMAT. This system worked well, even with undiluted human sera, and was able to detect allergen-specific and biologically active IgE in sera that had been previously tested low-responsive by basophil activation test (BAT).[24] Thus, hiPSC-MCs were suggested as an alternative and sensitive diagnostic tool to determine IgE/allergens in clinical routine. However, the analytical sensitivity and clinical relevance of the iMAT method need to be further proof studied. An appropriate inclusion of more well-documented patients and comparison with positive results for serum specificity IgE (sIgE), skin testing, and a CD63-based basophil activation test (BAT) will be critical for robust analyses. The larger collaborative studies are required to confirm these promising observations and to allow mainstream use.

4.3.2 hiPSC-MC facilitate research on non IgE-mediated disorders

In the last 10 years, investigating non-IgE-mediated mechanisms of MC degranulation and immune host response has gained more immense interest. MRGPRX2 has emerged as a significant MC receptor in IgE-independent drug reactions and the pathogenesis of MC-driven skin diseases such as chronic urticaria.[38-39] It is highly expressed on MCs in the skin and believed to be responsible for various non-IgE-mediated pseudo-allergic reactions by triggering MC activation and promoting multicellular signaling cascades by the release of proinflammatory mediators.[40] MCs have been demonstrated to interact with neuronal cells via MRGPRX2, which induces itch. In patients with chronic spontaneous urticaria and atopic dermatitis, increased levels of MRGPRX2 agonists (e.g. Sub.P, major basic protein, eosinophil peroxidase) have recently been reported.[40] Therefore, MRGPRX2 and its agonists are potential candidates for surrogate markers in assessing progression of inflammatory diseases and targets for treatment of signs and symptoms in patients with chronic inflammatory skin diseases and/or drug hypersensitivity reactions. Our study demonstrated, for the first time, that mature hiPSC-MCs highly express MRGPRX2 and degranulate in response to the MRGPRX2 agonist SP in a concentration-

dependent manner. Therefore, hiPSC-MCs seem to be a proper tool for future MRGPRX2-related research and new drug discovery investigations.

4.3.3 hiPSCs enable MC-driven disease-specific *in vitro* models

In vitro studies aiming to investigate biological MC functions have taken on several challenges in the past, including the lack of sufficient amounts of cells, donor-dependent heterogeneity and, in particular, missing access to patient-derived MCs. Because of their pluripotency and self-renewal capacity, the use of iPS cells for the generation and differentiation of mature human MCs might remove past hurdles and become a powerful tool for studying disease-specific features of MC-driven disorders. hiPSCs allow for the development of disease-specific models by generating patient-derived hiPSC lines. For a long time, MC-driven disease models were based on animal studies and immortalized or neoplastic cell lines. However, the problem of inter-species differences has often nullified exciting novel findings and, thus, shattered promising drug targets and therapeutic study results. Studies based on immortalized or neoplastic cell lines often neglect pathophysiological issues compared to the real patient. Besides, neither immortalized cell lines nor animal models, could as of yet cover the entire genetic background or the species-specificity of disease-defining genetic and epigenetic alterations.[42] hiPSC-MCs enable their generation from patients' cells, thereby, transferring clinical phenotypes. In fact, yet no disease-specific *in vitro* model for mastocytosis, a prototype MC-driven disorder, is available that sufficiently resamples the heterogeneity of the actual clinical settings. Thus, hiPSC-MCs could enable us to generate disease-specific *in vitro* models that additionally harbor phenotypes to be linked to the clinical presentations based on MC-related genetic alterations, such as the mastocytosis-defining KIT D816V mutation[43], combined with the patient-specific background. In future, the generation of mutant patient-derived hiPSC lines might hold great promise for a better understanding of disease pathologies, novel drug discoveries, and biomedical research.

In contrast to the existing MC lines, the hiPSC-MC model allows us to study human diseases at different developmental stages and to evaluate drugs designed for targeting MC progenitors and/or HSCs. Since MCs derive from the bone marrow, they are released into the circulation as immature MC progenitors and do not fulfill their maturation until they have entered their target tissue to undergo terminal differentiation. Researchers require detailed knowledge about the hematopoiesis altered by mutated

stem cells to better understand the pathogenesis of MC-driven diseases and the respective clinical phenotypes. Thus, in addition to mature MCs, future *in vitro* drug testing will also require the use of MCp/HSC. For instance, in mastocytosis, the common presence of the KIT D816V mutation has led to large research efforts towards novel selective tyrosine kinase inhibitors targeting mutant MC growth and/or activation.[44-45] The oral multikinase inhibitor midostaurin (PKC412) produces a >50% reduction in MC burden in patients with systemic mastocytosis.[46] However, the detailed mechanism of action is yet not fully understood. Based on hiPSCs derived from patients with advanced systemic mastocytosis, Zenke *et al*. conformed that midostaurin significantly reduce the viability of both patients' mutant HSC and MCp.[32]

