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Regulation of adult hematopoiesis by the a disintegrin and metalloproteinase 10 (ADAM10)

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ABSTRACT

Adult hematopoiesis requires tightly regulated cell–cell interactions between hematopoietic cells and the bone marrow stromal microenvironment. We addressed the question if the ectodomain sheddase ADAM10 is essential to regulate adult hematopoiesis. Induced ADAM10 deletion in hematopoietic cells resulted in morphological and histological abnormalities that resemble an unclassified myeloproliferative disorder (MPD). The MPD was characterized by an expansion of granulocytic subpopulations and their infiltration of peripheral hematopoietic tissues, the development of hepatosplenomegaly with extramedullary erythropoiesis, lymphnodepathy and death of the mice around 20 weeks after induction. ADAM10 expression analysis during the different stages of the MPD revealed that non-targeted hematopoietic cells repopulated the immune system of the ADAM10-deficient mice. Examination of mice with a myeloid- or epidermis-specific deletion of ADAM10 and bone marrow transplantation (BMT) experiments indicated that the development of the MPD can be triggered by non-cell autonomous effects. We found that plasma levels of clinical markers for MPD such as G-CSF, TIMP-1 and IL-16 were significantly elevated in ADAM10-deficient mice. Our findings indicate that a tightly controlled ADAM10 expression is needed to balance hematopoietic cell-fate decisions in adult mice.

1. Introduction

In recent studies emerging evidence for the crucial role of tightly controlled cell–cell communication between the bone marrow microenvironment and hematopoietic cells during hematopoiesis has been established. However the detailed molecular mechanisms regulating this process have remained elusive so far. A rapid and irreversible posttranslational modification of cell surface protein density can be achieved by an extracellular proteolytic cleavage step which is referred to as "ectodomain shedding" [1–4]. ADAM10 is an ubiquitously expressed protein which is capable of cleaving numerous transmembrane proteins such as Notch receptors [5] that are implicated for proper regulation of hematopoietic development and homeostasis. In order to study the consequence of ADAM10 downregulation during adult hematopoiesis, we conditionally inactivated its expression using the inducible Mx-Cre strain [6] and a myeloid-specific deleter strain [7], respectively. The Mx promoter becomes activated by interferon response or synthetic double-stranded RNA in most cells of immunologically important tissues including bone marrow stroma cells [6]. A similarly induced knockout of the Notch1 receptor and the putative sole downstream signaling mediator *Rbp-j*, led to *de novo* ectopic B-cell development in the thymus from common lymphoid progenitors [8] thus underlining the importance of Notch receptor signaling for cell-fate decisions during lymphopoiesis.

In this study, similar to a recently published report [9], we show that the induced loss of ADAM10 leads to the development and progression of a MPD with concomitant loss of mature B- and T-lymphocytes in the bone marrow, spleen and peripheral blood. The expanded myeloid population mainly consists of $CD11b^+$/$Gr1^+$ cells with normal ADAM10 expression levels. Additionally bone marrow transplantation (BMT) experiments strongly support a non-cell autonomous origin of the MPD which is possibly triggered and maintained by the excessive secretion of G-CSF, TIMP-1 and IL-16.

2. Materials and methods

2.1. Generation of inducible conditional ADAM10 (cKO) mice

The *ADAM10*$^{flox/flox}$ mice were crossed with *Mx*-Cre mice [10], K5-tTA-Cre mice [11] or LysM-Cre mice [7]. To induce

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Mx-mediated deletion of *ADAM10* during adult hematopoiesis, 6-week old *ADAM10*$^{flox/flox}$/Mx-Cre$^{+/-}$ (*ADAM10*$^{-/-}$) and *ADAM10*-$^{flox/flox}$ (Ctr) mice were injected three times in two-day intervals with 250 μg polyinosinic–polycytidylic acid (pI–pC).

2.2. Western blot of tissue lysates

Tissues were uniformly lysed (150 mM NaCl, 2 mM EDTA, 0.8 mM EGTA, 10 mM Tris–HCl, pH 7.4 and Complete Protease Inhibitors (1:25, Roche)). ADAM10 was detected using a polyclonal antiserum (B42.1) (kind gift of W. Annaert, University Leuven), EEF-2 was detected by the Abcam antibody 40812 and actin by a polyclonal antibody from Sigma–Aldrich. The HRP-labeled secondary antibodies were from Dianova, Hamburg, Germany, and detection of chemiluminescence was performed with Amersham ECL Advance Western Blotting Detection Reagent (GE Healthcare, Uppsala, Sweden).

2.3. Histology and immunohistochemistry

Bones, spleen, lymph nodes, liver and skin were fixed by immersion in 4% buffered formalin. For decalcification bones were incubated for 24 h incubation in 1% acetic acid. After overnight postfixation, dehydratation and paraffin embedding 5-μm-sections were either stained with Hematoxylin and Eosin (H&E), Giemsa or processed for immunohistochemistry to show B220 (Immunotools) or Ki-67 (Abcam).

