Different Protein Expression between Human Eosinophilic Leukemia Cells, EoL-1 and Imatinib-resistant EoL-1 Cells, EoL-1-IR

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Chronic eosinophilic leukemia (CEL) is characterized by eosinophilia and organ damage. Imatinib is widely used for treating CEL, chronic myeloid leukemia (CML) and acute myeloid leukemia (AML). Unfortunately, the cancer cells gain resistance against the drug after prolonged molecular-targeted therapies. Imatinib-resistant EoL-1 (EoL-1-IR) cells were produced from chronic eosinophilic leukemia cells (EoL-1) after treatment with imatinib for a long duration. Two-dimensional electrophoresis (2-DE) analysis revealed numerous protein variations in the EoL-1 and EoL-1-IR sub-types. Compared to the EoL-1 cells, expression levels of TIP49, RBBP7, α -enolase, adenosine deaminase, C protein, galactokinase, eukaryotic translation initiation factor, IFN- γ , and human protein homologous to DROER were increased, whereas core I protein, proteasome subunit p42, heterogeneous ribonuclear particle protein, chain B, and nucleoside diphosphate were decreased in the EoL-1-IR cells. Taken together, these results contribute to understanding the pathogenic mechanism of drug-resistant diseases.

Key Words: Chronic eosinophilic leukemia, Imatinib, Drug resistance

Chronic eosinophilic leukemia (CEL) is a chronic myeloproliferative neoplasm characterized by a clonal proliferation of eosinophilic precursors that lead to increase eosinophils in the peripheral blood, the bone marrow, and possibly peripheral tissues (Qu et al., 2016; Kim et al., 2017). The blood shows $> 1.5 \times 10^9/L$ mature eosinophils and often increases the percentage of blasts in the blood or marrow up to and even exceeding 20%. *FIP1L1-platelet-derived growth factor receptor- α (PDGFR α)* fusion gene is very significant for the diagnosis and treatment of CEL. If there is no increase in the number of blasts and no evidence of mono-

clonality, the term hypereosinophilic syndrome (HES) is recommended (Antoniou, 2010; Klion, 2015). CEL is also called myeloid/lymphoid neoplasm with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, *FGFR1* or *PCMI-JAK2* based on World Health Organization (WHO) classification (Reiter and Gotlib, 2017). Imatinib is a small-molecule inhibitor of breakpoint cluster region-abelson (BCR-ABL) kinase with additional activity against receptor tyrosine kinases such as c-KIT, PDGFR α , and PDGFRB. Although imatinib is highly effective in cancer including CEL, cancer cells can have imatinib-resistant characteristics after a long

Received: September 14, 2018 / Revised: November 7, 2018 / Accepted: November 12, 2018

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term treatment. Therefore, we investigated whether there are differences between imatinib-sensitive and resistant CEL cells or not.

Human eosinophilic leukemia cells, EoL-1 cells were purchased from RIKEN BRC Cell Bank (Tsukuba, Japan). The imatinib-resistant EoL-1 (EoL-1-IR) cells were established

by culturing with increasing imatinib concentration (from 1 to 100 nM) for 6 months (Nishioka et al., 2010). EoL-1 and EoL-1-IR cells were cultured in RPMI 1640 including fetal bovine serum (FBS) and antibiotics. The cells were incubated at 37 °C in a 5% CO₂ incubator. For two-dimensional electrophoresis, cell lysates in sample buffer were applied

Fig. 1. Two-dimensional electrophoresis with EoL-1 and EoL-1-IR cells. EoL-1 and EoL-1-IR were analyzed by 2-DE. (A) 2-DE image for comparison between EoL-1 and EoL-1-IR. (B) The arrow marks on EoL-1-IR indicate spots for proteins differentially expressed by more than 2-fold compared with EoL-1. (C) The arrow marks on EoL-1-IR indicate spots for proteins differentially expressed by less than 2-fold compared with EoL-1.

to pH 3-10 nonlinear gradient strips (Amersham Biosciences, Uppsala, Sweden) and isoelectric focusing (IEF) was carried out. The second dimension was analyzed on gradient polyacrylamide gel at 40 mA for 5 h. After fixation, the gels were stained with CBB G-250 for 12 h. The gels were destained, scanned in a Bio-Rad GS710 densitometer (Richmond, CA, USA) and converted into electronic files. The spots were analyzed with Image Master Platinum 5.0 image analysis program (Amersham Biosciences). For matrix associated laser desorption ionization-time of flight mass spectrometry

(MALDI-TOF/TOF MS) analysis, samples were applied to the R2, R3 column and eluted with an elution buffer. Mass spectra were acquired on a 4800 proteomics analyzer (Applied Biosystems, Foster, CA, USA) operated in MS and MS/MS modes. Peptide fragmentation was conducted by collision-induced dissociation (CID). For MS and MS/MS analysis, the 800~4,000 m/z mass range was used with 1,000 shots per spectrum, and a maximum of 15 precursors with minimum S/N of 50 were chosen. The MASCOT algorithm (Matrix Science, Boston, MA, USA) was used for protein

