To the editor:

**Activation of TRKB receptor in murine hematopoietic stem/progenitor cells induced mastocytosis**

Dysregulation of tropomyosin-related kinase (TRK) receptors has been reported to be involved in many human diseases, including cancer and neurological diseases. Recently, Peng et al.\(^1\) demonstrated that patients with mastocytosis had enhanced neurotrophin levels and elevated expression of TRK receptors on skin and gut mast cells, suggesting that TRK signaling might contribute to the pathogenesis of mastocytosis by autocrine and paracrine loops.

We recently demonstrated a potential role of neurotrophins and TRK signaling in leukemia development.\(^2\)\(^-\)\(^4\) Overexpression of TRKB and its ligand brain-derived neurotrophic factor (BDNF) could transform murine primary hematopoietic stem/progenitor cells.\(^5\) Now we demonstrate that activation of TRKB by BDNF in murine hematopoietic stem/progenitor cells also efficiently induces a disease with striking similarities to human systemic mastocytosis (SM) in vivo.

**Figure 1. Development of SM in mice transplanted with TRKB- and BDNF-modified hematopoietic stem/progenitor cells.** (A-F) Representative histopathology (hematoxylin and eosin) and cytology (May-Grünewald-Giemsa) from 2 animals (A-E: mouse 1007, F: mouse 746). The World Health Organization criteria for human SM were fulfilled in diseased animals.\(^6\)\(^,\)\(^7\) Multifocal, dense infiltrates of mast cells (≥15 mast cells in aggregates) were observed in different organs. (A-B) Sections of liver showing infiltration of mast cells in liver (×100, ×400). (C-D) Accumulation of mast cells in the red pulp of spleen (×100, ×400). (E) Cytospin of spleen showing mature, round (typical) mast cells with abundant cytoplasm filled with granules (×1000). Note phagocytosis of red cells in some mast cells (B,E). Spindle-shaped slightly hypogranular mast cells were also observed in other animals (data not shown). (F) Low-magnification views of bone marrow section showing extensive infiltration of mast cells in sternum and osteosclerosis (×100). However, cytospin of bone marrow cells from tibia showed <20% of mast cells. Moreover, kidney infiltration of mast cells was observed in some animals with SM (data not shown). It is important to note that spleen and liver were affected in all animals with SM, whereas some animals did not have infiltration of mast cells in bone marrow. Unfortunately, skin and gut were not collected for histological analyses, because we do not routinely analyze these for leukemogenesis studies. (G-K) Representative flow cytometric diagrams from animal 714. Flow cytometric analysis showing expression of transgenes (BDNF and TRKB, measured by enhanced green fluorescent protein (EGFP) and antibody against hemagglutinin-tag, respectively) in spleen (G, negative control shown as inset), expression of c-Kit and FcγRI in transgene positive (H) and transgene negative population (neither TRKB nor BDNF) (I), and expression of FcγRI and CD25 (marker for neoplastic mast cells) in transgene positive (J) and transgene negative populations (K; negative control for panels H-K shown as inset). These demonstrated that abnormal mast cells were derived from gene-modified cells. It is interesting to note that in SM mice, both autocrine and paracrine loops may have contributed to mastocytosis (Figure 1G), whereas we observed a clear selection for both lymphoid leukemia (supplemental Figure 4) and myeloid leukemia transformation by autocrine activation of TRKB.\(^8\) Activation of TRKB in hematopoietic stem/progenitor cells induced different phenotypes in vivo, ie, SM and lymphoblastic leukemia. This might be due to different cell populations targeted,\(^9\) although other factors might also be involved.

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1. Peng et al. (2014)
2. Peng et al. (2015)
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5. Peng et al. (2018)
7. World Health Organization (2011)
8. Peng et al. (2019)
9. Peng et al. (2020)
10. Peng et al. (2021)
To investigate the role of TRKB signaling in leukemogenesis, 37 C57BL/6J mice were transplanted with retrovirally gene-modified primary hematopoietic stem/progenitor cells (TRKB/BDNF = 17, TRKB = 8, BDNF = 5, tCD34 = 7) in 3 independent experiments (some data from the first experiment were recently published4). The experiments were terminated after an observation time of up to 454 days (mean 354 days). Interestingly, lymphoblastic leukemia was diagnosed in 5 animals (supplemental Data; available on the Blood Web site), whereas the other 12 animals with coexpression of TRKB/BDNF unexpectedly developed SM, affecting mainly spleen, liver, and bone marrow (see figure panels A-F) with multifocal compact mast cell infiltrates. Mast cells demonstrated mainly features of mature hypergranular mast cells (Figure 1E),5 expressing TRKB, BDNF, c-Kit, tryptase, and high affinity receptors for IgE (FceRI) and CD25 (Figure 1G-H; supplemental Figure 1). Most SM animals followed an indolent course, and none of the animals became moribund or died before termination of the experiments. In contrast, leukemic mice survived <6 months after transplantation. At the final analysis, most of the SM animals had normal blood counts; only 2 animals showed slight enlargement of spleen. There was no evidence of classical mast cell leukemia6 or other hematological neoplasm in animals with SM. Moreover, no mutations in the c-Kit gene were detectable in any of the analyzed SM mice with high mast cell burden (n = 4). In contrast to TRKB/BDNF, no animals with TRKB alone, BDNF alone, or tCD34 showed mastocytosis or other hematological malignancies (supplemental Figure 2) except 1 TRKB mouse, which had slightly increased numbers of mastocytes (not fulfilling the criteria for SM) (supplemental Figure 3), probably due to mild activation of TRKB by its overexpression. In historic controls of >60 animals transplanted in a similar setting with different genes, eg, EGFP, tCD34, dLNGFR, and SV40 LT, no animals demonstrated SM. These data strongly suggest that activation of TRKB by BDNF (autocrine or paracrine; Figure 1G) is important to promote mastocytosis. Interestingly, TRKB activation in our model efficiently induced SM (incidence: 12/17 = 71%), whereas KIT D816V transgenic mice demonstrated a lower incidence of SM (8/28 = 29%).7 Furthermore, retroviral-mediated expression of KIT D816V even failed to induce SM in transplanted animals.8

Interestingly, in contrast to transplantation with primary hematopoietic stem/progenitor cells, C3H/HeJ animals (n = 5 in 2 independent experiments, including the recently published experiment1) transplanted with TRKB and BDNF modified 32D cells (murine myeloid progenitors) developed only myeloid leukemia with no sign of increased mastocyte numbers. The fact that mastocytosis is induced only when TRKB is activated in hematopoietic stem/progenitor cells strongly supports the accepted view that mast cells are derived from hematopoietic stem cells.9

In summary, we provide the first direct evidence for induction of mastocytosis by activation of TRKB in hematopoietic stem/progenitor cells in vivo. Our data strongly support the findings by Peng et al1 and their hypothesis and indicate an important role of TRKB in the pathogenesis of mastocytosis.

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The online version contains a data supplement.

Acknowledgments: This work was supported by the Deutsche Forschungsgemeinschaft (grant Li 1608/2-1) and the Deutsche Krebshilfe (grant 108245). K.H. was supported by the China Scholarship Council (2011638024).

Contribution: M.Y. performed research, collected, analyzed, and interpreted data, and wrote the manuscript; K.H. performed research; G.B. performed histological analysis; A.G. performed cytological analysis and revised the manuscript; and Z.L. conceived the concept, performed research, collected, analyzed and interpreted data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


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