Modern Pathology (2018) 31, 553–561
© 2018 USCAP, Inc All rights reserved 0893-3952/18 $32.00

Expression of enhancer of zeste homolog 2 (EZH2) protein in histiocytic and dendritic cell neoplasms with evidence for p-ERK1/2-related, but not MYC- or p-STAT3-related cell signaling

Xuejun Tian[1,2], Jie Xu[1], Christopher Fletcher[1], Jason L Hornick[1] and David M Dorfman[1]

[1]Brigham and Women's Hospital and Harvard Medical School, Department of Pathology, Boston, MA, USA and [2]Montefiore Medical Center, Albert Einstein College of Medicine, Department of Pathology, Bronx, NY, USA

EZH2 is an important enzymatic subunit of the epigenetic regulator polycomb repressive complex 2 (PRC2), which controls gene silencing through post-translational modification, and is overexpressed in various carcinomas and hematopoietic neoplasms. We found that the majority of cases of histiocytic and dendritic cell neoplasms, including histiocytic sarcoma, follicular dendritic cell sarcoma, Langerhans cell histiocytosis, and interdigitating dendritic cell sarcoma, show strong EZH2 expression by immunohistochemical staining, in contrast to benign histiocytic lesions and normal cellular counterparts, which did not show EZH2 expression, suggesting that this molecule may function as an oncogenic protein in these neoplasms. We correlated EZH2 expression with that of p-ERK1/2, MYC, and p-STAT3, potential regulators of EZH2, and found that 60–80% of these cases showed strong p-ERK1/2 expression, and only a minority of cases showed positivity for MYC or p-STAT3 in neoplastic cells. In cases of follicular dendritic cell sarcoma, Langerhans cell histiocytosis, histiocytic sarcoma, and interdigitating dendritic cell sarcoma with strong EZH2 expression, 90%, 89%, 70%, and 100% of cases showed co-expression of p-ERK1/2 with EZH2, respectively, while only a small percentage of these cases showed MYC or p-STAT3 co-expression with EZH2 (≤30%). These findings suggest that the p-ERK1/2 signaling cascade, but not the p-STAT3 and MYC signaling cascades, may regulate EZH2 expression in histiocytic and dendritic cell neoplasms, and that EZH2 and the p-ERK1/2 signaling cascade could serve as therapeutic targets for the treatment of these neoplasms. Interestingly, only a minority of cases of blastic plasmacytoid dendritic cell neoplasm exhibited high EZH2 expression, and only a minority of these cases showed p-ERK1/2 co-expression, suggesting that alternative mechanisms may contribute to tumorigenesis in this aggressive neoplasm.
Modern Pathology (2018) **31,** 553–561; doi:10.1038/modpathol.2017.174; published 12 January 2018

EZH2 is an enzymatic subunit of the polycomb repressive complex 2 (PRC2), an important epigenetic regulator. EZH2 functions as a methytransferase that targets the lysine 27 of histone H3, leading to trimethylation (H3K27me3), a mechanism of post-translational modification that leads to transcriptional repression of PRC2 target genes.[1] EZH2, which is expressed in stem cells and proliferating cells and downregulated in differentiated cells, is overexpressed in a wide range of non-hematopoietic and hematopoietic neoplasms, and its overexpression is associated with tumor cell proliferation, metastasis, and poor prognosis. Neoplasms with increased EZH2 expression include a range of carcinomas, including breast, non-small cell lung, prostate, hepatocellular, ovarian, colorectal, renal and endometrial carcinomas, glioblastoma multiforme, other solid tumors, as well as hematopoietic neoplasms, including aggressive B-cell lymphomas, plasma cell neoplasms, myeloid neoplasms, including acute myeloid leukemia, and a range of T-cell lymphomas.[2–5]

EZH2 overexpression in various neoplasms has been shown to be due to various mechanisms, including intracytoplasmic oncogenic signaling molecules, transcription factors, gene amplification, gain of function mutations, and other mechanisms reviewed in reference.[6] For example, in a subset of

Correspondence: Dr DM Dorfman, MD, PhD, Department of Pathology Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA.
E-mail: ddorfman@bwh.harvard.edu
Received 26 May 2017; revised 17 October 2017; accepted 18 October 2017; published online 12 January 2018

follicular lymphomas and diffuse large B-cell lymphomas of germinal center B-cell type, a change of amino acid tyrosine 641 (Y641) has been identified as a recurrent somatic mutation in the *EZH2* gene, leading to increased enzymatic activity.[7] The oncogenic role of the Y641 mutation was further confirmed in an engineered mouse model in which conditional expression of mutant EZH2 in germinal center B cells induced germinal center hyperplasia and promoted lymphomagenesis in cooperation with BCL2 overexpression.[8] In addition to the Y641 mutation, A687V and A677G mutations have been identified as gain of function mutations of *EZH2* in B-cell lymphomas.[9,10] Small-molecule inhibitors of EZH2, which selectively block EZH2 methyltransferase activity and reduce global H3K27 methylation, have been shown to block tumor cell proliferation and induce cell cycle arrest and apoptosis in B-cell lymphomas.[11,12]

Another mechanism by which EZH2 promotes oncogenesis is through loss of its tumor suppressor function. Frequent homozygous and heterozygous *EZH2* deletions or inactivating mutations have been found in myeloid malignancies, including myelodysplastic syndromes, myeloproliferative neoplasms, and myelodysplastic/myeloproliferative neoplasms, such as chronic myelomonocytic leukemia,[13–16] and are predictive of poor survival.[17,18] In addition, inactivation of the *EZH2* gene through loss of function mutations and gene deletions leading to inactivation of the PRC2 complex were found in a significant number of cases of T-cell acute lymphoblastic leukemia, suggesting that loss rather than overexpression of EZH2 may also contribute to tumorigenesis in this T-cell neoplasm.[19] A third mechanism by which EZH2 promotes oncogenesis is by switching to a transcriptional activator independent of its methytransferase activity, a noncanonical function of EZH2 reported in NK/T-cell lymphoma as a result of JAK3 phosphorylation of EZH2 at Y244.[20,21] EZH2 action as a transcriptional activator able to stimulate cell growth may also occur in carcinomas of breast, colon, and other organs.[22,23]

