ARID1A mutation in blastic plasmacytoid dendritic cell neoplasm

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare and aggressive hematologic disease originating from plasmacytoid dendritic cell precursors.[1] According to the WHO 2008 classification of hematopoietic tumors, BPDCN belongs to the category "acute myeloid leukemia (AML) and related precursor neoplasms".[2] Patients with BPDCN usually present cutaneous lesions and bone marrow infiltration. Diagnosis of BPDCN relies on the immunophenotype of blast cells characterized by $CD4^+$, $CD56^+$, $CD123^+$, $HLA\text{-}DR^+$, negative for lineage specific markers (Lin-).[3] BDCA2 and TCL1 expression further supports the diagnosis.[3] Cytogenetic and molecular abnormalities are frequent in BPDCN cases.[4] Recently, whole-exome-sequencing identified recurrent mutations in BPDCN, including *TET2, ASXL1, RAS, NPM1*.[5,6] Until now, the molecular mechanism of BPDCN remains largely unknown and new drivers constantly emerge.

The SWI/SNF complex is composed of ARID1A, ARID1B, SMARCA4 as well as SMARCB1, and it remodels chromatin architecture by sliding or ejecting nucleosomes from DNA to modulate its replication, transcription and repair.[7,8] The SWI/SNF complex plays a tumor suppressive role, and its subunits are mutated in 19.2% of all human tumors.[9] Among the SWI/SNF complex subunits, ARID1A mutation is the most frequent and typical by nonsense or frame-shift leading to premature translation termination codons and loss of function.[8,9] Defect in ARID1A function plays a driving role in the pathogenesis of various cancers including GCB-cell original lymphoma,[10] but how it functions in BPDCN remains to be clarified.

Herein, we report a 29-year-old man presenting with progressive cutaneous erythema on his back (Figure 1A) for almost one year; constantly elevated blasts with anemia as well as thrombocytopenia in peripheral blood (Blood counts at diagnosis: WBC $7.1x10^9$/L, Hb 91g/L, PLT $55x10^9$/L, blasts 72%) had appeared. Bone marrow examination revealed that monomorphic, poorly differentiated blasts accounted for 90.5% (Figure 1B). Immunophenotype of the blast cells was defined as $CD4^+$, $CD56^+$, $CD123^+$, $HLA\text{-}DR^+$, $CD7^+$, $CD5^-$, $CD2^-$, $CD34^-$, $CD117^-$, $CD33^-$, and $CD13^-$ by flow cytometry (Figure 1C). Thus, BPDCN was diagnosed. In addition to bone marrow, cutaneous involvement was demonstrated by morphology examination and immunohistochemistry (Figure 1D). Karyotype analysis of bone marrow cells showed complex abnormality with 45,X, –Y, der(1)(q24), –5, der(9)(q34), der(13)(q34), der(16)(q24), der(22)(q11), +mar[13] (Figure 1E). FISH (Fluorescence *in situ* hybridization) was then displayed in skin samples, and the karyotype was positive for –Y (345/400, 86.25%) but negative for –5 (0/400, 0%) (Figure 1F). Subsequently, the patient underwent one cycle of Hyper-CVAD A regimen (Cyclophosphamide 500mg q12h d1-3; Vindesine 4mg qd d4, 11; Liposomal doxorubicin 60mg qd d4; Dexamethasone 20mg qd d1-4, 11-14). Four weeks after chemotherapy onset, bone marrow complete remission was achieved and cutaneous erythema diminished remarkably.

In this patient, targeted exome sequencing of 390 genes recurrently mutated in cancers was performed in bone marrow and skin biopsy samples at diagnosis. Oral exfoliative cells were collected and then subjected to sequencing as germline control. Average amplicon coverage was greater than 5000 in each sample. After excluding the pseudo-mutations caused by frameshift and normal polymorphisms, the mutation with ≥1% variant allele frequency (VAF) in tumor samples and more than 600 read depth in tumor and control samples was considered significant. RNA sequencing was also displayed in bone marrow and skin samples to confirm the mutation detected by exome sequencing. Data volume of each sample reached 30M reads. DNA and RNA were extracted from fresh samples. Sequencing was displayed by Hiseq (Illumina). Written informed consent has been obtained before all above performance.

