Brief Report

blood

MYELOID NEOPLASIA

Functional evidence for derivation of systemic histiocytic neoplasms from hematopoietic stem/progenitor cells

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Key Points

- *BRAF*[V600E] mutations are detectable in hematopoietic stem and progenitors in adults with histiocytosis.
- Transplantation of CD34+ cells from histiocytosis patients can give rise to genetically and phenotypically accurate xenografts.

Langerhans cell histiocytosis (LCH) and the non-LCH neoplasm Erdheim-Chester disease (ECD) are heterogeneous neoplastic disorders marked by infiltration of pathologic macrophage-, dendritic cell–, or monocyte-derived cells in tissues driven by recurrent mutations activating MAPK signaling. Although recent data indicate that at least a proportion of LCH and ECD patients have detectable activating kinase mutations in circulating hematopoietic cells and bone marrow–based hematopoietic progenitors, functional evidence of the cell of origin of histiocytosis from actual patient materials has long been elusive. Here, we provide evidence for mutations in MAPK signaling intermediates in CD34+ cells from patients with ECD and LCH/ECD, including detection of shared origin of LCH and acute myelomonocytic leukemia driven by *TET2*-mutant CD34+ cell progenitors in one patient. We also demonstrate functional self-renewal capacity for CD34+ cells to drive the development of histiocytosis in xenotransplantation assays in vivo. These data indicate that the cell of origin of at least a proportion of patients with systemic histiocytoses resides in hematopoietic progenitor cells prior to committed monocyte/macrophage or dendritic cell differentiation and provide the first example of a patient-derived xenotransplantation model for a human histiocytic neoplasm. (*Blood*. 2017;130(2):176-180)

Introduction

Histiocytic neoplasms are heterogeneous disorders marked by tissue infiltration and accumulation of pathologic macrophage-, dendritic cell (DC)–, or monocyte-derived cells.[1] Over the last 5 years, recurrent mutations activating MAPK signaling have been identified in adults and children affected by Langerhans cell histiocytosis (LCH)[2-7] or Erdheim-Chester disease (ECD).[7,8] While the discovery of these mutations has impacted treatment,[7,9-13] these discoveries may also be useful in identifying the cellular origins of LCH and ECD, which have been debated for decades.[14,15]

LCH was historically thought to arise from epidermal Langerhans cells[14,16] due to phenotypic similarities between LCH and normal Langerhans cells. Recent data however suggest that LCH may be derived from myeloid precursor cells bearing somatic mutations in

