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BRIEF REPORT

Clonal evolution of chronic lymphocytic leukemia to Langerhans cell histiocytosis: a case report

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Abstract

The traditional concept of unidirectional maturation of hematopoietic cells has been called into question due to the recognition of lineage plasticity, which is increasingly found also in the clonal evolution of hematopoietic and lymphoid malignancies. Here we present an unusual case of a patient with *TP53*-mutated chronic lymphocytic leukemia (CLL) treated with a PI3Kδ inhibitor evolving to clonally related Langerhans cell histiocytosis (LCH) with acquired *BRAF* V600E and *STK11* mutations and loss of expression of PAX-5 and other examined B cell markers. In indolent B cell lymphoma, transformation to a more aggressive high-grade lymphoma occurs frequently during the course of disease and is thought to be caused by clonal evolution. Our case further supports the concept of significant lineage plasticity in lymphomas and raises the question of a potential role of novel pharmacologic agents in clonal evolution.

Keywords Langerhans cell histiocytosis · Chronic lymphocytic leukemia · TP53 · Transdifferentiation

Background

The traditional concept of unidirectional maturation of hematopoietic cells has been called into question due to the recognition of lineage plasticity, which is increasingly found also in the clonal evolution of hematopoietic and lymphoid malignancies. In indolent B cell lymphoma, transformation to a more aggressive high-grade lymphoma occurs frequently during the course of disease and is thought to be caused by clonal evolution. Whereas conventional high-grade transformation retains the B cell lineage, recent reports have documented that neoplasms with histiocytic or dendritic cell morphology and phenotype arising in the setting of an indolent B-NHL frequently are clonally related, confirmed by the presence of identical immunoglobulin heavy chain (*IGH*) rearrangements and/or translocations, such as the

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t(14;18)(q32;q21) in both cell populations [1]. Three possible explanations have been provided for this phenomenon: (1) The clonal B cells of the established lymphoma acquire mutations triggering a different hematopoietic lineage differentiation; this has been called transdifferentiation. (2) Clonal B cells dedifferentiate to precursor cells and re-differentiate to histiocytes sharing the identical clonal *IGH* rearrangement with the underlying B cell neoplasia. (3) Both neoplasms arise from a pluripotent transformed stem cell; this latter explanation, however, does not explain the presence of complete IG gene rearrangements of translocations involving the *IGH* locus, since these events only occur in cells committed to the B cell lineage [2, 3]. Of note, until now, it remains unknown how transdifferentiation occurs and which mechanisms could trigger it. The majority of cases which showed transdifferentiation to a histiocytic/dendritic cell neoplasia were described in follicular lymphoma and chronic lymphocytic leukemia (CLL), predominantly to histiocytic and follicular dendritic cell sarcomas [4–6]. Langerhans cell histiocytosis (LCH) is a rare histiocytic disorder characterized by a clonal proliferation of Langerhans-type cells which in the systemic form is considered to derive from myeloid stem cells rather than epidermal Langerhans cells [7]. LCH shows the *BRAF* V600E mutation in approximately 50% of cases, and *MAP2K1* mutations in 25–30% [8]. Clonally related LCH and Langerhans cell sarcoma have been rarely reported to occur in patients with CLL, follicular lymphoma, or marginal zone lymphoma, but data on the potential molecular mechanisms of transdifferentiation are sparse [6].