Previously, we found that EZH2 is overexpressed in T-cell lymphomas, and that this overexpression is associated with aggressive behavior and higher proliferation rates.[4] In a wide range of low- and high-grade B-cell lymphoproliferative disorders, EZH2 expression correlates with aggressive behavior and proliferation rate, suggesting that EZH2 may function as an oncogenic protein in these neoplasms as well.[5] There is evidence for regulation of EZH2 by different signaling cascades in different types of aggressive B-cell lymphomas: p-ERK-related signaling in diffuse large B-cell lymphoma, and MYC-related signaling in Burkitt lymphoma and double hit lymphoma.[5] Because of the complicated role of EZH2 in the development of non-hematologic as well as hematologic malignancies, here we investigated the expression of EZH2 in different types of histiocytic and dendritic cell neoplasms. We further investigated the expression of different intracellular signaling molecules, including p-ERK1/2, MYC, and p-STAT3, as potential regulators of EZH2 expression in these neoplasms.

Materials and methods

Case Selection

Histiocytic and dendritic cell neoplasm cases were obtained from the files of the Department of Pathology, Brigham and Women's Hospital, Boston, MA, from 1990 to 2015, with the institutional internal review board's approval. Sixty-five cases of histiocytic and dendritic cell neoplasms were collected in the study, including 12 cases of blastic plasmacytoid dendritic cell neoplasm, 17 cases of histiocytic sarcoma, 15 cases of follicular dendritic cell sarcoma, 16 cases of Langerhans cell histiocytosis, and 5 cases of interdigitating dendritic cell sarcoma (Table 1). Nine cases of benign histiocytic diseases, including six cases of sinus histiocytosis with massive lymphadenopathy and three cases of juvenile xanthogranuloma, were also included in the study. The pathologic diagnoses were established according to the criteria of the 2008 World Health Organization (WHO) classification and 2016 revision of the WHO classification, based on morphologic, immunohistochemistry, cytogenetics, and molecular findings.[24] Diagnostic slides from all cases were reviewed and agreed upon by more than two pathologists in the department.

Immunohistochemistry

Immunostaining for EZH2 (Cell Signaling 5246), pSTAT (Cell Signaling 9145), and MYC (Abcam ab32072) were performed on the Leica Bond automated stainer, using the Bond Polymer Refine Detection kit (cat #DS9800), as previously described.[5] Heat-induced epitope retrieval was completed using Bond Epitope Retrieval Solution 2 (cat #AR9640), which is an EDTA based pH 9.0 solution, for 20 min at 100 °C. Primary antibodies were

Table 1 EZH2, p-ERK1/2, MYC, and p-STAT3 expression in histiocytic and dendritic cell neoplasms

	EZH2 (POS/ Total)	*p-ERK (POS/ Total)*	*MYC (POS/ Total)*	*p-STAT3 (POS/Total)*
BPDCN	5/12 (41%)	3/11(27%)	2/11(18%)	0/11(0%)
HS	10/17 (59%)	9/15 (60%)	3/11 (27%)	3/12 (25%)
FDCS	10/15 (67%)	12/15 (80%)	2/13 (15%)	4/13 (30%)
LCH	9/16 (56%)	10/16 (63%)	0/14 (0%)	1/15 (6%)
IDCS	3/5 (60%)	3/5 (60%)	0/5 (0%)	0/5 (0%)

Abbreviations: BPDCN, blastic plasmacytoid dendritic cell neoplasm; FDCS, follicular dendritic cell sarcoma; HS, histiocytic sarcoma; IDCS, interdigitating dendritic cell sarcoma; LCH, Langerhans cell histiocytosis; POS, positive cases.

applied at the optimized dilution (1:100) for 30 min at ambient temperature. For p-ERK1/2 (Cell Signaling 4370) immunostaining, heat-induced epitope retrieval was completed using Bond Epitope Retrieval Solution 1 (cat #AR9961)—which is a citrate based pH 6.0 solution—for 30 min at 100 °C. Primary antibody was applied at the optimized dilution (1:150) for 30 min at ambient temperature. This was followed by a 10 min application of the post primary, 10 min of polymer, and 5 min of peroxide block. Staining was visualized with a DAB chromogen for 10 min, and counterstained with hematoxylin. The slides were then removed from the autostainer, rinsed in running tap water, dehydrated through alcohols and xylene, and coverslipped.

The cases were scored for the percentage of positive tumor cells (0–100%) and for staining intensity (0–3) by two hematopathologists (X.T. and D.M.D.). EZH2 staining was considered to be overexpressed if $\geq 60\%$ of the neoplastic cells exhibited 2 + or 3+ staining intensity.[4] P-ERK1/2, MYC, and p-STAT3 staining was considered positive if $\geq 5\%$ of neoplastic cells were positive, as described previously.[5] Statistical analysis was performed using Graphpad Prism (Graphpad Software, La Jolla, CA, USA).

Results

Immunohistochemical staining for EZH2 was first performed on a range of histiocytic and dendritic cell neoplasms and benign histiocytic diseases; the results are summarized in Table 1, with representative results shown in Figure 1. In 65 cases of histiocytic and dendritic cell neoplasms studied, including 12 cases of blastic plasmacytoid dendritic cell neoplasm, 17 cases of histiocytic sarcoma, 15 cases of follicular dendritic cell sarcoma, 16 cases of Langerhans cell histiocytosis, and 5 cases of interdigitating dendritic cell sarcoma, 37/65 cases (57%), showed overexpression of EZH2. The majority of cases studied in all disease categories showed overexpression of EZH2, ranging from 56% of Langerhans cell histiocytosis cases to 67% of follicular dendritic cell sarcoma cases, with the exception of blastic plasmacytoid dendritic cell neoplasm, which was positive for EZH2 in a minority of cases (41%; Table 1 and Figure 1). Neoplastic cell positivity in EZH2-positive cases ranged from 67 to 81%, averaging 70% in histiocytic sarcoma, 75% in follicular dendritic cell sarcoma, 67% in Langerhans cell histiocytosis, 71% in interdigitating dendritic cell sarcoma, and 81% in blastic plasmacytoid dendritic cell neoplasm. In cases that did not overexpress EZH2, staining ranged from < 5 to 40% of neoplastic cells. In contrast, all nine cases of benign histiocytic diseases, including six cases of sinus histiocytosis with massive lymphadenopathy and three cases of juvenile xanthogranuloma, did not show EZH2 expression (Figure 1). Normal cellular counterparts of histiocytic and dendritic cell neoplasms in reactive lymphoid tissue, including follicular dendritic cells, interdigitating dendritic cells, and macrophages, did not exhibit significant EZH2 expression, nor did Langerhans cells in oral squamous mucosa (data not shown).