MAPK pathway members,[17] including *BRAF*[V600E] mutations in ∼50% of patients, as well as mutations in *NRAS*,[18] *KRAS*,[18] *ARAF*,[3,5,7] and *MAP2K1*.[4,5,7,19] Gene expression profiling of CD207+ DCs from LCH patients revealed that LCH cells are more akin to bone marrow (BM)–derived, immature myeloid DCs than epidermal Langerhans cells.[20] Moreover, *BRAF*[V600E] mutations have been identified in CD11c+ myeloid DC precursors in LCH, CD14+ monocytes in LCH and ECD, and BM CD34+ hematopoietic stem/progenitor cells (HSPCs) in a proportion of LCH patients. Finally, expression of the *BRAF*[V600E] mutation in committed DC precursors is sufficient to drive LCH-like disease in mice.[17] Despite these data, however, functional evidence linking the cell of origin for histiocytosis to HSPCs in actual patient materials does not currently exist. Therefore, we attempted to
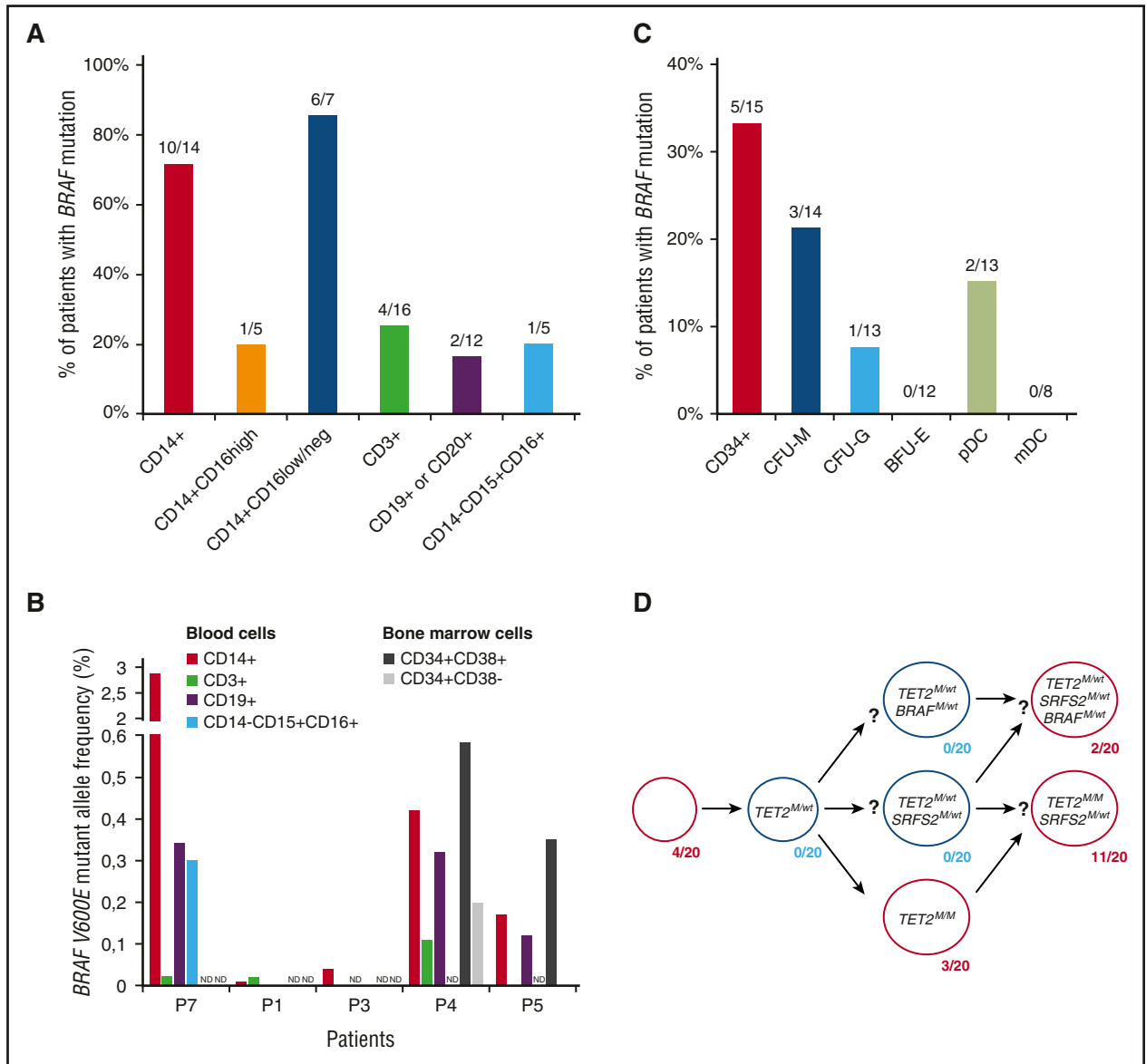
Figure 1. The $BRAF^{V600E}$ mutation is detectable in mature and immature hematopoietic cells from patients with histiocytosis. (A) Percentage of patients for whom $BRAF^{V600E}$ mutation was detected in mature PB cells. (B) $BRAF^{V600E}$ mutant allele frequency in fluorescence-activated cell sorting–purified cells from the BM or PB of 5 histiocytosis patients. ND indicates populations not studied. Patient numbering per supplemental Table 1. (C) Percentage of patients for whom the $BRAF^{V600E}$ mutation was detected in CD34$^+$ BM cells or in CD34-derived CFU-M, CFU-G, BFU-E, and plasmacytoid DCs (pDC) or mDCs. (D) $BRAF^{V600E}$, $TET2^{L1819X}$, and $SRSF2^{L95P}$ status of 20 colonies derived from single CD34$^+$ BM cells of a patient with $BRAF^{V600E}$-mutant MH (#P10). The results suggest that histiocytosis and AMML cells of this patient derived from a common $TET2$-mutated HSPC. In panels A and C, each denominator indicates the total number of patients for whom results were obtained.

understand the cell of origin of histiocytic neoplasms using genetic and functional analyses of HSPCs from histiocytosis patients. We identify that CD34$^+$ cells in histiocytosis patients have functional self-renewal potential and describe the first successful patient-derived xenograft of a histiocytic neoplasm.

