Coexistence of *EZH2*, *NOTCH1*, *IL7R*, and *PHF6* Mutations in Adult T-cell Acute Lymphoblastic Leukemia

Erişkin T-hücre Akut Lenfoblastik Lösemi'sinde *EZH2, NOTCH1, IL7R* ve *PHF6* Mutasyonlarının Birlikteliği

Xilian Zhou[1], Yan Gu[1], Qi Han[1], Mario Soliman[2], Chunhua Song[2], Zheng Ge[1]

[1] *Zhongda Hospital, Medical School of Southeast University Department of Hematology Nanjing, China*
[2] *Pennsylvania State University, Department of Pediatrics, Pennsylvania, USA*

To the Editor,

Enhancer of zestehomolog 2 (*EZH2*) mutations are reported in solid tumors [1,2,3] as well as leukemia, and they are most commonly detected in early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) [4,5,6,7,8], which is an extraordinarily aggressive malignancy of enigmatic genetic basis [9]. We screened *EZH2* mutations in 146 Chinese adult ALL patients, among which 24.7% (36/146) cases were T-cell acute lymphoblastic leukemia (T-ALL) and 12.9% (4/31) T-ALL cases were identified as ETP-ALL. We found three *EZH2* mutations in two patients with T-ALL. One patient had Mu#1:D730fs*1, a truncation mutation that was previously reported in acute myeloid leukemia, and the another patient had two new *EZH2* mutations, Mu#2:K466T and Mu#3:T467fs*>3 (Figure 1). We also screened the mutations in other genes (Table 1). Strikingly, the *EZH2* mutations coexisted with mutations of *NOTCH1*, *IL7R*, and *PHF6* in the two patients and they responded poorly to chemotherapy and experienced difficult clinical histories and inferior outcomes (Table 1). Patient 1 was diagnosed with T-ALL with myeloid expression based on his bone marrow (BM) smear and immunophenotypes (Table 1). With the first inductive therapy (Table 1), the patient achieved complete remission (CR) with 0.1% blasts in the peripheral blood (PB) and 0.8% in BM. One year later, the patient relapsed with 90.4% lymphoblasts in the BM and 1.0% in the PB, and CR was achieved after the first chemotherapy. During the following treatment, he underwent an intramedullary and an extramedullary relapse infiltrating his left tonsil and then endured three more relapses. On the fifth relapse, the BM blast rate was 50.4%. Although the patient was treated with nelarabine, no CR was achieved in the subsequent treatments. Even though the BM blast rate was 5.2%, the patient died of infection during the BM suppression period after he received the last chemotherapy. We examined the *EZH2* mutational status in the BM samples of the 1st relapse, 5th relapse, and 6 weeks after his 5th relapse; the *EZH2* and *NOTCH1* mutation status remained the same as in the first diagnosis even after the nelarabine treatment (Figure 1D). Patient 2 presented with 80.0% lymphoblasts in the PB and 78.0% blasts in the BM (Table 1). Two somatic mutations, K466T and T467fs*>3 in