We were able to compare EZH2 overexpression with Ki-67 proliferation index in a subset of histiocytic sarcoma and blastic plasmacytoid dendritic cell neoplasm cases, and found that in general overexpression of EZH2 did not correlate with a high proliferation index for these neoplasms: 1/5 histiocytic sarcoma cases with EZH2 overexpression exhibited a high proliferation index, and 1/2 histiocytic sarcoma cases with low level EZH2 expression exhibited a high proliferation index; similarly, 1/5 blastic plasmacytoid dendritic cell neoplasm cases with EZH2 overexpression exhibited a high proliferation index, and 3/3 blastic plasmacytoid dendritic cell neoplasm cases with low level EZH2 expression exhibited a high proliferation index.

Immunohistochemical staining was performed for a number of signaling cascade-associated molecules that have been shown to up-regulate EZH2 expression in various neoplasms, including p-ERK1/2, MYC, and p-STAT3. We found that the majority of histiocytic and dendritic cell neoplasms in all disease categories showed strong p-ERK1/2 expression, ranging from 60% of histiocytic sarcoma and interdigitating dendritic cell sarcoma cases to 80% of follicular dendritic cell sarcoma cases, with the exception of blastic plasmacytoid dendritic cell neoplasm, which was positive for p-ERK1/2 in a minority of cases (27%; Table 1 and Figure 1), although the association of p-ERK1/2 expression with EZH2 expression was not statistically significant for any tumor category, except Langerhans cell histiocytosis ($P = 0.03497$). The percentage of positive neoplastic cells in p-ERK1/2-positive cases ranged from 63 to 73%, averaging 67% in histiocytic sarcoma, 72% in follicular dendritic cell sarcoma, 68% in Langerhans cell histiocytosis, 73% in interdigitating dendritic cell sarcoma, and 63% in blastic plasmacytoid dendritic cell neoplasm. In contrast with p-ERK1/2 expression, only a minority of cases ($< 27\%$) showed positivity for MYC in neoplastic cells, with no staining for MYC in any Langerhans cell histiocytosis or interdigitating dendritic cell sarcoma cases. Similarly, only a minority of cases ($< 30\%$) showed positivity for p-STAT3, with no staining for p-STAT3 in any blastic plasmacytoid dendritic cell neoplasm or interdigitating dendritic cell sarcoma cases (Table 1 and Figure 1).

To further understand the intracellular signaling cascades that potentially contribute to up-regulation of EZH2, we next focused on those histiocytic and dendritic cell neoplasm cases with EZH2 overexpression to investigate the association of EZH2 expression with p-ERK1/2, MYC, and/or p-STAT3 co-expression. In follicular dendritic cell sarcoma,

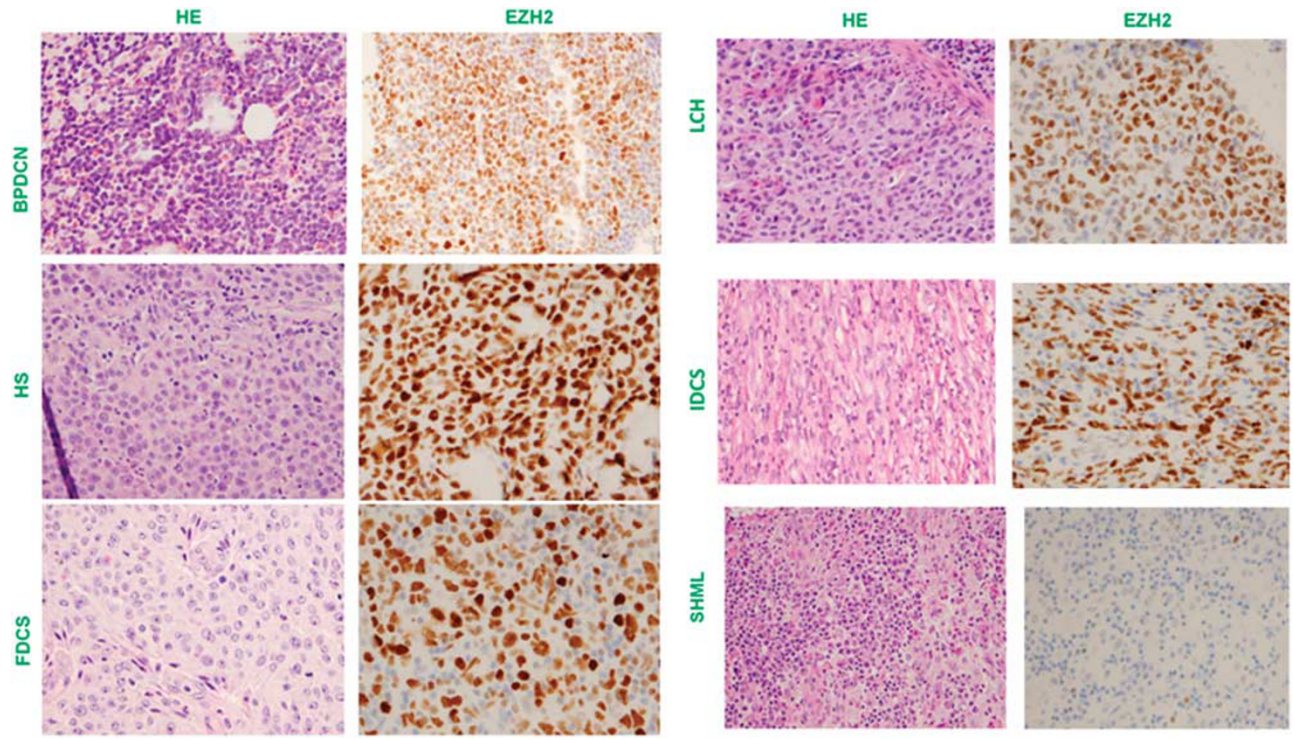

Figure 1 EZH2 immunohistochemical staining of representative cases of different histiocytic and dendritic cell neoplasms. BPDCN: blastic plasmacytoid dendritic cell neoplasm; FDCS: follicular dendritic cell sarcoma; HS: histiocytic sarcoma; IDCS: interdigitating dendritic cell sarcoma; LCH: Langerhans cell histiocytosis; SHML: sinus histiocytosis with massive lymphadenopathy. All images are ×400 original magnification.

