

Identification of signature genes by microarray for acute myeloid leukemia without maturation and acute promyelocytic leukemia with t(15;17)(q22;q12)(PML/RAR α)

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Abstract. Acute myeloid leukemia (AML) has distinct subgroups characterized by different maturation and specific chromosomal translocation. In order to gain insight into the gene expression activities in AML, we carried out a gene expression profiling study with 21 AML samples using cDNA microarrays, focusing on acute promyelocytic leukemia with specific translocation t(15;17)(q22;q12) [French-American-British or FAB-M3 with t(15;17)] and AML without maturation (FAB-M1) characterized by morphologically and phenotypically immature AML blasts and no recurrent chromosomal abnormalities. Using a multivariate σ -classifier algorithm, we identified 33 strong feature genes that distinguish FAB-M3 with t(15;17) from other AML samples, and 24 strong feature genes that classify FAB-M1. A direct comparison between FAB-M3 with t(15;17) and FAB-M1 led to selection of 13 strong feature genes. Those genes include some known to be related to leukemogenesis and cell differentiation. RIN1, a gene in the ras pathway, was up-regulated in FAB-M3 with t(15;17). Growth factor-binding protein 2 gene was down-regulated in FAB-M1. Huntingtin gene was up-regulated in FAB-M1. Others include syndecan 4, interleukin-2 receptor β ,

folate receptor β , low affinity immunoglobulin γ , Fc receptor IIC precursor, insulin-like growth factor binding protein 2, and myeloperoxidase, which are involved in cell differentiation. Overexpression of myeloperoxidase in FAB-M3 cells with t(15;17) compared to FAB-M1 cells is consistent with the conventional cytochemical staining pattern. Thus, the study revealed that a morphologically-defined FAB-M1 subtype has a distinct gene expression signature that contributes to its cell differentiation and proliferation as well as FAB-M3 with a recurrent cytogenetic abnormality t(15;17)(q22;q12).

Introduction

Acute myeloid leukemia (AML) is a malignant and clonal disease manifested with myeloid blast expansion in bone marrow and blood. The French-American-British (FAB) classification (1) or the World Health Organization (WHO) classification based on FAB classification of AML (2) is widely used for diagnosis of AML subtypes. Acute promyelocytic leukemia (FAB-M3) is an AML subtype in which abnormal promyelocytes predominate (1,2). More than 90% of patients with FAB-M3 have specific chromosomal translocation of t(15;17)(q22;q12) (1,2). Morphological and cytochemical characteristics of the FAB-M3 blasts with t(15;17) include numerous azurophilic granules and Auer rods in the cytoplasm and strongly positive myeloperoxidase reaction (1,2). Most FAB-M3 cases are clinically associated with disseminated intravascular coagulation (1,2). The chromosomal translocation t(15;17) results in a gene fusion between PML (15q22) and RAR α (17q12). All trans-retinoic acid (ATRA) has been found to be an effective differentiating agent for FAB-M3 with t(15;17) by its action on releasing the dominant negative transcriptional repression of PML/RAR α (3). The discovery of ATRA as an effective agent targeting PML/RAR α overcoming disseminated intravascular coagulation, and inducing cell differentiation, has dramatically improved the prognosis of FAB-M3 patients with PML/RAR α (4-6). In contrast, the prognosis of AML without maturation

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Table I. FAB classification and karyotypes of AML cells.

Case	WHO classification (FAB classification)	Karyotype
AML01-M0	AML minimally differentiated (FAB-M0)	46,XY
AML02-M1	AML without maturation (FAB-M1)	47,XX,+21
AML03-M1	AML without maturation (FAB-M1)	46,XX
AML04-M1	AML without maturation (FAB-M1)	46,XX
AML05-M1	AML without maturation (FAB-M1)	46,XX
AML06-M1	AML without maturation (FAB-M1)	nt
AML07-M1	AML without maturation (FAB-M1)	46,XY,del(7)(q?)
AML08-M2	AML with maturation (FAB-M2)	nt
AML09-M2	AML with maturation (FAB-M2)	46,XX
AML10-M2	AML with maturation (FAB-M2)	46,XY
AML11-M3	APL (FAB-M3)	t(15;17)(q22;q12)
AML12-M3	APL (FAB-M3)	t(15;17)(q22;q12)
AML13-M3	APL (FAB-M3)	t(15;17)(q22;q12)
AML14-M3	APL (FAB-M3)	t(15;17)(q22;q12)
AML15-M3	APL (FAB-M3)	t(15;17)(q22;q12)
AML16-M3	APL (FAB-M3)	t(15;17)(q22;q12)
AML17-M3	APL (FAB-M3)	t(15;17)(q22;q12)
AML18-M4	AMMoL (FAB-M4)	46,XY,-16,-20,5q-,12p-
AML19-M4	AMMoL (FAB-M4)	46,XY
AML20-M4	AMMoL (FAB-M4)	51,XY,+5,+6,+10,+13,+21
AML21-M5	AMoL (FAB-M5)	45,XY,-7