4.3.4 Genome editing in hiPSCs for MC-driven disease models

The combination of the hiPSC-MC and the CRISPR/Cas9 gene editing technologies provides an extraordinary opportunity to study MC-driven diseases. CRISPR (clustered regularly interspaced short palindromic repeats) technology has emerged as a powerful technology for genome editing. Compared with other gene editing tools, it is an easy-to-use, highly specific, and efficient genome editing tool that has been now increasingly applied to the studies in basic biomedical research to explore gene function and generate disease models. The CRISPR/Cas9 system consists of two parts: the Cas9 nuclease and a guide RNA (sgRNA). The Cas9 enzyme introduces the sequence-specific targeted mutation into the genome by sgRNA precisely directing.[43] Since the discovery of this system, it has been considered as the most efficient accurate gene editing tool. However, an efficient gene editing is based on the successful delivery of sgRNA and Cas9 into cells. That is often dependent on the cell type and the structure of the target molecule.[46] Unfortunately, some cell types are particularly difficult to transfect, for instance, CD34+ HSCs and MCs, due to their low rate of cell division and/or a combination of defined and undefined cellular features.[47-49] That is why the use of gene editing technologies in research on MC biology and MC-driven diseases is currently limited. In fact, we have tested a variety of strategies with limited success in PSCMCs and CD34+ HSCs in previous studies. In contrast, hiPSCs are easy-to-transfect cells. Moreover, the transfected hiPSCs can be selected and banked as a cell line. In our ongoing research, we achieved 75-92% efficient genome editing via CRISPR/Cas9 in hiPSCs and established a gene edited KIT D816V mutant hiPSC line, which enables for an *in vitro*

model, which might lead to a better understanding of the disease pathophysiology in mastocytosis in the future.

4.4 Strengths and weaknesses of the study

hiPSC-MCs are not meant to (and cannot) replace primary human MCs but are complementary for *in vitro* studies. Major limitations of hiPSC-MCs include the complexity of the hiPSC technology that requires extended experimental and time-consuming conditions as compared to existing *in vitro* MC models, e.g. hsMCs and PSCMCs. Thus, the costs for hiPSC-MC differentiation are similar as for PSCMCs; most of the costs for generating hiPSC-MCs derive from personnel and consumables.

On the other hand, cellular heterogeneity is a critical issue that cannot and should not be ignored when exploring tissue/organ-specific MC features and functions. The impressive heterogeneity of MCs often brings multiple challenges and interferences to *in vitro* experiments. MC heterogeneity is presumed to be caused by its ontogeny, tissue microenvironment, and donors. In fact, even MCs derived from the same tissue or culture dish could exhibit variation in the level of receptor expression, cytochemical features, epigenetic controls on gene expression, responses to stimuli and functions. There are still large gaps in our knowledge of the ontogeny and heterogeneity of MCs. Until now, it is not clear how MCs achieve their heterogeneity in peripheral tissues.[51] Studies on different human MC populations are needed. Working with hiPSC-MCs will complement the work that has already been done with primary human MCs (e.g. derived from lung and skin tissue).

Of note, hiPS cell lines also exhibit obvious donor-specific heterogeneity. Though the variable genetic and phenotypic traits could restrict human hiPSC applications, which remains a challenge, it is also a potential opportunity for MC-driven disease research. The hiPSC lines transferring genetic background differences could be useful for studying the role and relevance of genetic variants in different diseases or phenotypic traits.[52-53] To overcome the heterogeneity of cell sources and investigate donor-specific phenotypes and pathologies, there is a need for cell banks, which enable to provide large healthy and patient-derived disease-affected cell lines and provide extended services for cell differentiation. Recently, the rapid development of hiPSC banks provides an essential resource for researchers around the globe to gain access to cell lines with different

genetic background and, especially, disease-specific features. Such developments will tremendously speed up hiPSC technologies in basic research and clinical applications.

4.5 Implications for practice and/or future research

We believe that hiPSC-MCs represent a valuable and useful addition for *in vitro* MC biology research. This approach offers the ability to generate functional human MCs derived from hiPS cell lines with multiple application opportunities, including high-throughput screening assays, drug discovery and evaluation, gene editing and routine clinical diagnostics. It allows for the generation of disease- and patient-specific MC populations and, thus, opens the door for personalized precision medicine and management for MC-driven diseases.