Table 2 Co-expression of EZH2 with p-ERK1/2, MYC, and p-STAT3 proteins

	EZH2-positive cases (% positivity)	*p-ERK/EZH2*	*MYC/EZH2*	*p-STAT3/EZH2*
BPDCN	5 (81 ± 10%)	2/5 (40%)	2/5 (40%)	0/5 (0%)
HS	10 (70 ± 13%))	7/10 (70%)	3/10 (30%)	2/10 (20%)
FDCS	10 (75 ± 12%)	9/10 (90%)	1/10 (10%)	3/10 (30%)
LCH	9 (67 ± 10%)	8/9 (89%)	0/9 (0%)	1/9 (11%)
IDCS	3 (71 ± 13%)	3/3 (100%)	0/3 (0%)	0/3 (0%)

Abbreviations: BPDCN, blastic plasmacytoid dendritic cell neoplasm; FDCS, follicular dendritic cell sarcoma; HS, histiocytic sarcoma; IDCS, interdigitating dendritic cell sarcoma; LCH, Langerhans cell histiocytosis.

Langerhans cell histiocytosis, histiocytic sarcoma, and interdigitating dendritic cell sarcoma cases that overexpressed EZH2, the vast majority of cases (90%, 89%, 70%, and 100% of cases, respectively), showed co-expression of p-ERK1/2 with EZH2, in contrast to blastic plasmacytoid dendritic cell neoplasm, in which only a minority of EZH2-overexpressing cases (40%) showed co-expression of p-ERK1/2 (Table 2 and Figure 2). This association was statistically significant in cases of histiocytic sarcoma ($P = 0.004$), follicular dendritic sarcoma ($P = 0.002$), and Langerhans cell histiocytosis ($P = 0.004$). In contrast to the finding of frequent EZH2/p-ERK1/2 co-expression in these histiocytic and dendritic cell neoplasm cases, only a small percentage of cases showed MYC co-expression with EZH2 (< 30%, except blastic plasmacytoid dendritic cell neoplasm), with no cases of Langerhans cell histiocytosis or interdigitating dendritic cell sarcoma showing EZH2/MYC co-expression (Table 2 and Figure 2). Similarly, only a small percentage of cases showed p-STAT3 co-expression with EZH2 (< 30%, with no cases of blastic plasmacytoid dendritic cell neoplasm or interdigitating dendritic cell sarcoma showing EZH2/p-STAT3 co-expression (Table 2 and Figure 2).

We next focused on EZH2 overexpressing histiocytic and dendritic cell neoplasm cases to investigate how many of these cases have increased expression of more than one signaling cascade-associated molecule. In EZH2-positive histiocytic sarcoma cases, one case showed p-ERK1/2, MYC, and p-STAT3 co-expression, and one case showed p-ERK1/2 and MYC expression. In EZH2-positive follicular dendritic cell sarcoma cases, one case showed p-ERK1/2, MYC, and p-STAT3 co-expression, and two cases showed p-ERK1/2 and p-STAT3 co-expression. In EZH2-positive Langerhans cell histiocytosis cases, only one case showed p-STAT3 and p-ERK1/2 co-expression. In EZH2-positive interdigitating dendritic cell sarcoma cases, none showed any pattern of co-expression of p-ERK1/2 with p-STAT3 or MYC. These results suggest that the majority of EZH2-overexpressing histiocytic and dendritic cell neoplasms cases have associated p-ERK1/2 expression alone. Only a small number