APL, acute promyelocytic leukemia; AMMoL, acute myelomonocytic leukemia; AMoL, acute monoblastic and monocytic leukemia.

(FAB-M1) is much poorer and there is no specific agent that uniquely targets FAB-M1 blasts. FAB-M1 is characterized by proliferation of bone marrow blasts without maturation to more mature neutrophils (1,2). Morphological and cytoplasmic characteristics of FAB-M1 blasts include small amount or lack of azurophilic granules and/or unequivocal Auer rods in the cytoplasm and low myeloperoxidase positivity (1,2). There has been no demonstrated association between FAB-M1 and specific recurrent chromosomal abnormalities (2).

The recent breakthroughs in leukemia treatment are derived from the recognition of specific molecular targets and development of drugs inhibiting those targets. In addition to the afore-mentioned ATRA, the ABL tyrosine kinase inhibitor, STI571, has shown impressive effect against BCR/ABL-positive leukemia (7-9). Other specific drugs include anti-CD33 gemtuzumab ozogamicin (10,11), and inhibitors for histone deacetylase for patients with abnormal core-binding factor (CBF) leukemia; FAB-M2 with t(8;21)(q22;q22) (*AML1/ETO*) (12-14). Furthermore, new signal transduction inhibitors (15,16), specific tyrosine kinase inhibitors (15,17), and BCL-2 antisense (15,18) are now in clinical trials.

The success of targeted therapy in leukemia has prompted expanded effort in molecular characterization of different subtypes of AML. One of the approaches is gene expression profiling with cDNA microarray, which has revealed distinct gene expression signatures for AML and acute lymphoblastic leukemia (19) and for subtypes of acute leukemias (20-23). Recent reports showed that AML with t(8;21)(q22;q22),

t(15;17)(q22;q12), and inv(16)(p13q22) are distinguished by specific gene expression profiles (20). Genes on chromosome 8 are overexpressed in AML blasts with trisomy 8 compared with the AML blasts with normal cytogenetics (21). Expression profiling of CD34-positive hematopoietic cells revealed distinct subtypes of therapy-related AML (22). Acute lymphoblastic leukemias with MLL translocation are separated from other acute leukemias (23). However, gene expression profiles of AML subtypes which are classified to 'AML not otherwise categorized' in WHO classification are not reported yet.

The FAB-M1 cells are diagnosed by morphologic and cytochemical features. They are heterogeneous in genetic background and categorized as 'AML not otherwise categorized' in WHO classification (2). To identify signature genes of AML, especially of FAB-M1, gene expression profiling of 21 AML cases with different subtypes was performed. The analysis revealed strong feature genes that distinguish FAB-M1 from others as well as genes that distinguish cases of FAB-M3 with t(15;17) from others.

Patients and methods

Patients/samples. Clinical samples were obtained from 21 patients with AML (1 patient with FAB-M0, 6 patients with FAB-M1, 3 patients with FAB-M2, 7 patients with FAB-M3, 3 patients with FAB-M4 and 1 patient with FAB-M5). FAB classification and karyotypes of AML cells are shown in Table I.

The diagnoses were made according to the revised FAB classification (1) and the World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues (2). Total RNA was extracted from mononuclear cell pellets of which more than 70% were leukemic cells.

DNA microarray studies using specimens of patients with hematopoietic malignancies were approved by Institutional Review Committee in Mie University School of Medicine (24).