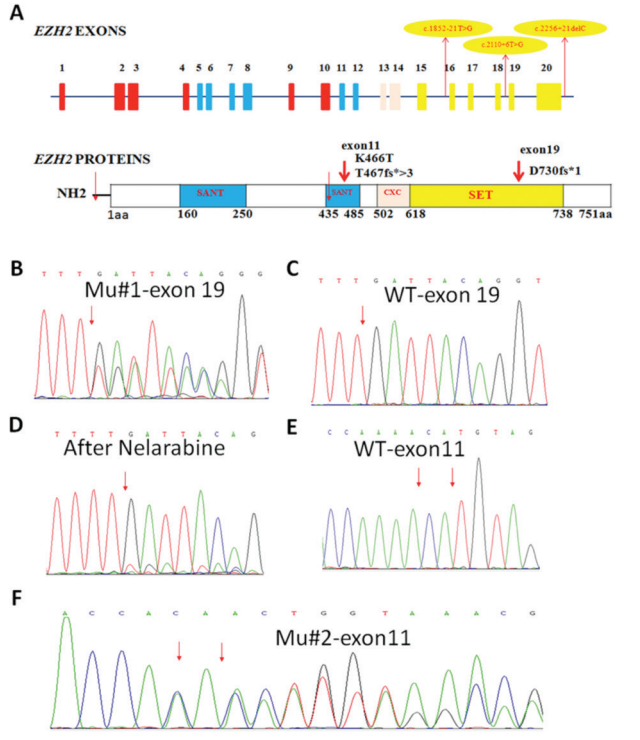

Figure 1. Location and sequencing data of the *EZH2* mutations. A) Mutation 1 (Mu#1:D730fs*1), located in exon 19, is a frame shift-creating insertion; on the protein level, it leads to a truncated protein with a length of 731 amino acids, which is located in the conserved catalytic SET domain(amino acids 618-731). This domain is critical for the methyltransferase activity of *EZH2*. The other two mutations (Mu#2:K466T; Mu#3:T467fs*>3) located within exon 11 are anon-synonymous single-nucleotide substitution and a frame shift-creating deletion, respectively; on the protein level, they result in the substitution of *EZH2* lysine466 to tyrosine and a truncation of the *EZH2* protein, respectively. Mu#2 and Mu#3 are novel *EZH2* mutations; both of them are located in the SANT domain of the *EZH2* protein (amino acids 435-485), which is known to be incharge of the DNA binding. Mu#1 was detected in patient 1 and the other two in patient 2. Blue, pink, and yellow bars correspond to exons encoding the SANT domains, the cysteine-rich CXC domain, and the SET domain, respectively. The red arrows show *EZH2* mutations. B-F) The direct sequencing data of *EZH2* mutations (B, D, F) and wild-type (C, E). B: c.2187_2188insT p.D730fs*1; C: *EZH2* exon 19 wild-type; D: Mu#1 after nelarabine treatment; E: *EZH2* exon 11 wild-type; F: c.1397A>C; 1399delA p.K466T; T467fs*>3.

Table 1. Clinical features of patients with *EZH2* mutations.

				Patient 1	Patient 2
Age (years)/Sex				49/Male	41/Female
WBC, x10^9/L				9.7	64.9
Hemoglobin (g/L)				128	71
Platelets (x10^9/L)				96	38
BM/PB blasts (%)				50.4/0	78.0/80.0
Mutation screening				Patient 1 (nucleotide/mutant ID)	Patient 2 (nucleotide/mutant ID)
Gene	ALL (%) (n=146)	T-ALL (%) (n=36)	Exon		
EZH2	1.4	5.6	Exon 19	2187_2188insT/COSM52999	
			Exon 11		1397A>C / (new) 1399delA / (new)
NOTCH1	75.0	74.4	Exon 34	7541_7542delCT/COSM13065	7329_7330delinsCCCA / COSM5752017
			Exon 27		5033T>A / COSM21907
PHF6	33.3	33.3	Exon 2	134delG+insCC/(new)	
IL7R	3.4	9.7	Exon 2	197T>C/COSM149813	197T>C / COSM149813
			Exon 4	254G>A/COSM149814	254G>A / COSM149814
			Exon 6		755_756ins9 / (new)
			Exon 8	1066A>G/rs3194051	
FBXW7	4.6	16.7	Exons 5-12	Negative	Negative
PTEN	12.1	12.5	Exons 1-9	Negative	Negative
CRLF2	27.7	17.2	Exons 1-6	Negative	33C>G / (new)
SH2B3	21.2	16.0	Exons 1-7	Negative	Negative
DNM2	14.7	15.2	Exon 6	789G>A/rs199976453	
			Exon 20	2139T>C/rs2229920	2139T>C / rs2229920
TP53	6.9	11.1	Exons 4-9	Negative	Negative
JAK1	7.0	14.8	Exons 13,14,16-19	Negative	Negative
Immunophenotype (%)				Patient 1	Patient2
CD34				64.3%	91%
CD13				28%	98%
CD33				97%	90%
CD3				32.5%	-
CD5				99.4%	38%
CD7				99.5%	63%
Hepatomegaly				Negative	Negative
Splenomegaly				Negative	Positive
Lymphadenopathy				Negative	Positive
IKZF1 deletion				Negative	Negative
BCR/ABL1				Negative	Negative
Complex karyotype				Negative	Negative
Treatment				1xHyperCVAD+2xIDA+FLAG+1xFLAG +1xBFM2002-HR-1+1xMOAP+4xCA G+Methylprednisolone+1xICE+3xNel arabine+1xDecitabine+0.5xCAG	1xHyper-CVAD+1xMA; no nelarabine treatment
1st CR time				42 days to achieve CR	Unknown
Relapse time after CR1				21 months	Unknown
Total relapse time				5	Lost to follow-up
Outcome				Death	Lost to follow-up

WBC: White blood cell, BM: bone marrow, PB: peripheral blood, CR: complete remission.

EZH2 exon 11, were detected in her BM sample (Figure 1). No CR was achieved with the first induction therapy. Finally, the patient was administered methotrexate and cytarabine and endured a long period of BM suppression. Unfortunately, the patient was lost to follow-up. Our data indicated the oncogenic and poor prognostic effect of *EZH2* mutations on T-ALL. The coexistence of *EZH2* mutations with mutations in the *NOTCH1*, *PHF6*, and *IL7R* genes suggested a new mechanism underlying the tumorigenesis of *EZH2* mutations in T-ALL. T-ALL and particularly ETP-ALL still have largely negative outcomes. In the past years, the effect of the use of nelarabine for relapsed and refractory T-ALL seemed to be negligible [10]. In our cohort, the first patient's relapse, even after nelarabine treatment, revealed the insensitivity of patients with multiple mutations to such treatment. Moreover, our case report suggested that the gene mutations may be the cause of the failure of the drug treatment and emphasized the importance of developing more effective therapies as well as more active and tailored treatments for aggressive T-ALL.