Case presentation

Here we present an unusual case of a patient with *TP53*-mutated CLL treated with a PI3Kδ inhibitor evolving to clonally related LCH with acquired *BRAF* V600E and *STK11* mutations and loss of expression of PAX-5 and other examined B cell markers. A 52-year-old female patient was first diagnosed with CLL in clinical stage Binet A in 2004, cytogenetic examination at that time point revealed del17p. At diagnosis, the bone marrow biopsy showed a typical interstitial, non-paratrabecular lymphoid infiltrate, positive for CD20, CD5, CD23, and LEF-1. After initial watch-and-wait approach, the patient developed increasing leukocytosis, anemia, splenomegaly, and progressive lymphadenopathy, and was treated in the following years with various (immune-) chemotherapy regimens, including R-CHOP, ibrutinib, and finally, the PI3Kδ inhibitor idelalisib, which was given in combination with rituximab for 7 cycles between October 2014 and January 2015. In March 2015, 1 month before planned allogeneic hematopoietic stem cell transplantation as salvage therapy, an osteolytic lesion in the right distal femur suspicious for Richter's transformation was identified. The peripheral blood at this time showed almost normal cell counts after treatment (leukocytes 4230/μl, lymphocytes 1.500/μl, hemoglobin 12.5 mg/dl, and platelets 122,000/μl). CT-guided biopsy revealed an infiltration by classical LCH with large cells with abundant cytoplasm and oval to elongated, grooved nuclei, arranged in clusters. Immunohistochemically, the cells were strongly positive for Langerin, CD1a, and S100 and negative for PAX-5 and other B cell markers and only very rare, isolated B cells were detected in the biopsy. Staining with the mutation-specific *BRAF* V600E antibody VE1 was positive. Comparative B cell clonality analysis following macrodissection revealed an identical, strong biallelic immunoglobulin heavy chain (*IGH*) rearrangement with BIOMED-2 FR2 and FR3 primer sets in the CLL from the 2004 bone marrow biopsy and the osteolytic lesion (LCH) (Fig. 1). Furthermore, next-generation sequencing (NGS) using the Ion AmpliSeq™ Colon and Lung Cancer Panel (Life Technologies) on the Ion Torrent PGM™ Platform detected both shared and private mutations. In both the CLL and the LCH, an identical *TP53* c.572_574del/p.P191del mutation with variant allele frequencies (VAF) of 48% in the CLL and 3% in the LCH was found. The high VAF in the CLL is explained by the presence of the deletion 17p, whereas the low VAF in the LCH is probably due to rare—estimated at 3%, given the 17p deletion—contaminating CLL cells. Moreover, the LCH showed an additional *TP53* c.275C>T/p.P92L (VAF 6%) mutation, the *BRAF* c.1799T>A/p.V600E (VAF 19%) mutation in line with the positivity in immunohistochemistry for p53 and BRAF V600E, and a further point mutation of *STK11* c.1004T>C/p.Met335Thr (VAF 12%). Fluorescence in situ hybridization detected a deletion 17p in both the CLL and the LCH.

Discussion and conclusions

Transdifferentiation of low-grade B-NHL to neoplasms of histiocytic and dendritic cell lineage including LCH has been recognized repeatedly in the last years, and comparative molecular studies using clonal markers such as immunoglobulin gene rearrangements and translocations have established the clonal relationship in these cases. Our case adds to this growing body of evidence for transdifferentiation as a special form of clonal evolution and indicates that certain mutations such as the *BRAF* V600E, a genetic hallmark lesion of LCH, may drive transdifferentiation, and that genetic instability associated with *TP53* mutations may represent a contributing factor.

Mutations of *TP53* play an important role in intratumoral heterogeneity of CLL and progression to high-grade lymphoma [9]. Of note, 17p deletions have also been described as a common alteration in histiocytic and dendritic cell tumors associated with B-NHL [6], supporting a potential role in transdifferentiation, whereas *TP53* alterations in conventional