Microarray production. A total of 2,142 known human cDNAs were prepared by PCR from the Research Genetics cDNA clone library using the two primers, purified using MultiScreen PCR plates (Millipore Corp., Bedford, MA) and verified by sequencing at Cancer Genomics Core Lab. (M.D. Anderson Cancer Center) before printing (25). The DNA clones, in 384-well plates, were spotted onto poly-L-lysine-coated microscope slides using an arrayer (Genomic Solutions, Ann Arbor, MI).

RNA amplification and target labeling. AML cell pellets were lysed in the TRI reagent (MRC, Cincinnati, OH). Control RNA was prepared by mixing the same amount of total RNA extracted from these 6 cell lines; K562, HL60, NB4, BV173, KBM7 and Jurkat cells (24). The labeling reaction was performed as described previously (24,26,27).

Microarray hybridization and image scanning. Methods of hybridization were described previously (24). Hybridized arrays were scanned at 10 μ m resolution on a GeneTAC LS-IV scanner (Genomic Solution), and the obtained signal intensities were quantified with ArrayVision (Imaging Research Inc., St. Catherines, Ontario, Canada).

Assessment of replicability of the data. As mentioned above, each gene is duplicated on the array. Thus, the variation between the replicate spots can be used to assess the reliability of the measurement of that gene's expression. Informally, if the two replicate measurements are close to each other, then the estimate of that gene's expression can be obtained by a combination (e.g., average) of the two replicates. If, on the other hand, the two replicates are quite different, then that gene should be flagged as unreliable and the measurements should not be used in subsequent analysis. We use the following simple method to flag unreliable measurements. For each array (patient), we compute the standard deviation of the absolute values of the differences between the corresponding replicates. Then, any absolute value of the difference that exceeds three times the standard deviation is flagged as being unreliable. Using our procedure, about 3% of the genes, on average, are deemed unreliable.

Algorithm for finding strong feature (gene) sets. We desire classifiers that categorize sample tissues based on the expression values of a gene set. Because the number of samples for clinical studies is often small, we use a simple classifier and a small number of genes (at most three in this study) to form the classifier. This helps mitigate the likelihood that a classifier that does well on the sample data, but is not good relative to the populations. As for a specific algorithm to

design the classifier, we used a recently developed σ -classifier algorithm (28,29). σ -classifier is designed from a probability distribution resulting from spreading the mass of the sample points via a circular distribution to make classification more difficult, while maintaining sample geometry. The simple logic is that the noise would increase when more cases are included. Since we do not have a large number of cases, a controlled increase of noise to some extent mimics the scenario of more cases. The algorithm is parameterized by the standard deviation (σ) of the circular distribution. By considering increasing standard deviations, the algorithm finds gene sets whose classification accuracy remains strong relative to greater spreading of the sample. In addition, the error of a classifier is not estimated from the sample points, but is instead measured as the error the classifier makes in trying to spatially separate the distributions, which is a much more difficult task if the spread is large.

A classifier that has a small error for a large variance is desirable, since its performance is likely to be more robust relative to new data. If a classifier has an extremely small error (≈ 0) for a small σ but a large error for large σ , then we do not consider it sufficiently strong. Intuitively, this method mitigates overfitting the data, since it favors solutions for which little changes in the data lead to little changes in the classifier. Additionally, the method improves the robustness of the classification in regard to the inaccuracy of the inputs.

The second feature of the algorithm is that it searches for gene combinations that separate the classes. This feature is especially attractive in biological settings where heterogeneity is the norm and no single gene can dictate classifications.

All of the computations that search and analyze more than 16 billions classifiers in this study were done on a Beowulf-based supercomputer at the Center for Information Technology at NIH. This system is a distributed memory parallel computer consisting of a total of 780 XP/Athlon and Pentium III processors interconnected through a high-speed network.

Results

The WHO (FAB) classification and karyotypes for the AML patients used in this study are shown in Table I. All patients with FAB-M3 have t(15;17)(q22;q12) and other AML patients have heterogeneous karyotypes.