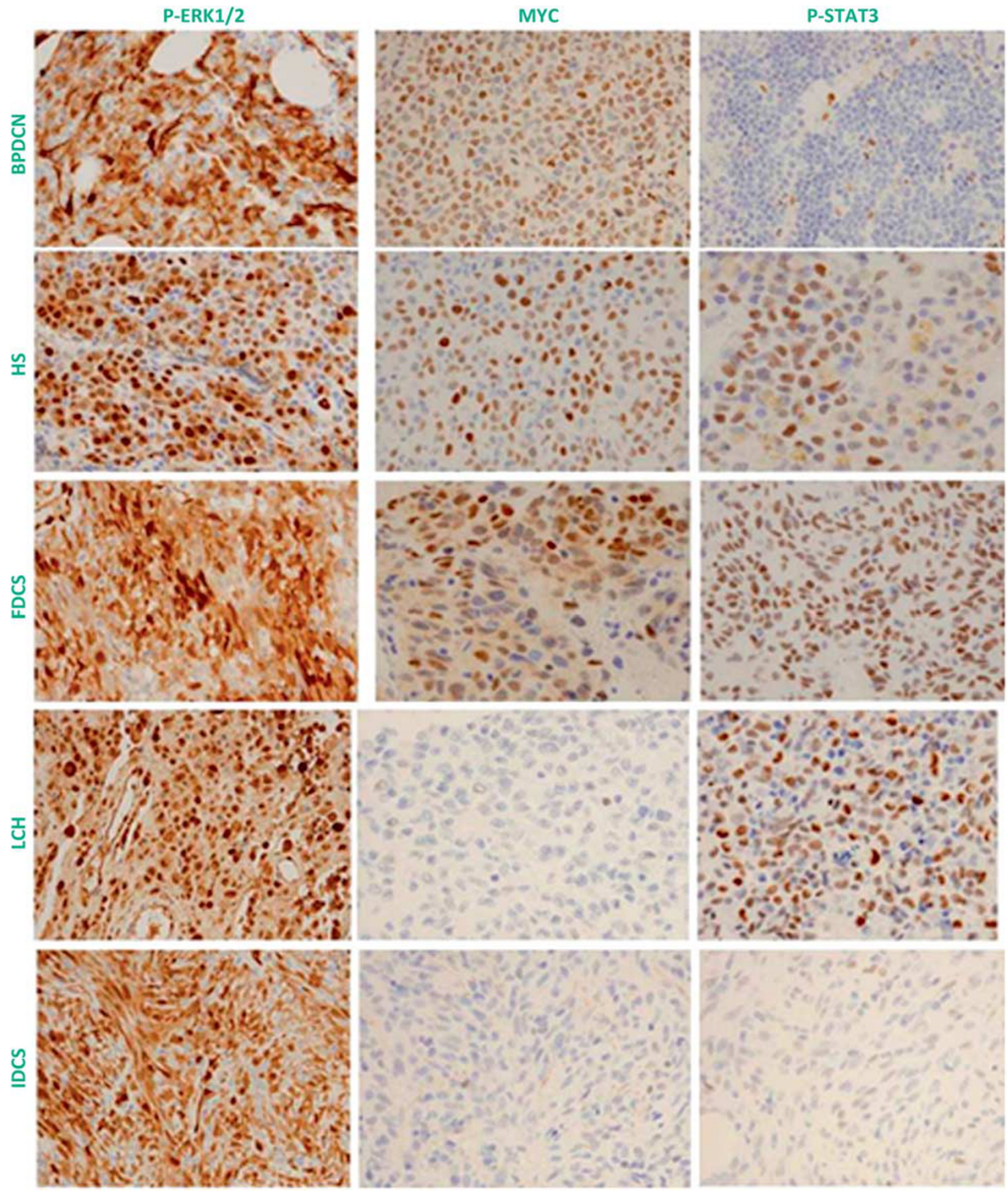

Figure 2 Representative EZH2-positive histiocytic and dendritic cell neoplasm cases showing co-expression of p-ERK1/2, MYC, and/or p-STAT3. All images are ×400 original magnification.

of these neoplasms exhibit expression of molecules corresponding to more than one intracellular signaling cascade that could contribute to EZH2 overexpression and tumorigenesis.

Interestingly, in blastic plasmacytoid dendritic cell neoplasm, a particularly aggressive neoplasm, only a minority of EZH2-overexpressing cases exhibited p-ERK1/2 co-expression. In addition, blastic plasmacytoid dendritic cell neoplasm had the highest percentage of EZH2/MYC co-expression (40%) among histiocytic and dendritic cell neoplasms studied. None of these cases showed p-STAT3/EZH2 co-expression.

Discussion

Histiocytic and dendritic cell neoplasms are rare, with limited data on intracellular signaling cascades that may contribute to tumorigenesis in these neoplasms. Here we report that the majority of histiocytic and dendritic cell neoplasms show overexpression of EZH2 by immunohistochemical staining, with the exception of blastic plasmacytoid dendritic cell neoplasm, in contrast with benign histiocytic diseases and normal cellular counterparts, which do not overexpress EZH2. This finding suggests that EZH2 plays a role in tumorigenesis in the majority of cases of histiocytic and dendritic neoplasms through overexpression, similar to its role in T-cell neoplasms,[4] high-grade B-cell lymphomas,[5] and non-hematologic solid tumors such as breast, colorectal, prostate, and hepatocellular carcinomas.[2]

A number of intracellular signaling cascade-associated molecules, including p-ERK1/2, MYC, and p-STAT3, have been shown to up-regulate EZH2 expression in hematologic neoplasms. Our previous work indicated that EZH2 overexpression, associated with aggressive behavior and higher proliferation rate in B-cell neoplasms,[5] appears to be regulated by different signaling cascades in different types of aggressive B-cell lymphomas. We found a high frequency of EZH2/p-ERK1/2 co-expression in diffuse large B-cell lymphoma, suggesting p-ERK1/2-related signaling of EZH2 expression in these neoplasms, and a high frequency of EZH2/MYC co-expression in Burkitt lymphoma and double hit lymphoma, suggesting MYC-related signaling of EZH2 expression in these neoplasms.[5] Similarly, in our study of T-cell lymphomas, we found that EZH2 overexpression correlated with high proliferation rate in these neoplasms, with MYC and/or p-STAT3 co-expression in subsets of T-cell lymphomas.[4]

In this study, we focused on these three well-known intracellular signaling molecules that are known to contribute to tumorigenesis and found that the majority of histiocytic and dendritic cell neoplasms cases in all disease categories showed strong p-ERK1/2 expression, with the exception of blastic plasmacytoid dendritic cell neoplasm. In contrast with p-ERK1/2 expression, only a minority of cases showed positivity for MYC or p-STAT3 in neoplastic cells. We further studied the association of these molecules with EZH2 overexpression and found that the vast majority of histiocytic and dendritic cell neoplasm cases that overexpress EZH2 showed strong co-expression of p-ERK1/2 with EZH2, with the exception of blastic plasmacytoid dendritic cell neoplasm, in which only a minority of EZH2-overexpressing cases showed co-expression of p-ERK1/2. In contrast to the finding of frequent EZH2/p-ERK1/2 co-expression in histiocytic and dendritic cell neoplasms, only a small percentage of cases showed MYC co-expression with EZH2 or p-STAT3 co-expression with EZH2. Furthermore, there were very few cases of histiocytic and dendritic cell neoplasms that showed a pattern of co-expression of more than one of these signaling molecules. These results suggest that the p-ERK1/2 signaling pathway may drive EZH2 overexpression in histiocytic and dendritic cell neoplasms (Figure 3), with the exception of blastic plasmacytoid dendritic cell neoplasm, although this model is speculative in the absence of functional data. Interestingly, in blastic plasmacytoid dendritic cell neoplasm a minority of cases showed EZH2 overexpression, and among those cases a minority showed co-expression of p-ERK1/2. A relatively increased number of blastic plasmacytoid dendritic cell neoplasm cases showed MYC/EZH2 co-expression, suggesting a different mechanism of tumorigenesis than in other histiocytic and dendritic cell neoplasms. The MYC signaling cascade may play a role in tumorigenesis in blastic plasmacytoid dendritic cell neoplasm, similar to our findings in a subset of high-grade B-cell lymphomas.[5]