Table 1. List of proteins increased in EoL-1-IR cells

Spot ID	NCBI accession no.	Protein name	Nominal mass	MAS COT score	No. of matched peptides	TAMRA-fold change	emP AI
286	gil3132308	TIP49 (RUVBL1, Pontin)	50,538	878	29 (18)	2.0	4.00
297	gil11935049	RBBP7 (RbAp46)	66,198	827	20 (13)	2.2	1.56
347	gil119339	Alpha-enolase	47,481	889	26 (19)	2.2	4.01
394	gil28380	Adenosine deaminase	41,024	411	14 (8)	2.6	1.01
402	gil306875	C protein (ribonuclear protein particle c)	32,004	571	20 (10)	2.3	2.84
421	gil1002507	Galactokinase	42,702	599	17 (11)	2.9	2.09
444	gil124200	Eukaryotic translation initiation factor	36,374	791	22 (15)	2.4	6.13
560	gil186513	Interferon-gamma	28,876	545	22 (14)	2.5	7.58
764	gil1374695	Human protein homologous to DROER protein	12,422	216	9 (2)	3.3	1.14

Ions score is $-10 \times \log(P)$, where P is the probability that the observed match is a random event. Individual ions scores > 34 indicate identity or extensive homology ($P < 0.05$)

Table 2. List of proteins decreased in EoL-1-IR cells

Spot ID	NCBI accession no.	Protein name	Nominal mass	MAS COT score	No. of matched peptides	TAMRA-fold change	emP AI
321	gil468935	Core I protein (core I protein subunit of human ubiquinol-cytochrome C reductase)	53,297	622	22 (13)	2.0	1.95
392	gil1526426	Proteasome subunit p42	44,418	519	19 (7)	3.8	1.15
519	gil87651	Heterogeneous ribonuclear particle protein	34,289	742	23 (14)	2.9	4.33
628	gil28252	Unnamed protein product	42,052	543	18 (9)	3.0	1.81
701	gil1025735596	Chain B	18,642	408	16 (10)	5.0	5.05
721	gil127983	Nucleoside diphosphate	17,401	390	19 (11)	2.1	8.08
740	gil34343	Unnamed protein product	15,048	236	14 (6)	2.1	3.89
772	gil34773	Unnamed protein product	10,885	248	10 (5)	2.1	.58

Ions score is $-10 \times \log(P)$, where P is the probability that the observed match is a random event. Individual ions scores > 34 indicate identity or extensive homology ($P < 0.05$)

identification.

Here, we investigated the different protein expression between EoL-1 and EoL-1-IR cells. Two-dimensional electrophoresis analysis was used for examining different proteins between EoL-1 and EoL-1-IR cells. After separation in the second dimension, 511 spots were routinely detected on two-dimensional electrophoresis (2-DE) gels of EoL-1 cell lysates, and 479 spots were detected on 2-DE gels of EoL-1-IR cell lysates (Fig. 1A). Molecular mass and pH values were also indicated. The arrow marks in 2DE of EoL-1-IR lysate indicate spots that were differentially expressed by more than 2-fold compared to spots in 2DE of EoL-1 cell lysate. This analysis identified an increase (Fig. 1B) or a reduction (Fig. 1C) of more than 2-fold spots with a significant difference in EoL-1-IR cells compared to EoL-1 cells. Table 1 describes the names of the proteins, which increased more than 2-fold in EoL-1-IR compared to EoL-1. TIP49 (RUVBL1, Pontin), RBBP7 (RbAp46), Alpha-enolase, adenosine deaminase, C protein (ribonuclear protein particle C), Eukaryotic translation initiation factor, interferon-gamma and human protein homologous to DROER protein were detected MALDI-TOF/TOF. Both TIP49 and RBBP7 proteins have been known to be involved in cancer pathogenesis (Si et al., 2010; Yeh et al., 2015). The names of the proteins, which decreased more than 2-fold in EoL-1-IR compared to EoL-1 cells are described in Table 2. Core I protein, Proteasome subunit p-42, Heterogeneous ribonuclear particle protein, Unnamed protein product Chain B, Nucleoside diphosphate were detected by MALDI-TOF/TOF. Resistance was observed in various situations as CEL patients take imatinib for a long time. Although we unveiled the proteins increased or decreased by imatinib resistance, their exact mechanisms remain to be unknown. Further study is required to elucidate the exact relationship of the proteins with drug resistance.

ACKNOWLEDGEMENT

This paper was supported by Wonkwang Health Science University in 2018.

CONFLICT OF INTEREST

The authors have no conflicts of interest, financial or

otherwise, to declare.

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<https://doi.org/10.15616/BSL.2018.24.4.426>

Cite this article as: Sung KH, Kim IS, Lee JS. Different Protein Expression between Human Eosinophilic Leukemia Cells, EoL-1 and Imatinib-resistant EoL-1 Cells, EoL-1-IR. *Biomedical Science Letters*. 2018. 24: 426-429.