Sequencing showed that *ARID1A* (G64fsX99, Q67R) was mutated in both bone marrow (VAF=42.5%) and skin (VAF=44.6%) samples (Table 1). Surprisingly, we found that *BCORL1* mutation (A1100fsX1128, C1101Y, VAF=26.7%; R1196X, VAF=5.4%) was only present in the bone marrow sample, while *K-RAS* mutation (A146T, VAF=45%) was only present in the skin sample (Table 1). The results indicated that BCORL1 mutation and K-RAS mutation played important roles in bone marrow and skin infiltration, respectively. At the same time, clonal evolution was observed in bone marrow though the consequence of the two *BCORL1* mutations was sim-

Table 1. Targeted exome sequencing and RNA sequencing results from this BPDCN patient.

Gene	Mutation	Consequence	Exome-Seq in BM	Exome-Seq in skin	RNA-Seq in BM	RNA-Seq in skin
ARID1A	c.191_194delGGCC, c.A200G	G64fsX99, Q67R	42.5%	44.6%	37.1%	27.5%
BCORL1	c.3299_3300insT, c.G3302A	A1100fsX1128, C1101Y	26.7%	ND	33.3%	ND
BCORL1	c.C3586T	R1196X	5.4%	ND	20%	ND
BCORL1	c.C2508G	Y836X	1.2%	ND	ND	ND
BCORL1	c.3331_3332insCGGCC	T1111fsX1121	21.5%	ND	ND	ND
FLT3	c.G1471C	V419L	2%	ND	ND	ND
FLT3	c.T2505G	D835E	1.2%	ND	ND	ND
K-RAS	c.G436A	A146T	ND	45%	ND	48.9%
K-RAS	c.G35A	G12D	3.6%	ND	ND	ND

Gene mutations identified in bone marrow sample and skin sample, and their predicted consequences on the amino acid sequences as well as variant allele frequencies are listed. Exome Sequencing, Exome-seq; RNA sequencing, RNA-Seq; Bone marrow, BM; Variant allele frequency, VAF; Not detected, ND.