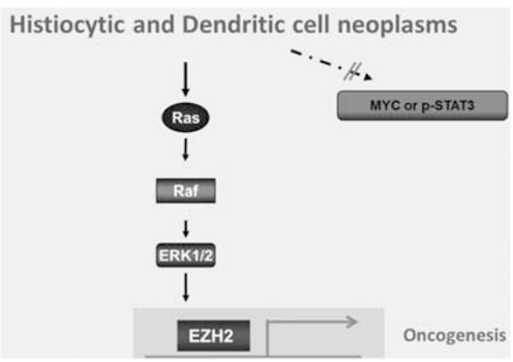

Figure 3 A proposed model for EZH2's contribution to tumorigenesis in histiocytic and dendritic neoplasms. The p-ERK1/2 signaling cascade, but not those of MYC or p-STAT3, appears to play an important role in histiocytic and dendritic cell neoplasms, possibly through up-regulation of EZH2 expression.

A number of publications have reported the presence of T-cell receptor or immunoglobulin gene rearrangements in histiocytic and dendritic neoplasms, as well as chromosomal aberrations associated with lymphoid neoplasms, and/or a history of a preceding B-cell or T-cell lymphoproliferative disorder.[25,26] For example, a case was recently reported of a 53-year-old man with a history of follicular lymphoma that transformed to diffuse large B-cell lymphoma, both showing the t(14;18) chromosomal translocation, who subsequently developed a histiocytic sarcoma which showed the t (14;18) chromosomal translocation characteristic of follicular lymphoma.[26] These findings suggest that B-cell or T-cell neoplasms may transdifferentiate to histiocytic and dendritic neoplasms.[25,26] Our finding that histiocytic and dendritic cell neoplasms overexpress EZH2 with co-expression of p-ERK1/2, MYC, and/or p-STAT3, similar to our prior observations in T-cell and B-cell lymphomas,[4,5] is consistent with the close molecular relationship that has been

observed between histiocytic and dendritic cell neoplasms and B-cell and T-cell lymphomas.

The mechanism of EZH2 overexpression has been extensively studied in non-hematologic and hematologic malignancies. One of the prototypic oncogenic signaling pathways, the RAS/MEK/ERK/ELK pathway, which is upregulated in many cancer cells, is linked to the overexpression of EZH2 in triple-negative and ERBB2-overexpressing subtypes of breast cancer.[27] MEK inhibitor and Elk-1 siRNA successfully blocked EZH2 mRNA and protein expression in cells from aggressive breast cancers,[28] suggesting that the p-ERK1/2 signaling cascade is upstream of EZH2 and regulates its expression. Recently we found that high-grade B-cell lymphomas, including diffuse large B-cell lymphoma, double hit lymphoma, and Burkitt lymphoma, overexpress EZH2, and that the vast majority of diffuse large B-cell lymphoma cases are positive for p-ERK1-/2, while cases of double hit lymphoma and Burkitt lymphoma show weak to negative p-ERK1/2 staining and positive staining for MYC.[5] These results suggest that the RAS/MEK/ERK/ELK pathway may preferentially up-regulate EZH2 expression in diffuse large B-cell lymphoma, compared with Burkitt lymphoma and double hit lymphoma, similar to the current findings in the majority of cases of histiocytic and dendritic cell neoplasms. Additional, *in vitro* studies are needed to further investigate the role of p-ERK1/2 signaling in EZH2 overexpression in histiocytic and dendritic cell neoplasms.

p-ERK1/2 activation could be the result of mutations in upstream signaling molecules such as BRAF, and, in fact, a number of studies have reported the *BRAF* V600E mutation in Langerhans cell histiocytosis, at an overall rate of 77/174 cases (44%; 29 – 33). While these studies found that only a minority of other histiocytic and dendritic cell neoplasms harbor *BRAF* mutations, 1/18 dendritic cell sarcomas (6%), 0/6 cases of histiocytic sarcoma, and 0/3 cases of blastic plasmacytoid dendritic cell sarcoma,[29 – 33] another study found the *BRAF* V600E mutation in 5/8 cases of histiocytic sarcoma and 5/27 cases of follicular dendritic sarcoma, but it was not present in 7 cases of blastic plasmacytoid dendritic cell neoplasm, 1 case of interdigitating dendritic cell sarcoma, 5 cases of sinus histiocytosis with massive lymphadenopathy, and 10 cases of xanthogranuloma.[34] Another study found *BRAF* mutations other than V600E in 3/5 cases of histiocytic sarcoma,[35] and case reports have reported *BRAF* V600E in two cases of histiocytic sarcoma and two cases of interdigitating dendritic cell sarcoma.[36 – 39] MAP2K1 mutations have been reported in 11/40 cases (27.5%) of Langerhans cell histiocytosis in one study[40] and 7/21 cases (33%) in another study,[41] which were negative for the BRAF V600E mutation. These findings suggest that the BRAF V600E mutation and MAP2K1 mutations may be responsible for p-ERK1/2 activation in a subset of histiocytic and dendritic cell neoplasms.

In a previous study of Langerhans' cell histiocytosis, p-ERK1/2 expression was assessed by immunohistochemical staining of 22 cases, with variable staining.[29] The total number of cases showing at least some 2+ or greater staining, 17/22, accounted for 77% of cases studied, similar to our immunohistochemical staining findings for p-ERK1/2 in Langerhans cell histiocytosis and other histiocytic and dendritic cell neoplasms, with the exception of blastic plasmacytoid dendritic cell neoplasm. Differences in p-ERK1/2 staining in the two studies may also be due to differences in staining methodology, tissue fixation, and the fact that our cases of these rare neoplasms were collected over a number of years and included tissue from outside institutions.