Using the σ -classifier algorithm (28,29), we first set out to identify strong feature genes that are robustly associated with FAB-M3 with t(15;17) or with FAB-M1 subtype of AML by finding the best classifiers that separate one type from the others in the experimental group. We also identified classifier genes that separate the FAB-M1 from FAB-M3 with t(15;17). Because of the limited sample size, we limit our search to two-gene classifier and three-gene classifier. Gene sets selected by two-gene classifier clustered patients with FAB-M1 and FAB-M3 with t(15;17) with very low σ -errors and leave-one-out (LOO) errors. We assembled the genes that appeared repeatedly in the best two-gene classifiers in Table II. Thirty-three strong feature genes were identified to appear at least five times for the separation of FAB-M3 with t(15;17) from the others, 24 strong feature genes appeared at least twice for FAB-M1 separation, and 13 strong feature genes showed at least five times for the cases of FAB-M3 with t(15;17) and

Table II. Strong feature genes that appeared repeatedly in the best two gene classifiers.

A, FAB-M3 with t(15;17) vs. others		
Accession no.	Gene name	Expression in FAB-M3 with t(15;17)
M37190	Human ras inhibitor mRNA, 3'-end	+
H67274	KIAA0088 gene	+
AA148736	Syndecan 4 (amphiglycan, ryudocan)	+
M96739	NSCL-1	+
R97340	Prostate carcinoma tumor antigen (pcta-1)	+
AA057156	Interleukin-2 receptor β chain	+
W88899	Sulfotransferase	+
R00833	Solute carrier family 2, member 2	+
AA608576	Novel T-cell activation protein	-
AA598401	Coatomer δ subunit	-
T68336	Complement component 8, β	-
T50633	V-ski avian sarcoma viral oncogene homolog	-
T71976	Phosphatidic acid phosphatase type 2B	-
T55801	TFIIA γ	-
AA488718	Human BRCA2	-
AA460830	(clone mf. 18) RNA polymerase II	-
AA486313	Low density lipoprotein-related protein-associated protein 1	-
T47815	Interferon- γ up-regulated I-5111	-
AA196465	Sarcophilin (SLN)	-
AA486919	Human ribosomal protein L28 mRNA	-
H11346	Pyrroline-5-carboxylate dehydrogenase (P5CDh)	-
AA401853	Proteasome subunit p27	-
NM_014606	Hect domain and RLD 3 (HERC3)	-
L77566	<i>Homo sapiens</i> DGS-I	-
R24635	Folate receptor 1 (adult)	-
AA453816	Folate receptor β precursor	-
N52835	Coagulation factor XI (plasma thromboplastin antecedent)	-
T64192	T-cell receptor, β cluster	-
R53406	Recepin	-
NM_003118	Osteonectin, SPARC	-
T60111	Fatty acid-binding protein, epidermal	-
N70349	ART4 gene	-
D80002	KIAA0180 gene	-
B, FAB-M1 vs. others		
Accession no.	Gene name	Expression in FAB-M1
AA452278	Sodium bicarbonate cotransporter (HNBC1)	+
W38923	Transmembrane receptor (ror2)	+
NM_014396	Vacuolar protein sorting 41 (yeast homolog) (VPS41)	+
AA489609	Clone HH109	+
AF099032	Embryonic ectoderm development protein short isoform	+
T53907	Coatomer β subunit	+
T64094	Huntingtin (Huntington disease)	+
AL132857	Chromosome 14 DNA sequence	+
AA600190	KIAA0225 gene	+
W95118	KIAA0161 gene	+
AA143509	Pyrroline-5-carboxylate synthetase	+
AF091078	Clone 559 unknown mRNA	+
AA625666	Pig7 (PIG7)	-

Table II. Continued.