2.4. Magnetic cell separation

HSCs and progenitor cells from bone marrow aspirates were enriched by a negative depletion using the Miltenyi Biotech Lineage Negative Depletion kit according to the manufacture's instructions.

2.5. Flow cytometry

Single-cell suspensions of bone marrow, spleen, lymph nodes were prepared and 5×10^5 cells were stained with the respective antibodies for 45 min at 4 °C. Monoclonal antibodies were FITC-conjugated (CD3e, Gr-1, CD23, CD71), PE-conjugated (CD4, CD11b, CD21, Ter-119) and APC-conjugated (CD8a, F4/80, B220) or unconjugated (ADAM10, BD Biosciences; detection with secondary antibody anti-rat IgG2a, Jackson ImmunoResearch).

2.6. Preparation of bone marrow derived macrophages (BMDM)

Total bone marrow cells were flushed from long bones (tibiae and femur), passed through a nylon sieve (BD Biosciences) and cultured with macrophage growth medium (50% DMEM, 50% PAA Macrophage Growth Medium, 20% FCS, 50 μg/ml Pen/Strep and 20 ng/ml M-CSF (Immunotools, Friesoythe, Germany). For analysis 7-day old BMDMs were harvested using accutase (PAA) and stained for F4/80 (BD Biosciences) to show maturation and enrichment of mature macrophages.

2.7. Serology

Plasma granulocyte-colony stimulating factor (G-CSF), tissue-inhibitor of Metalloprotease 1 (TIMP-1) and thymic stromal lymphopoietin (TSLP) levels were measured according to the instructions in the corresponding DuoSet mouse ELISA kits (R&D Systems).

2.8. Myeloperoxidase (MPO) activity measurement

Myeloperoxidase (MPO) activity was measured in cell suspension of bone marrow, spleen, liver, lymph nodes and skin. After freezing in liquid nitrogen, equal amounts of tissue samples were homogenized and incubated at 60 °C for 2 h and centrifuged for 5 min at 10,000*g*. 10 μl of the supernatant was mixed with 50 μl peroxidase substrate (BM blue POD, Roche). After 10–20 min the reaction was stopped by adding 50 μl 2 M H_2SO_4 and absorbance was recorded at 450 nm.

2.9. Bone marrow transplantations

Six week old *ADAM10*$^{flox/flox}$/Mx-Cre$^{+/-}$ (*ADAM10*$^{-/-}$) and *ADAM10*$^{flox/flox}$ (Ctr) mice were injected three times in two-day intervals with 250 μg polyinosinic–polycytidylic acid (pI–pC) to induce ADAM10 deletion. Two weeks after the last injection recipient mice (3 females and 2 males per group) were lethally irradiated with 6 Gy the day before and the day of transplantation. Control mice were transplanted with 10×10^6 bone marrow cells from littermate ADAM10$^{-/-}$ mice (1 male, 1 female), and vice versa for transplantation of ADAM10$^{-/-}$ bone marrow into control mice. After 6 weeks recovery, mice were used for the analyses.

2.10. Statistical analysis

Statistical significance was performed as unpaired Student's *T*-test using Microsoft Excel software. Error bars indicate the mean ± standard deviation of the mean. *P* values: $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$.

3. Results

3.1. Lack of ADAM10 expression and activity in the adult hematopoietic system

We induced the deletion of the protease in *ADAM10*$^{flox/flox;\ Mx1Cre}$ mice and sacrificed mice 2 weeks after the last injection of double stranded RNA (pI–pC). Genomic deletion was observed in bone marrow, spleen, liver and skin (Fig. 1A). Immunoblot analysis revealed a strong downregulation of ADAM10 protein expression in bone marrow and spleen as well as of the proform of the protease in liver (Fig. 1B). Despite significantly downregulated ADAM10 protein expression (Fig. 1CI) in bone marrow derived macrophages (BMDMs), we could not observe overt alterations in cell morphology (Fig. 1CII) or differentiation status (Fig. 1CIII). Isolated B-lymphocytes from spleens of wild type and induced ADAM10 knock-out mice revealed the downregulation of ADAM10 protein expression and concomitantly diminished CD23 and Notch2 receptor processing (data not shown). Lineage depletion of bone marrow cells to obtain hematopoietic stem (HSC) and progenitor cells was performed. A clear absence of ADAM10 expression in a subset of HSCs (Fig. 1DIII) was demonstrated.

3.2. Phenotypical analysis of pI–pC induced *ADAM10*$^{-/-}$ mice

About 6–12 weeks following the last injection ADAM10-depleted mice start to show fatigue, an apathic behavior and loss of hair especially in facial regions (Fig. 2A). 12 weeks after induction the mice became moribund showing an enlarged liver with thrombocytosis (Fig. 2AI, I'), splenomegaly (Fig. 2AII), lymphnodepathy (Fig. 2AIII) and severe thymic atrophy (Fig. 2AIV). Histological examination revealed an increase of white blood cells in the cavity of long bones (Fig. 2BI, I'), extramedullary hematopoiesis