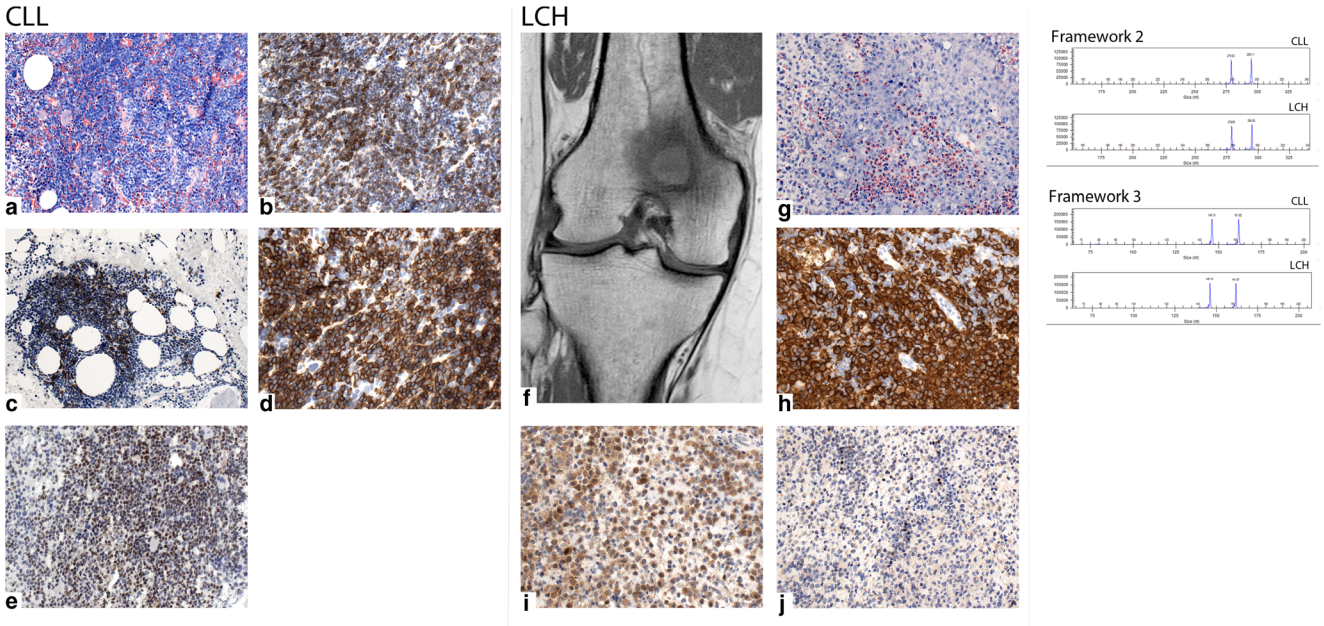

Fig. 1 Morphology and immunohistochemistry of the bone marrow biopsy with the diagnosis of CLL and the biopsy of the right femur infiltrated by the Langerhans histiocytosis. Bone marrow/CLL: (**A**) Giemsa stain highlights the small cell lymphoid infiltrate. (**B**–**E**) All immunohistochemical analysis was performed on an automated immunostainer (Ventana Medical Systems, Tucson, AZ), following the manufacturer's protocols. (**B**) CD23 shows a positive membranous staining of the lymphoid cells. (**C**) The small cell infiltrate shows strong positivity for CD20. (**D**) The transcription factor PAX5 is positive in the specimen of the CLL. (**E**) CD5 is positive, as a marker for CLL all original magnification × 20. Femoral lesion/Langerhans histiocytosis: (**F**) MRI scan of the tumor. (**G**) Morphology in the Giemsa stain shows large polygonal cells with oval to grooved nuclei, arranged in clusters typical for a classical LCH original magnification × 40. Insert: Giemsa, original magnification × 20. (**H**–**J**) (**H**) CD1a is found in the surface of Langerhans cells. (**I**) S100 shows cytoplasmic and nuclear expression. (**J**) The transcription factor PAX5, which was positive in the specimen of the CLL, is lost in the LCH. Rare positive B cells are interspersed. All original magnification × 200. (**K**) Identical biallelic immunoglobulin heavy chain (*IGH*) rearrangement in both biopsies identified with BIOMED-2 FR2 and FR3 primer sets

LCH are virtually non-existent. This fact and the strong biallelic *IGH* rearrangement in the LCH sample despite an at best very limited number of contaminating CLL cells after macrodissection further support a true clonal relationship. The presence of del17p in both lesions indicates that it was an early event in pathogenesis. The additional *TP53* mutation of the LCH clone possibly represents an acquired secondary event during clonal evolution to LCH. This is supported by the absence of this mutation and persistence of the *TP53* c.572_574del/p.P191del mutation initially identified in the 2004 specimen in a bone marrow biopsy 1 year after allogeneic stem cell transplantation, when the patient suffered from relapsed CLL. The acquisition of the *BRAF* V600E mutation has been documented in a single LCH case with clonally related CLL, but not in other studies [9, 10].

STK11 mutations are very rarely found in hematological neoplasia, such as polycythemia vera [11]. In Peutz-Jeghers syndrome, however, these mutations seem to cooperate with *TP53* mutations in tumor progression, suggesting a potential role for clonal evolution in our case [12]. A typical feature of cases of transdifferentiation is the loss of all B cell markers in the histiocytic/dendritic cell neoplasm including transcription factors. In animal models, downregulation of transcription factor PAX-5 is able to induce transdifferentiation of mature B cells to histiocytic cells [13]. The role of the loss of PAX-5 expression is in line with the findings of Ambrosio et al. who showed in a case of marginal zone lymphoma with transdifferentiation to LCH that the loss of PAX5 was caused by aberrant methylation and subsequent silencing of the gene [2].

In patients with CLL, clonal evolution originating from subclones with driver mutations that expand over time occurs with increased frequency in patients treated with chemotherapy [14]. This could indicate that in particular, the PI3Kδ inhibitor idelalisib, which was added to the conventional chemotherapy before the development of LCH, might have played a role in clonal evolution. In vivo and in vitro studies have shown that idelalisib affects the PI3Kδ pathway, which regulates the activation-induced deaminase (AID) expression. The B cell-specific enzyme AID plays an important role in genomic stability of B cells. Thus, it has been shown that through affecting AID, idelalisib-treated murine B cells have increased somatic hypermutation and translocation frequency [15]. These findings suggest that idelalisib treatment in our case could have led to increased genomic instability, finally

resulting in transdifferentiation. Our case supports the concept of significant lineage plasticity in lymphomas and points to pharmacologic agents as potential factors of clonal evolution.

Authors' contributions BF and FF designed the study, analyzed the data, and wrote the paper. LF analyzed the data and wrote the paper. IB, JS, and BM performed the research and analyzed the data. WB and SW contributed with vital patient information. MH contributed with radiological analysis. All authors revised the manuscript.

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Compliance with ethical standards

Broad informed consent was obtained from the patient.

Conflict of interest The authors declare that they have no conflict of interests.

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