B, FAB-M1 vs. others		
Accession no.	Gene name	Expression in FAB-M1
H23459	TUP1-like enhancer of split gene 1	-
AA479981	Protein A alternatively spliced form 1 (A-1)	-
AA449831	Growth factor receptor-bound protein 2	-
R68106	Low affinity immunoglobulin γ FC receptor IIC	-
AA464731	Calgizzarin	-
M96739	NSCL-1	-
H53340	(clone 14VS) metallothionein-IG (MT1G) gene	-
AA150507	Interleukin-1 β	-
R70402	Heat shock 70 kDa protein 1	-
H39560	Selectin E (endothelial adhesion molecule 1)	-
NM_015847	Methyl-CpG binding domain protein 1 (MBD1)	-
C, FAB-M1 vs. FAB-M3 with t(15;17)		
Accession no.	Gene name	Expression in FAB-M1
W38923	Transmembrane receptor (ror2)	+
T57556	Putative protein kinase C inhibitor (PKCI-1)	+
T67053	Rearranged immunoglobulin λ light chain	+
NM_004856	Kinesin-like 5 (mitotic kinesin-like protein 1) (KNSL5)	+
T47815	Interferon- γ up-regulated I-5111	+
NM_014396	Vacuolar protein sorting 41 (yeast homolog) (VPS41)	+
AA057156	Interleukin-2 receptor β chain	-
AA464731	Calgizzarin	-
H79047	Insulin-like growth factor binding protein 2 (36 kDa)	-
NM_000250	Myeloperoxidase (MPO) precursor	-
M96739	NSCL-1	-
R97340	Prostate carcinoma tumor antigen (pcta-1)	-
AA127100	Ribophorin I	-

+, represents the overexpression of gene in the category and -, the down-regulation.

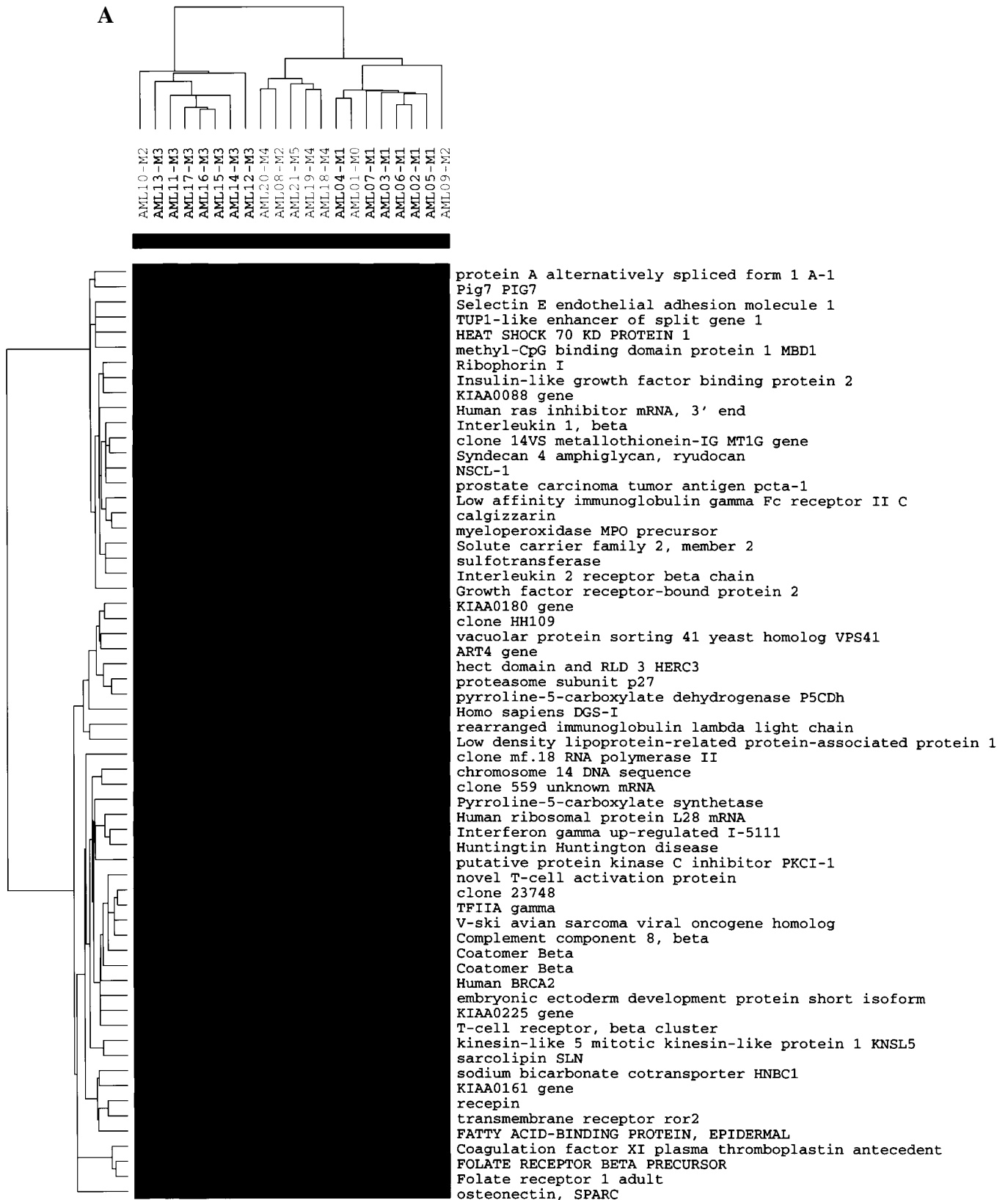
FAB-M1 separation. Interestingly, myeloperoxidase was selected as one of strong feature genes to distinguish between FAB-M1 and FAB-M3 with t(15;17), a result that is consistent with conventional cytochemical staining pattern that intrinsic myeloperoxidase positivity of FAB-M3 blasts is very strong whereas that of FAB-M1 blasts is relatively low (1,2). The separations of the comparison groups by those genes were confirmed by three hierarchical clusterings (figure not shown). We then put together all identified strong genes and performed a hierarchical clustering (Fig. 1A) and multi-dimensional scaling (MDS) analysis (Fig. 1B). From the hierarchical clustering, we observed three main clusters where FAB-M1, FAB-M3 with t(15;17), and the others are clustered together, except AML10-M2 being clustered together with FAB-M3 and AML01-M0 with FAB-M1. Also, MDS shows a similar separation among three groups. Notably, the fact that AML10-M2 lies between FAB-M3 with t(15;17) and the others, and AML01-M0 and AML09-M2 lie between FAB-M1 and the others may suggest that these three patients are in transition between the subtypes. These results suggest that the selected