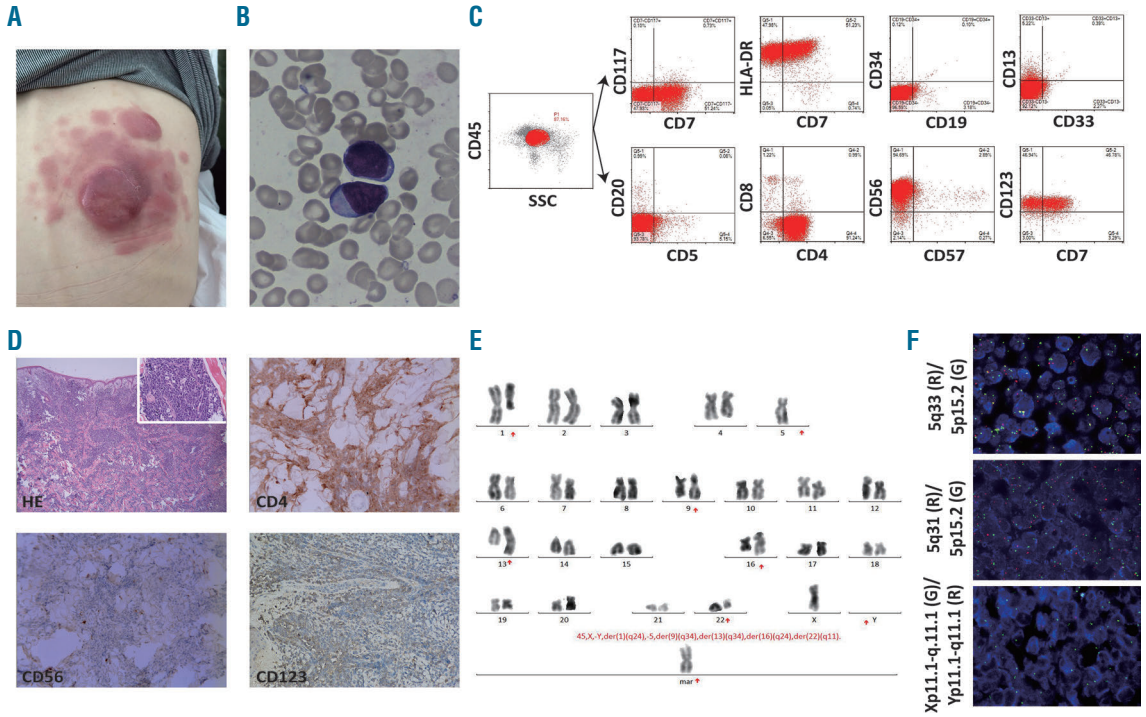

Figure 1. Clinical, cytological, histological and cytogenetical features of this BPDCN patient. (A) Skin lesion in this patient. (B) The morphology of bone marrow (BM) cells. (C) The immunophenotype of BM cells was determined by flow cytometry at diagnosis. (D) The morphology and immunohistochemistry analysis was displayed in skin biopsy sample. HE, CD4, CD56, and CD123 staining shown respectively. (E) The karyotype of BM cells. (F) Chromosomal aberrations such as –5 and –Y were examined by Fluorescence *in situ* hybridization in skin sample. R, red; G, green.

ilar. In addition, we also found two *BCORL1* mutations (Y836X, VAF=1.2%; T1111fsX1121, VAF=21.5%), two FLT3 mutations (V419L, VAF=2%; D835E, VAF=1.2%), and N-RAS mutation (G12D, VAF=3.6%) in the DNA but not the RNA sample of bone marrow (Table 1), indicating that there were many sub-clones in the bone marrow, and that these mutations were below the detection threshold of RNA sequencing. Notably, *BCORL1* mutation (T1111fsX1121) with high VAF was not detected in RNA sequencing, which could be explained by defects in transcription.

Of note, we first identified loss-of-function mutation of *ARID1A* in BPDCN. ARID1A^{G64fsX99}, Q67R retains only the N-terminal 98 amino acids of full-length ARID1A, which is similar to complete loss of its expression. It has been reported that *ARID1A* knockout causes chromatin decatenation defect, leading to chromosomal instability.[7,9] BCORL1 is a transcriptional corepressor which inhibits the expression of tumor suppressor genes, and can interact with CtBP corepressor as well as class II histone deacetylases.[11] *BCORL1* mutation has previously been detected in other myeloid neoplasms such as AML or MDS,[12,13] but not in BPDCN. The truncated BCORL1 protein in this patient only retained its CtBP binding site at N-terminal, and might lose its inhibitory effect on prooncogenes. K-RAS belongs to the small GTPase family, and constitutively activated K-RAS functions as a prooncogene in a wide range of cancers. *K-RAS* mutation accounts for 8% in BPDCN patients. We identified K-RAS^{A146T} in this patient, which has similar but relatively inferior function in activating downstream signaling pathway such as c-RAF-MEK-ERK and PI3K-AKT compared to K-RAS^{G12D}.[14]

Cytogenetic abnormality is common in BPDCN. It is reported that complex chromosomal aberrations are present in 66% of BPDCN patients, and chromosomes 5q (72%), 12p (64%), 13q (64%), 6q (50%), 15q (43%) and 9 (28%) are the major recurrent targets.[15] However, no single chromosomal aberration was specific to BPDCN. In our patient, one complex karyotype was identified in BM cells, including aberrations involving with hotspot chromosome 5q and 13q. Surprisingly, FISH results showed that –Y but not –5 existed in the skin sample, indicating that BPDCN cells in BM and skin probably share one clonal origin, but clonal evolution occurred at different times. Chromosomal abnormalities arise from aberrant mitotic behaviour, and loss of ARID1A function leads to chromatin decatenation defect.[7,9] Therefore, we considered that the emergence of complex karyotype was secondary to *ARID1A* mutation.