Methods

BM and/or peripheral blood (PB) cells were obtained from 41 patients with ECD or mixed histiocytosis (MH). A total of 37 patients had a $BRAF^{V600E}$ mutation, and 1 patient had $KRAS^{G12S}$ mutation within histiocytosis-infiltrated tissues (supplemental Table 1, available on the *Blood* Web site); 10 patients had a concurrent

myeloid neoplasm (chronic myelomonocytic leukemia or myeloproliferative neoplasm) in addition to histiocytosis (a recently identified clinical overlap[21]).

BM and PB cell subsets were purified by fluorescence-activated cell sorting or immunomagnetic selection. Purity was consistently >90% (supplemental Methods). CD34$^+$ BM cells were assayed in methylcellulose (Methocult GF+ H4435, STEMCELL Technologies) and/or grown in liquid culture conditions allowing differentiation into myeloid dendritic cells (mDCs) or plasmacytoid dendritic cells.[22] In some experiments, single CD34$^+$ cells were expanded in liquid culture for 10 days.[23]

Detection of $BRAF^{V600E}$ and $BRAF^{WT}$ alleles within DNA extracted from BM, PB, or colonies was performed by real-time polymerase chain reaction (PCR) and/or picodroplet digital PCR as described previously.[18] Mutations in colony-forming unit-macrophage (CFU-M), colony-forming unit-granulocyte (CFU-G), and burst-forming unit-erythroid (BFU-E) were analyzed by pools of 10 colonies.
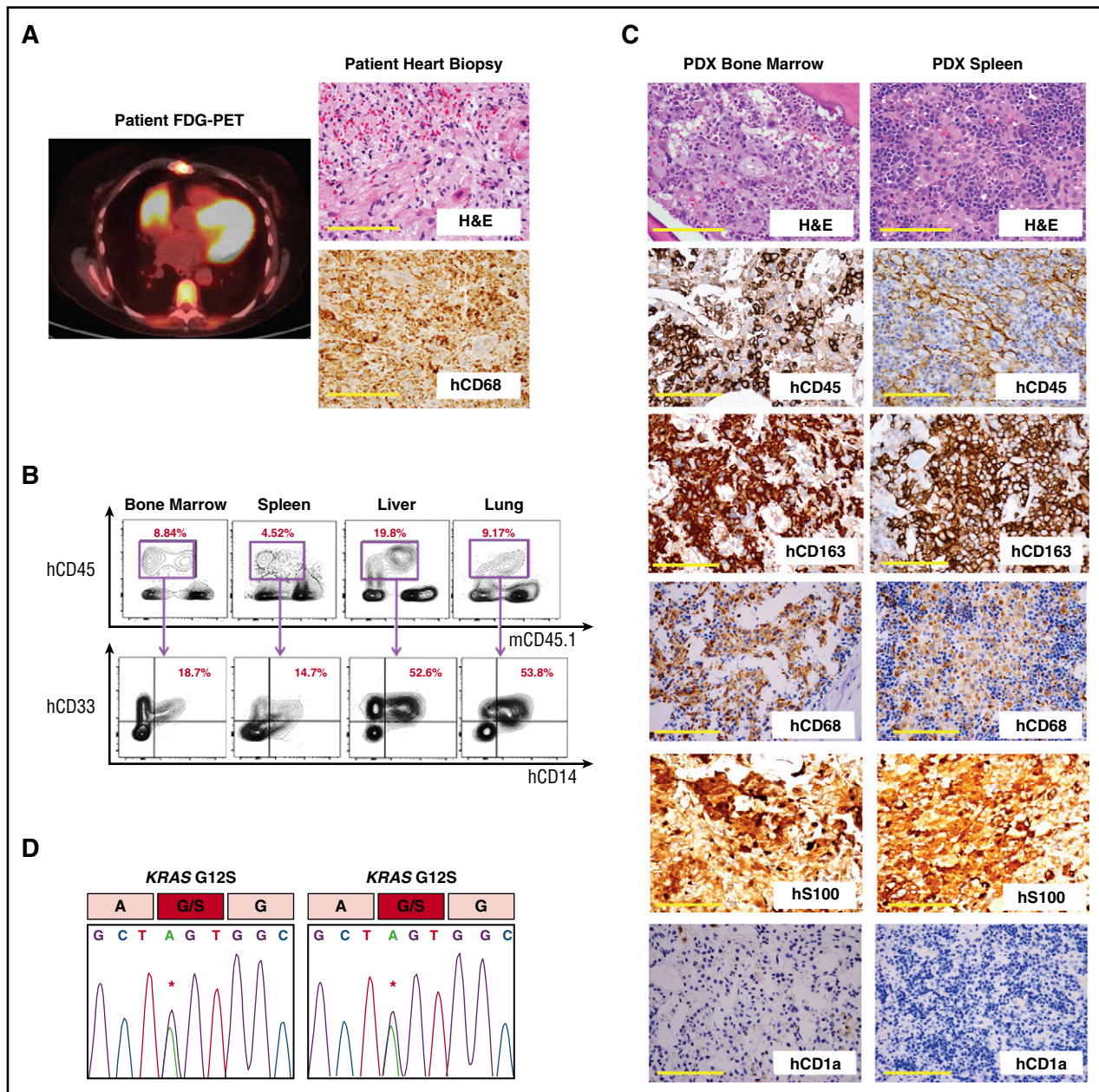
Figure 2. Analysis of a patient-derived xenograft from a _KRAS_^{G12S}-mutant patient with ECD. (A) Fludeoxyglucose positron emission tomography (FDG-PET) imaging and histologic and immunohistochemical analysis of the _KRAS_^{G12S}-mutant ECD patient's heart biopsy specimen (scale bars represent 200 μm). (B) Flow cytometric analysis of BM, spleen, liver, and lung of recipient NSGS mouse 90 days postxenotransplantation with CD34$^+$ cells from the same _KRAS_^{G12S}-mutant ECD patient. (C) Histologic and immunohistochemical analysis of engrafted tissue from the recipient mouse (scale bars represent 200 μm). (D) Sanger sequencing of genomic DNA from hCD45$^+$ cells purified from recipient mouse BM (left) and spleen (right) revealing a _KRAS_^{G12S} mutation in engrafted human hematopoietic cells. H&E, hematoxylin and eosin.