genes possess genetic signatures for FAB-M1 and FAB-M3 with t(15;17).

Discussion

AML consists of several subtypes with respect to morphology, cytochemistry, immunophenotype, cytogenetics, and clinical course. FAB-M3 with t(15;17) is a typical subtype of acute promyelocytic leukemia, which expresses *PML/RAR α* fusion gene and responds to ATRA. In contrast, FAB-M1 is a subgroup of AMLs with heterogeneous cytogenetics and poses challenge to targeted therapy. To improve the outcome of AML, it is important to understand the molecular mechanisms of leukemogenesis in AMLs both with and without recurrent cytogenetic abnormalities. In this section, we will discuss some of the classifier genes we identified in the context of their known information in literature.

We first compared between FAB-M3 with t(15;17) as a typical subtype of AML with recurrent cytogenetic abnormalities and others. Among strong feature genes to



classify cases of FAB-M3 with t(15;17) from others (Table II, Fig. 1A) is the ras inhibitor, also called RIN1 (Ras and Rab interactor 1), which was named by its function to interfere with activated Ras in yeast (30). The role of RIN1 in mammalian cells is likely to be very different from that in yeast. It was reported that RIN1 accelerates BCR-ABL-

induced leukemias in mice (30), and overexpression of tyrosine phosphatase by fusing the catalytic domain of SHP1 to ABL binding domain of RIN1 inhibited growth of K562 human BCR-ABL-positive cells (31). Our analysis found that RIN1 was overexpressed in FAB-M3 cases with t(15;17). Although FAB-M3 cells with t(15;17) do not have BCR-