In this patient, our results provided a model for the initiation and development of BPDCN. First, *ARID1A* mutation (G64fsX99, Q67R) of BPDCN was acquired, then chromosomal aberrations, at least –Y but not –5, emerged in the original clone and an extremely complex karyotype was generated as a result. *BCORL1* (A1100fsX1128, C1101Y; R1196X) as well as deletion of chromosome 5 and *K-RAS* (A146T) mutation were then acquired when BPDCN cells disseminated to bone marrow and skin, respectively, and evident BPDCN then developed. At the same time, clonal evolution was dynamic in bone marrow. Alternatively, mutation hierar-

chy could be explained by another model: evident BPDCN infiltrated both skin and bone marrow after *ARID1A* mutation had occurred following complex chromosomal aberrations including –Y but not –5. Subsequently, *BCORL1* mutation as well as deletion of chromosome 5 or *K-RAS* mutation were acquired when BPDCN progressed in bone marrow or skin, respectively. In our data, we found that the VAF of *ARID1A* mutation was no more than the total VAF of other gene mutations in the same sample, indicating that *ARID1A* mutation needed to cooperate with other gene mutations in order to develop to evident BPDCN in skin or bone marrow. Therefore, the possibility of the second model was relatively low.

In conclusion, we have reported *ARID1A* mutation in BPDCN, and provided a possible evolutional model for this patient in order to illustrate how BPDCN transformed and progressed.

Lei Wang,[1,2,3#] Min Yang,[1,2,3#] Xiang Zhang,[1,2,3#] Chunmei Yang,[1,2,3] Xin Huang,[2,3] Zhaoming Wang[4] and Jie Jin[1,2,3]

[1]Department of Hematology, The First Affiliated Hospital, Zhejiang University College of Medicine; [2]Institute of Hematology, Zhejiang University; [3]Key Laboratory of Hematologic Malignancies, Diagnosis and Treatment, Zhejiang, and [4]Department of Pathology, The First Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, China.

#LW, MY, and XZ contributed equally to this work

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Correspondence: jiej0503@zju.edu.cn
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References

1. Herling M, Jones D. CD4+/CD56+ hematodermic tumor: the features of an evolving entity and its relationship to dendritic cells. Am J Clin Pathol. 2007;127(5):687-700.
2. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
3. Marafioti T, Paterson JC, Ballabio E, et al. Novel markers of normal and neoplastic human plasmacytoid dendritic cells. Blood. 2008;111(7):3778-3792.
4. Pagano L, Valentini CG, Grammatico S, Pulsoni A. Blastic plasmacytoid dendritic cell neoplasm: diagnostic criteria and therapeutical approaches. Br J Haematol. 2016;174(2):188-202.
5. Menezes J, Acquadro F, Wiseman M, et al. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. Leukemia. 2014;28(4):823-829.
6. Stenzinger A, Endris V, Pfarr N, et al. Targeted ultra-deep sequencing reveals recurrent and mutually exclusive mutations of cancer genes in blastic plasmacytoid dendritic cell neoplasm. Oncotarget. 2014;5(15):6404-6413.
7. Williamson CT, Miller R, Pemberton HN, et al. ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. Nat Commun. 2016;7:13837.
8. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer. 2011;11(7):481-492.
9. Kadoch C, Hargreaves DC, Hodges C, et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. Nat Genet. 2013;45(6):592-601.
10. Lunning MA, Green MR. Mutation of chromatin modifiers; an emerging hallmark of germinal center B-cell lymphomas. Blood Cancer J. 2015;5:e361.
11. Pagan JK, Arnold J, Hanchard KJ, et al. A novel corepressor, BCoR-L1, represses transcription through an interaction with CtBP. J Biol Chem. 2007;282(20):15248-15257.
12. Li M, Collins R, Jiao Y, et al. Somatic mutations in the transcriptional corepressor gene BCORL1 in adult acute myelogenous leukemia. Blood. 2011;118(22):5914-5917.
13. Damm F, Chesnais V, Nagata Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. Blood. 2013;122(18):3169-3177.
14. Tyner JW, Erickson H, Deininger MW, et al. High-throughput sequencing screen reveals novel, transforming RAS mutations in myeloid leukemia patients. Blood. 2009;113(8):1749-1755.
15. Leroux D, Mugneret F, Callanan M, et al. CD4(+), CD56(+) DC2 acute leukemia is characterized by recurrent clonal chromosomal changes affecting 6 major targets: a study of 21 cases by the Groupe Francais de Cytogenetique Hematologique. Blood. 2002;99(11):4154-4159.