Xenografts were performed by direct intrafemoral injection[24] of a variable number of CD34$^+$ cells from BM mononuclear cells from histiocytosis patients into sublethally irradiated (200 cGy) nonobese diabetic severe combined immunodeficiency IL2Rgamma-null mice with transgenic expression of human interleukin-3, stem cell factor, and granulocyte-macrophage colony-stimulating factor (NSGS)[25] mice. Human engraftment was monitored by monthly flow cytometric analysis of PB until mice became moribund. Thereafter, animals were sacrificed, and tissues were analyzed using flow cytometry, histology, immunohistochemistry, and sequencing to detect driver mutations identified in the histiocytosis/co-occurring myeloid neoplasm. Studies were approved by the ethics committees of University Hospital La Pitié-Salpêtrière and Memorial Sloan Kettering Cancer Center and by the Institutional Review Board of the French Regulatory Agency and conducted in accordance to the Declaration of Helsinki. All patients signed informed consent.

Results and discussion

We first performed genotyping for the _BRAF_^{V600E} mutation in blood cells from ECD or MH known to harbor the _BRAF_^{V600E} mutations within infiltrated tissues. _BRAF_^{V600E} mutations were detected in 71% (10/14) of blood CD14$^+$ monocytes (Figure 1A). The _BRAF_^{V600E} mutation was more frequent within CD14$^+$ CD16^{low} classical[26] than CD14$^+$ CD16^{high} nonclassical monocytes. These results confirm prior detection of MAPK pathway mutations in CD14$^+$ cells of children with systemic LCH[17] and in 1 patient with ECD.[18] The _BRAF_^{V600E} mutation was also detected in T (CD3$^+$) and B lymphocytes (CD19$^+$ or CD20$^+$) and/or polymorphonuclear (CD14$^-$ CD15$^+$ CD16$^+$) cells in some

patients (Figure 1A). The $BRAF^{V600E}$ mutant allele frequency (BRAF-MAF) was low in all cell populations and ranged from 0.01 to 2.9 within monocytes (Figure 1B). The detection of the $BRAF^{V600E}$ mutation in multiple mature blood cells suggests that the oncogenic event involved a multipotent BM progenitor.