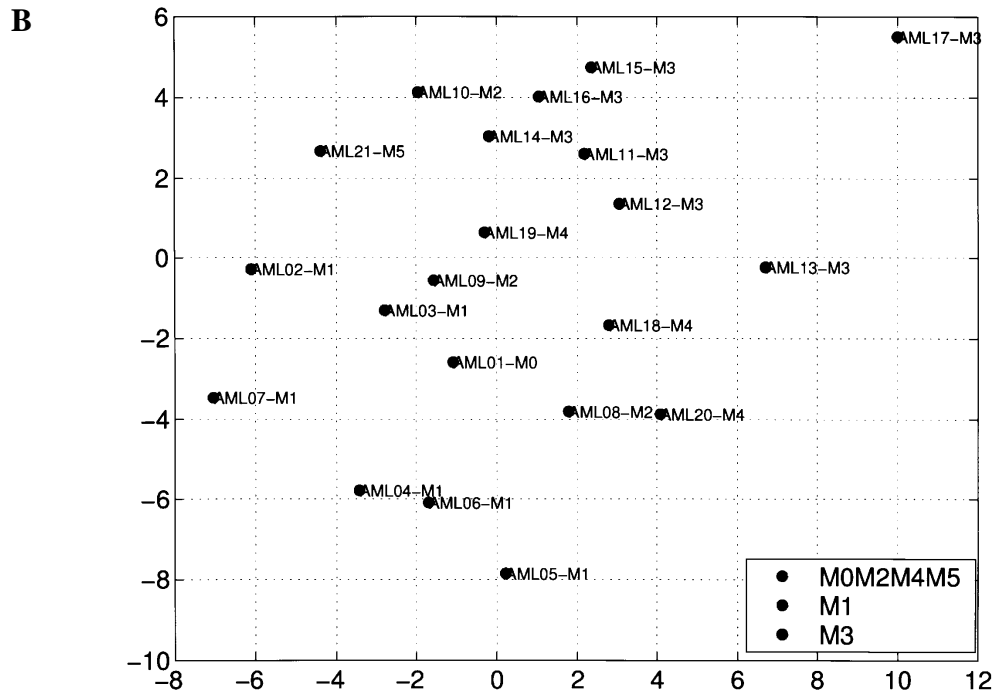


Figure 1. A, Hierarchical clustering of AMLs with strong feature genes. All the genes that are identified as strong features are put together and the hierarchical clustering with Pearson's correlation as similarity measure are applied. Each row represents a gene and each column represents a patient sample. The dendrogram on the left illustrates the final clustering tree, when genes close to each other have high similarity in their standardized expression values across the AML samples. A similar procedure is used to cluster AML samples. In the display, the primary data table is represented graphically by coloring each cell on the basis of the measured fluorescence ratio. Cells with log ratios of 0 (ratios of 1.0-genes unchanged) are colored black, increasingly positive log ratios with reds of increasing intensity, and increasingly negative log ratios with greens of increasing intensity. Patients with FAB-M3 with t(15;17) are in blue, FAB-M1 in red, and the rest of patients in green. All FAB-M3 patients with t(15;17) are clustered together, with a patient with AML10-M2 being within that cluster, and all patients with FAB-M1 are also tightly clustered together, with a patient with AML01-M0 being part of the cluster. B, Multi dimensional scaling (MDS) analysis is performed using the same set of strong feature genes with Euclidean metric as similarity measure. Even though MDS shows no perfect separation among three classes, FAB-M1 and FAB-M3 with t(15;17) make reasonable clusters. Also, in consistency with the hierarchical clustering, a patient with AML10-M2 stays close to the patients with FAB-M3 with t(15;17) and a patient with AML01-M0 is near to FAB-M1 patients.

ABL, RIN1 may contribute to cell growth of FAB-M3 cells with t(15;17) through ras-mediated signal transduction pathway.

Syndecan 4 is also overexpressed in FAB-M3 cells with t(15;17) (Table II, Fig. 1A). Syndecan 4 has been reported to be an antithrombin receptor of human neutrophils (32,33). As syndecan 4 mRNA is overexpressed in FAB-M3 cells with t(15;17), which are more mature to neutrophils compared with others, syndecan 4 may be one of myeloid differentiation markers like myeloperoxidase.

It has been reported that IL-2R α , β , and γ chains are expressed in some AMLs (34), and IL-2R α chain expression of AML cells was correlated with poor prognosis of AML patients (35). However, the role of IL-2R β chain expression in clinical implication has not been well evaluated. Our data show that IL-2R β chain mRNA overexpression is mainly seen in FAB-M3 cases with t(15;17) (Table II, Fig. 1A). Future studies will tell whether expression of IL-2R β chain has any correlation with prognosis.

Folate receptor β protein is expressed in the myelomonocytic lineage and is differentially expressed in myeloid leukemias (36). In the present study, mRNAs of folate receptor 1 and folate receptor β precursor are relatively down-regulated in FAB-M3 cells with t(15;17) (Table II, Fig. 1A). Interestingly, the expression levels of folate receptor β after treatment with ATRA are considered to be more important,

because folate receptor β is transcriptionally upregulated in AML cells that are treated with ATRA (37), and overexpressed folate receptor β is considered to be a molecular target of therapeutic intervention (38).