5 Conclusions

Taken together, the findings of this doctoral thesis provide a novel approach, by a 4-step protocol, for the generation of MCs from human iPS cells. hiPSC-MCs exhibit phenotypic and functional characteristics of human skin MCs (hsMCs) and peripheral hematopoietic stem cell-derived MCs (PSCMCs), including MC-associated receptors such as CD45, CD117, FcεRIα, CD200R, Siglec-8 and MRGPRX2, and degranulate in response to IgE/anti-IgE and SP stimulation. The approach promises to be useful for providing a continuous and sustainable source of human MCs and additionally opens the gate for disease or patient-specific *in vitro* MC models. Thus, this novel approach could markedly contribute and facilitate the development and testing of new treatment strategies for MC-driven disorders and improve our understanding of MC biology.

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Statutory Declaration

“I, Yanyan Luo, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Generation of mast cells from human induced pluripotent stem cells: a novel strategy for studying mast cell-driven disorders (in English). Generierung von Mastzellen aus humanen induzierten pluripotenten Stammzellen: eine neue Strategie zur Untersuchung Mastzell-vermittelter Erkrankungen (in German)”, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other people.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

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Declaration of your own contribution to the publications

Yanyan Luo contributed the following to the below listed publications:

Publication: Publication: Luo Y, Vallone VF, He J, Frischbutter S, Kolkhir P, Romero SM, Stachelscheid H, Streu-Haddad V, Maurer M, Siebenhaar F, Scheffel J. A novel approach for studying mast cell-driven disorders: mast cells derived from induced pluripotent stem cells. J Allergy Clin Immunol. 2022; 149(3):1060-1068.e4. (Impact factor: 10.228)

Contribution (in detail): designed major experiment, performed the culture, differentiation, characterization, function observation and RNA extraction, as well as produced figures, graphs, tables, and written work for this publication. I also worked on Bioinformatics data analysis, study design, and wrote revisions prior to publication with the help of other co-authors. Figure 1, 2, 3, 4, 6A-C, E1 and table 1 were created based on my experimental data and analysis. Figure 5, 6D, E2 were created based on my experimental data and analysis with co-authors.

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Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

Excerpt from Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2019** Selected Editions: SCIE,SSCI Selected Categories: '**ALLERGY**' Selected Category Scheme: WoS Gesamtanzahl: **28 Journale**

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY	52,417	10.228	0.077410
2	ALLERGY	18,217	8.706	0.024340
3	Journal of Allergy and Clinical Immunology-In Practice	9,255	8.861	0.019690
4	CLINICAL REVIEWS IN ALLERGY & IMMUNOLOGY	3,317	6.437	0.005910
5	Clinical and Translational Allergy	1,163	5.129	0.011830
6	ANNALS OF ALLERGY ASTHMA & IMMUNOLOGY	8,213	4.969	0.011830
7	ALLERGOLOGY INTERNATIONAL	2,335	4.806	0.004120
8	PEDIATRIC ALLERGY AND IMMUNOLOGY	4,456	4.699	0.005920
9	CLINICAL AND EXPERIMENTAL ALLERGY	10,602	4.217	0.011490

10	Allergy Asthma & Immunology Research	1,589	4.157	0.003110
11	CONTACT DERMATITIS	6,326	3.952	0.003550
12	Journal of Asthma and Allergy	577	3.730	0.001450
13	CURRENT ALLERGY AND ASTHMA REPORTS	2,574	3.577	0.005490
14	World Allergy Organization Journal	1,872	3.506	0.002750
15	JOURNAL OF INVESTIGATIONAL ALLERGOLOGY AND CLINICAL IMMUNOLOGY	2,263	3.488	0.002200
16	Current Opinion in Allergy and Clinical Immunology	2,838	3.246	0.003820
17	IMMUNOLOGY AND ALLERGY CLINICS OF NORTH AMERICA	1,709	3.000	0.002840
18	Allergy Asthma and Clinical Immunology	1,784	3.406	0.002230
19	ALLERGY AND ASTHMA PROCEEDINGS	2,039	2.414	0.002620
20	Allergy Asthma and Clinical Immunology	1,226	2.104	0.002250
21	JOURNAL OF ASTHMA	3,738	1.899	0.004950
22	JOURNAL OF ASTHMA	5,004	2.515	0.004530

22	Postepy Dermatologii i Alergologii	815	1.361	0.001550
23	Postepy Dermatologii i Alergologii	1,308	1.837	0.001810
24	ALLERGOLOGIA ET IMMUNOPATHOLOGIA	1,190	1.276	0.001540
25	ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY	755	1.247	0.000720
26	Pediatric Allergy Immunology and Pulmonology	242	0.785	0.000420
27	Revue Francaise d Allergologie	278	0.254	0.000120
28	ALLERGOLOGIE	133	0.078	0.000050

Printing copy(s) of the publication(s)

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Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

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Yanyan Luo