MYC, an important transcription factor, cell cycle regulator, and oncogenic protein that drives EZH2 overexpression in solid tumors, is overexpressed in Burkitt lymphoma, double hit lymphoma, and some cases of diffuse large B-cell lymphoma, and correlates with high EZH2 expression in these neoplasms.[5] Only a minority of histiocytic and dendritic cell neoplasm cases studied show significant MYC positivity, and we observed minimal EZH2/MYC co-expression. These findings suggest that MYC may not have a significant role in EZH2 overexpression in histiocytic and dendritic cell neoplasms. Similarly, we found that p-STAT3, which has been show to up-regulate EZH2 expression in colorectal cancer,[42,43] was expressed by a minority of histiocytic and dendritic cell neoplasm cases, with minimal EZH2/p-STAT3 co-expression, suggesting that it may not have a significant role in EZH2 overexpression in histiocytic and dendritic cell neoplasms.

Interestingly, in blastic plasmacytoid dendritic cell neoplasm, only a minority of cases showed EZH2 overexpression. These findings suggest that, in contrast with other histiocytic and dendritic cell neoplasms, EZH2 may not play a significant role in blastic plasmacytoid dendritic cell neoplasm oncogenesis. Attempts to understand the molecular basis for blastic plasmacytoid dendritic cell neoplasm have identified chromosomal losses, inactivation of tumor suppressor genes, activation of oncogenes, and mutations in genes encoding epigenetic regulators, including TET2, TET1, DNMT3A, IDH1, and IHD2, but not EZH2.[31] Recently, the E-box transcription factor TCF4 was identified as the master regulator of the blastic plasmacytoid dendritic cell neoplasm oncogenic program.[44] MYC was identified as a TCF4-activated gene, but not EZH2, p-ERK1/2, or p-STAT3.[38] These findings support our hypothesis that the mechanism of oncogenesis may differ in at least some cases of blastic plasmacytoid dendritic cell neoplasm compared with other histiocytic and dendritic cell neoplasms, and may be related to MYC expression.

EZH2 has been shown to play a multi-faceted role in cancer progression, functioning as an oncogenic protein through overexpression in solid tumors as

well as a number of hematopoietic neoplasms and as a tumor suppressor gene in myeloid and some lymphoid neoplasms. Because of its complex role in cancer development, attempts to therapeutically target EZH2 and EZH2-mediated signaling are emerging as an important strategy for cancer treatment. Several inhibitors of enzymes controlling epigenetic modifications, such as DNA methyltransferases and histone deacetylases, have shown promising antitumor effects.[45,46] Recently, a small molecule inhibitor of EZH2 has been developed which selectively blocks EZH2 methyltransferase activity and reduces global H3K27 methylation.[11,12] An EZH2 inhibitor has been used in early clinical trials in patients with advanced solid tumors or relapsed/refractory diffuse large B-cell lymphoma and follicular lymphoma (Clinical trails.gov, ID:NCT01897571). Our findings suggest that the majority of histiocytic and dendritic cell neoplasms may be suitable targets for EZH2 inhibitor treatment. In addition, targeting of the p-ERK1/2 signaling pathway, which appears to upregulate EZH2 expression in histiocytic and dendritic neoplasms, is another potential strategy for the treatment of the majority of these rare malignant neoplasms.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 2009;10:697–708.
2 Jiang T, Wang Y, Zhou F, *et al.* Prognostic value of high EZH2 expression in patients with different types of cancer: a systematic review with meta-analysis. Oncotarget 2016;7:4584–4597.
3 Herviou L, Cavalli G, Carton G, *et al.* EZH2 in normal hematopoiesis and hematological malignancies. Oncotarget 2015;7:2284–2296.
4 Shi M, Shahsafaei A, Liu C, *et al.* Enhancer of zeste homolog 2 is widely expressed in T-cell neoplasms, is associated with high proliferation rate and correlates with MYC and p-STAT3 expression in a subset of cases. Leuk Lymphoma 2015;56:2087–2091.
5 Tian X, Pelton A, Shahsafaei A, *et al.* Differential expression of Enhancer of zeste homolog 2 (EZH2) protein in small cell and aggressive B-cell non-Hodgkin lymphomas and differential regulation of EZH2 expression by p-ERK1/2 and MYC in aggressive B-cell lymphomas. Mod Pathol 2016;29:1050–1057.
6 Dorfman DM, Tian X. New insights into the mechanisms of EZH2's promotion of oncogenesis. Transl Cancer Res 2016;5(Suppl 6):S1057–S1060.
7 Morin RD, Johnson NA, Severson TM, *et al.* Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 2010;42:181–185.
8 Beguelin W, Popovic R, Teater M, *et al.* EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. Cancer Cell 2013;23:677–692.
9 Majer CR, Jin L, Scott MP, *et al.* A687V EZH2 is a gain-of-function mutation found in lymphoma patients. FEBS Lett 2012;586:3448–3451.
10 McCabe MT, Graves AP, Ganji G, *et al.* Mutation of A677 in histone methyltransferase EZH2 in human B-cell lymphoma promotes hypertrimethylation of histone H3 on lysine 27 (H3K27). Proc Natl Acad Sci USA 2012;109:2989–2994.
11 Qi W, Chan H, Teng L, *et al.* Selective inhibition of Ezh2 by a small molecule inhibitor blocks tumor cells proliferation. Poc Natl Acad Sci USA 2012;109:21360–21365.
12 Garapaty-Rao S, Nasveschuk C, Gagnon A, *et al.* Identification of EZH2 and EZH1 small molecule inhibitors with selective impact on diffuse large B-cell lymphoma cell growth. Cell Chem Biol 2013;20:1329–1339.
13 Ernst T, Chase AJ, Score J, *et al.* Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 2010;42:722–726.
14 Makishima H, Jankowska AM, Tiu RV, *et al.* Novel homo- and hemizygous mutations in EZH2 in myeloid malignancies. Leukemia 2010;24:1799–1804.
15 Nikoloski G, Langemeijer SM, Kuiper RP, *et al.* Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet 2010;42:665–667.
16 Score J, Hidalgo-Curtis C, Jones AV, *et al.* Inactivation of polycomb repressive complex 2 components in myeloproliferative and myelodysplastic/myeloproliferative neoplasms. Blood 2012;119:1208–1213.
17 Bejar R, Stevenson K, Abdel-Wahab O, *et al.* Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364:2496–2506.
18 Guglielmelli P, Biamonte F, Score J, *et al.* EZH2 mutational status predicts poor survival in myelofibrosis. Blood 2011;118:5227–5234.
19 Ntziachristos P, Tsirigos A, Vlierberghe P, *et al.* Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. Nat Med 2012;18:298–301.
20 Yan J, Ng S-B, JL-S Tay, *et al.* EZH2 overexpression in natural killer/T-cell lymphoma confers growth advantage independently of histone methyltransferase activity. Blood 2013;121:4512–4520.
21 Yan J, Li B, Lee PT, *et al.* EZH2 phosphorylation by JAK3 mediates a switch to noncanonical function in natural killer/T-cell lymphoma. Blood 2016;128:948–958.
22 Shi B, Liang J, Yang X, *et al.* Integration of estrogen and Wnt signaling circuits by the polycomb group protein EZH2 in breast cancer cells. Mol Cell Biol 2007;27:5105–5119.
23 Lee ST, Li Z, Wu Z, *et al.* Context-specific regulation of NF- κB target gene expression by EZH2 in breast cancers. Mol Cell 2011;43:798–810.
24 Swerdlow SH, Campo E, Pileri SA, *et al.* The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016;127:2375–2390.
25 Huang W, Qiu T, Zeng L, *et al.* High frequency of clonal IG and T-cell receptor gene rearrangements in histiocytic and dendritic cell neoplasm. Oncotarget 2016;7:78355–78362.
26 Rjoop A, Pandey S, Levy RA. A rare case of follicular lymphoma with transformation to diffuse large B-cell