FAB-M1 is a typical subtype of AML without recurrent cytogenetic abnormalities. Thus identification of classifier genes for this type of AML is poised to provide important insight into the leukemogenesis process. Among strong feature genes to classify cases of FAB-M1 from others, Huntingtin mRNA was overexpressed (Table II, Fig. 1A). Although it is not clear what this means physiologically at present, it is intriguing that Huntingtin interacting protein 1 (HIP1) gene is involved in a specific translocation t(5;7)(q33;q11.2) in chronic myelomonocytic leukemia resulting in fusion of HIP1 to platelet-derived growth factor β receptor (39-41). Thus, Huntingtin gene pathway may be an important pathway for leukemogenesis either through gene expression changes or gene translocation. Further studies are warranted.

Growth factor-binding protein 2 (Grb2) is an adaptor protein that links multiple tyrosine kinases to Ras (42-44) and plays an important role in proliferation of BCR-ABL-positive cells (42-44). Interestingly, Grb2 mRNA was down-regulated in FAB-M1 (Table II, Fig. 1A) suggesting that the key molecular changes in FAB-M1 may be downstream of Grb2.

CD32 (Fc γ R2) is a differentiation marker in myelomonocytic lineage, and is expressed in monocytes and neutrophils.

Low expression levels of low affinity Fc γ RII precursor in FAB-M1 cells (Table II, Fig. 1A) likely reflect the immature stage of FAB-M1 cells in myelomonocytic differentiation pathway.

When FAB-M1 and FAB-M3 with t(15;17)(q22;q12) were compared directly, a number of genes were found to have consistent difference in expression. Among them, insulin-like growth factor binding protein 2 (IGFBP2) was overexpressed in FAB-M3 with t(15;17) (Table II, Fig. 1A). IGFBP2 is a modifier gene for IGF pathway and has been shown to be involved in many cancers. In glioblastoma, IGFBP2 is overexpressed comparing to low-grade gliomas and IGFBP2 may contribute to invasive nature of glioblastoma (45,46). However, in synovial sarcoma, IGFBP2 overexpression was associated with epithelial differentiation (47). The reason for lower expression in FAB-M1 is not clear, however, this may be consistent with the lower level expression of Grb2 (see above), which also mediates the IGF pathway.

Overexpression of rearranged immunoglobulin λ light chain in some cases of FAB-M1 (Table II, Fig. 1A) suggests that immature leukemic cells have bilineage genotypes of myeloid and lymphoid blasts. It is reasonable that its overexpression is rare in the FAB-M3 blasts, which are more mature than the blasts of FAB-M1.

It has been reported that AMLs with recurrent cytogenetic abnormalities, t(8;21)(q22;q22), t(15;17)(q22;q11-12), and inv(16)(p13q22) can be distinguished by specific genes (20). The present study also identified strong feature genes to classify cases of FAB-M3 with t(15;17), which is a distinct subtype defined by *PML/RAR α* fusion gene. On the other hand, FAB-M1 cases do not have recurrent chromosomal abnormalities. The present study, however, revealed strong feature genes to classify cases of FAB-M1 as well as FAB-M3 with t(15;17). These findings suggest that subtypes of 'AML not otherwise categorized' in WHO classification have common molecular-based characteristics as well as AMLs with recurrent cytogenetic abnormalities.

In the diagnosis of AMLs, recurrent cytogenetic abnormalities are considered to be one of very strong prognostic factors. AML patients with t(15;17), t(8;21) and inv16 show favorable prognosis compared to those with normal karyotype, whereas AML patients with chromosomal abnormalities other than the three listed types of translocations show poorer prognosis than those with normal karyotypes (2,48). Morphologically- and cytochemically-defined characteristics of AML are also strong prognostic factors. For example, AML with multilineage dysplasia was reported to show poor prognosis (49). The presence of Auer body and more than 50% myeloperoxidase-positive AML cells were favorable prognostic factors (48). The present study suggested that AMLs without recurrent cytogenetic abnormalities defined by morphology and cytochemistry have some common gene expression profiles and should be evaluated to find therapeutic molecular targets as well as AML subtypes with recurrent cytogenetic abnormalities in future studies.

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