We next investigated BM progenitor populations for $BRAF^{V600E}$ in patients with ECD (n = 22) or MH (n = 8). $BRAF^{V600E}$ was detected within purified CD34$^+$ BM cells from 5 out of 15 patients (33%) known to have $BRAF^{V600E}$ in lesional histiocytes (Figure 1C). The BRAF-MAF was always <1% and lower in the CD34$^+$ CD38$^-$ multipotent progenitor/long-term hematopoietic stem cell compartment than in the CD34$^+$ CD38$^+$ myeloid progenitor compartment (Figure 1B). Given these low BRAF-MAFs in HSPCs, we further sought to evaluate if mutant $BRAF$ could be detected in progeny derived from $BRAF$ mutant HSPCs. Through evaluation of colonies obtained upon seeding CD34$^+$ BM cells in methylcellulose clonogenic cultures, $BRAF^{V600E}$ was detected in CFU-M (3/14) and CFU-G (1/13), but not in BFU-E colonies. $BRAF^{V600E}$ mutations were also detected within in vitro–derived plasmacytoid dendritic cells (2/13), but not in mDCs. We also analyzed colonies derived from single CD34$^+$ BM cells of 6 patients with $BRAF^{V600E}$ ECD. Only 3 out of 459 colonies (0.65%) were mutated for $BRAF$ (supplemental Figure 1), which confirms the low BRAF-MAF detected by picodroplet digital PCR. The patient with MH who had 2 $BRAF$-mutated colonies (#P10) was further analyzed for $TET2$ and $SRSF2$ mutations, which were known to exist in a concurrent acute myelomonocytic leukemia (AMML) in this patient. Interestingly, the presence of colonies with $BRAF/TET2$ comutation, as well as with $TET2$ mutation alone, revealed that the mutation in $TET2$ occurred before the $BRAF$ mutation and suggests a common clonal origin for both the histiocytosis and the AMML (Figure 1D). This was further reinforced by the detection of the same $BRAF$, $TET2$, and $SRSF2$ mutations within LCH cells in a skin biopsy from this patient (supplemental Figure 2).

Next, to test the self-renewal and differentiation capacity of HSPCs from histiocytoses patients, CD34$^+$ cells were purified from 8 patients, including 6 with ECD or MH plus a concomitant myeloid neoplasm and 2 with ECD alone (supplemental Table 1). A total of 0.1 to 0.8 × 10^6 CD34$^+$ cells from each patient were transplanted into sublethally irradiated NSGS[24] mice. Successful engraftment of human myeloid cells was observed in blood of recipient mice xenografted from 50% of patients (4 out of 8) after a median of 90 days (range: 60-120 days; supplemental Figure 3A). Of these 4 samples, CD34$^+$ cells from 1 ECD patient sample induced histiocytosis-like lesions, as shown by infiltration of human CD45$^+$ (hCD45) cells coexpressing the human myeloid and monocyte lineage markers hCD33 and hCD14 in the BM, spleen, liver, and lung (Figure 2A-B; supplemental Figure 3B). Immunohistochemistry revealed that tissues were infiltrated by an hCD45$^+$ hCD163$^+$ hCD68$^+$ hS100$^+$ hCD1a$^-$ population of foamy histiocytes, characteristic of ECD (Figure 2C). Furthermore, genomic analysis of hCD45$^+$ cell DNA from the engrafted animal's BM and spleen revealed the same $KRAS^{G12S}$ mutation present in the patient's ECD lesion (Figure 2D). All other mice that successfully engrafted with human cells revealed either no evidence of histiocytosis histologically and/or no evidence of the histiocytosis-associated mutation.

This study provides the first functional evidence that CD34$^+$ cells from patients with histiocytosis can functionally initiate disease. Xenotransplantation successfully recapitulated the unique histologic, immunophenotypic, and genetic characteristics of human ECD. Moreover, these data confirm prior results suggesting that somatic mutations affecting MAPK signaling detectable in histiocytosis lesions are also detectable in the CD34$^+$ compartment and in mature blood cells of a proportion of histiocytosis patients. Further analysis of HSPCs from histiocytosis patients for additional somatic mutations in histiocytosis lesional cells may therefore be very helpful in clarifying the cells of origin of systemic histiocytoses. Moreover, these data, together the wide clinical heterogeneity of histiocytoses, also suggest the possibility of alternate cells of origin for these conditions beyond HSPCs.

Authorship

Contribution: B.H.D., D.R.-W., C.B., A.Y., M.M., Z.H.-R., N.T., N.O., A.D., C.Y.P., G.G., V.T., O.A.B., O.A.-W., F.M.L., and J.-F.E. performed the experiments; F.C.-A., M.P., R.R., F.U., L.L.F., E.L.D., T.P., F.C., Z.A., and J.H. collected patient material and clinical data; and B.H.D., O.A.-W., J.H., and J.-F.E. wrote the manuscript.

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