Acknowledgment

This work was supported in part by the National Natural Science Foundation of China (81270613,30973376); Jiangsu Province Key Medical Talents (RC2011077); the Scientific Research Foundation for the Returned Overseas Chinese Scholars; State Education Ministry (39th); China Postdoctoral Science Foundation (20090461134); special grade of financial support from the China Postdoctoral Science Foundation (201003598); the Six Great Talent Peak Plan of Jiangsu (2010-WS-024); the Nanjing Municipal Bureau of Personnel (2009); the Fundamental Research Funds for the Central Universities (2242017K40271, 2242016K40143) (ZG); and the Milstein Medical Asian American Partnership Foundation Research Project Award in Hematology (2017) (ZG and CS).

Keywords: *EZH2*, Adult, T-cell, Acute lymphoblastic leukemia

Anahtar Sözcükler: *EZH2*, Erişkin, T-hücre, Akut lenfoblastik lösemi

Conflict of Interest: The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

References

1. Wassef M, Michaud A, Margueron R. Association between *EZH2* expression, silencing of tumor suppressors and disease outcome in solid tumors. Cell Cycle 2016;15:2256-2262.
2. Gonzalez ME, Moore HM, Li X, Toy KA, Huang W, Sabel MS, Kidwell KM, Kleer CG. *EZH2* expands breast stem cells through activation of *NOTCH1* signaling. Proc Natl Acad Sci U S A 2014;111:3098-3103.
3. Katoh M. Mutation spectra of histone methyltransferases with canonical SET domains and *EZH2*-targeted therapy. Epigenomics-UK 2016;8:285-305.
4. Ernst T, Pflug A, Rinke J, Ernst J, Bierbach U, Beck JF, Hochhaus A, Gruhn B. A somatic *EZH2* mutation in childhood acute myeloid leukemia. Leukemia 2012;26:1701-1703.
5. Guglielmelli P, Biamonte F, Score J, Hidalgo-Curtis C, Cervantes F, Maffioli M, Fanelli T, Ernst T, Winkelman N, Jones AV, Zoi K, Reiter A, Duncombe A, Villani L, Bosi A, Barosi G, Cross NC, Vannucchi AM. *EZH2* mutational status predicts poor survival in myelofibrosis. Blood 2011;118:5227-5234.
6. Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tönnissen ER, van der Heijden A, Scheele TN, Vandenberghe P, de Witte T, van der Reijden BA, Jansen JH. Somatic mutations of the histone methyltransferase gene *EZH2* in myelodysplastic syndromes. Nat Genet 2010;42:665-667.
7. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, Yap D, Humphries RK, Griffith OL, Shah S, Zhu H, Kimbara M, Shashkin P, Charlot JF, Tcherpakov M, Corbett R, Tam A, Varhol R, Smailus D, Moksa M, Zhao Y, Delaney A, Qian H, Birol I, Schein J, Moore R, Holt R, Horsman DE, Connors JM, Jones S, Aparicio S, Hirst M, Gascoyne RD, Marra MA. Somatic mutations altering *EZH2* (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 2010;42:181-185.
8. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, Easton J, Chen X, Wang J, Rusch M, Lu C, Chen SC, Wei L, Collins-Underwood JR, Ma J, Roberts KG, Pounds SB, Ulyanov A, Becksfort J, Gupta P, Huether R, Kriwacki RW, Parker M, McGoldrick DJ, Zhao D, Alford D, Espy S, Bobba KC, Song G, Pei D, Cheng C, Roberts S, Barbato MI, Campana D, Coustan-Smith E, Shurtleff SA, Raimondi SC, Kleppe M, Cools J, Shimano KA, Hermiston ML, Doulatov S, Eppert K, Laurenti E, Notta F, Dick JE, Basso G, Hunger SP, Loh ML, Devidas M, Wood B, Winter S, Dunsmore KP, Fulton RS, Fulton LL, Hong X, Harris CC, Dooling DJ, Ochoa K, Johnson KJ, Obenauer JC, Evans WE, Pui CH, Naeve CW, Ley TJ, Mardis ER, Wilson RK, Downing JR, Mullighan CG. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 2012;481:157-163.
9. Schäfer V, Ernst J, Rinke J, Winkelmann N, Beck JF, Hochhaus A, Gruhn B, Ernst T. *EZH2* mutations and promoter hypermethylation in childhood acute lymphoblastic leukemia. J Cancer Res Clin Oncol 2016;142:1641-1650.
10. Litzow MR, Ferrando AA. How I treat T-cell acute lymphoblastic leukemia in adults. Blood 2015;126:833-841.

Address for Correspondence/Yazışma Adresi: Zheng GE, M.D.,
Zhongda Hospital, Medical School of Southeast University Department of Hematology Nanjing, China
Phone : 86-25-83262468
E-mail : janege879@hotmail.com **ORCID-ID:** orcid.org/0000-0001-8028-1612

Received/Geliş tarihi: May 16, 2017
Accepted/Kabul tarihi: July 26, 2017
DOI: 10.4274/tjh.2017.0194