lymphoma and trans-differentiation to histiocytic sarcoma. Case Reports Clin Pathol 2016;3:45–49.
27 Fujii S, Ito K, Ito Y, *et al.* Enhancer of zeste homologue 2 (EZH2) down-regulates RUNX3 by increasing histone H3 methylation. J Biol Chem 2008;283:17324–17332.
28 Fujii S, Tokita K, Wada N, *et al.* MEK–ERK pathway regulates EZH2 overexpression in association with aggressive breast cancer subtypes. Oncogene 2011;30: 4118–4128.
29 Badalian-Very G, Vergilio J-A, Degar BA, *et al.* Recurrent *BRAF* mutations in Langerhans' cell histiocytosis. Blood 2010;116:1919–1923.
30 Fedoriw Y, Kim YS, Vergilio J-A, *et al.* BRAF V600E mutation-specific immunohistochemistry is a rare finding and dendritic cell- and histiocytic-derived tumors. Leuk Lymph 2014;56:1132–1133.
31 Bubolz A-M, Weissinger SE, Stenzinger A, *et al.* Potential clinical implications of *BRAF* mutations in histiocytic proliferations. Oncotarget 2014;5:4060–4070.
32 Gatalica Z, Bilalovic N, Palazzo JP, *et al.* Disseminated histiocytoses biomarkers beyond BRAFV600 E: frequent expression of PD-L1. Oncotarget 2015;6: 19819–19825.
33 Haroche J, Charlotte F, Arnaud L, *et al.* High prevalence of BRAF V600E mutations in Erdheim-Chester disease but not in other non-Langerhans cell histiocytoses. Blood 2012;120:2700–2703.
34 Go H, Jeon YK, Huh J, *et al.* Frequent detection of *BRAF* V600 E mutations in histiocytic and dendritic cell neoplasms. Histopathology 2014;65:261–272.
35 Liu Q, Tomaszewicz K, Hutchinson L, *et al.* Somatic mutations in histiocytic sarcoma identified next generation sequencing. Virchows Arch 2016;469:233–241.
36 Vaughn JL, Freitag CE, Hemminger JA, *et al. BRAF* V600E expression in histiocytic sarcoma associated with splenic marginal zone lymphoma: a case report. J Med Case Rep 2017;11:92.
37 Idbaih H, Mokhtari K, Emile J–F, *et al.* Dramatic response of a *BRAF* V600E–mutated primary CNS histiocytic sarcoma to vemurafenib. Neurology 2014;83: 1478–1480.
38 Di Liso E, Pennelli N, Lodovichetti G, *et al.* The Braf mutation in interdigitating dendritic cell sarcoma: a case report and review of the literature. Cancer Biol Ther 2015;16:1128–1135.
39 O'Malley DP, Agarwal R, Grimm KE, *et al.* Evidence of *BRAF* V600 E in indeterminant cell tumor and interdigitating dendritic cell sarcoma. Ann Diagn Pathol 2015;19:113–116.
40 Brown NA, Furtado LV, Betz BL, *et al.* High prevalence of somatic MAP2K1 mutations in BRAF V600E-negative Langerhans' cell histiocytosis. Blood 2014;124: 1655–1658.
41 Chakraborty R, Hampton OA, Shen X, *et al.* Mutually exclusive recurrent somatic mutations in *MAP2K1* and *BRAF* support a central role for ERK activation in LCH pathogenesis. Blood 2014;124:3007–3015.
42 Xiong H, Zhang ZG, Tian XQ, *et al.* Inhibition of JAK1, 2/STAT3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells. Neoplasia 2008;10:287–297.
43 Lin YW, Ren LL, Xiong H, *et al.* Role of STAT3 and vitamin D receptor in EZH2-mediated invasion of human colorectal cancer. J Pathol 2013;230:277–290.
44 Ceribelli M, Hou ZE, Kelly PN, *et al.* A druggable TCF4- and BRD4-dependent transcriptional network sustains malignancy in blastic plasmacytoid dendritic cell neoplasm. Cancer Cell 2016;30:764–778.
45 Egger G, Liang G, Aparicio A, *et al.* Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004;429:457–463.
46 Wee S, Dhanak D, Li H, *et al.* Targeting epigenetic regulators for cancer therapy. Ann NY Acad Sci 2014;1309:30–36.