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ORIGINAL ARTICLE

AML with translocation t(8;16)(p11;p13) demonstrates unique cytomorphological, cytogenetic, molecular and prognostic features

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Balanced chromosomal rearrangements define distinct entities in acute myeloid leukemia (AML). Here, we present 13 AML cases with t(8;16)(p11;p13) with observed low incidence (13/6124 patients), but more frequent presentation in therapy-related AML than in *de novo* AML (7/438 versus 6/5686, $P=0.00001$). Prognosis was poor with median overall survival of 4.7 months. Cytomorphology was characterized by parallel positive myeloperoxidase and non-specific esterase staining, therefore, French–American–British (FAB)-classification was impossible and origin of the AML with t(8;16) from an early stem cell with myeloid and monoblastic potential is hypothesized. Erythrophagocytosis was observed in 7/13 cases. Using gene expression profiling on 407 cases, patients with t(8;16) were compared to AML FAB subtypes with normal karyotype. Principal component analyses demonstrated that AML with t(8;16) were distinct from FAB subtypes M1, M4, M5a/b. When further compared to AML showing balanced rearrangements, that is, current WHO categories t(15;17), t(8;21), inv(16) and t(11q23)/*MLL*, AML with t(8;16) cases were clustered close to t(11q23)/*MLL* sharing commonly expressed genes. Subsequently, a pairwise comparison discriminated AML with t(8;16) from AML with t(11q23)/*MLL*, thus defining a highly unique signature for AML with t(8;16). In conclusion, AML with t(8;16) demonstrates unique cytomorphological, cytogenetic, molecular and prognostic features and is a specific subtype of AML.
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Introduction

Reciprocal chromosomal rearrangements lead to specific molecular fusion genes and define distinct subclasses with biologic and prognostic relevance in acute myeloid leukemia (AML).[1–3] Recurrent balanced translocations such as t(15;17)/*PML-RARA*, t(8;21)/*RUNX1-RUNX1T1*, inv(16)/*CBFB-MYH11* and t(11q23)/*MLL* demonstrate a close correlation between cytomorphology, cytogenetics and molecular biology and also have been demonstrated in microarray analyses to harbor characteristic gene expression signatures.[4–6] From a functional point of view these reciprocal rearrangements interfere directly or indirectly with transcription and lead to a stop of cellular differentiation.[7] In 2001, these entities were combined into the first hierarchical category of the WHO classification of AML.[8] Yet, even combined, these entities only account for a minority of cases in adult AML (20–25%) and further subclassification of AML with biological or prognostic relevance would be desirable to achieve a more detailed classification of this heterogeneous disease.

The reciprocal chromosomal rearrangement t(8;16)(p11;p13) is an interesting candidate for further investigation, in particular because the pathways of leukemogenesis in AML with t(8;16) are not fully understood. This rare translocation with less than 100 cases described so far,[9] is characterized by disruption and fusion of *MYST3* (alias *MOZ*) and *CREBBP* (alias *CBP*) genes. *MYST3* is localized on 8p11 and encodes the monocytic leukemia zinc finger protein, a histone acetyltransferase of the MYST family that modulates gene transcription through activation of the AML1 transcription factor complex.[10–12] Its fusion partner *CREBBP* on chromosome 16p13 encodes the cAMP response element-binding protein CBP, another histone acetyltransferase.[10] CREBBP modulates gene transcription by histone acetyltransferase activity and by binding to several proteins with central cell-cycle functions.[11]

Thus far, several different transcript types in AML with t(8;16) have been identified by reverse transcription (RT)–PCR. However, the question of the relevant leukemogenic fusion is still under debate.[13] Moreover, both genes involved in the translocation t(8;16) are known to be involved in other rare balanced rearrangements in AML, all associated with an adverse prognosis: *MYST3* in t(8;19)(p11;q13),[14] t(6;8)(q27;p11), t(8;22)(p11;q13)[15] and inv(8)(p11q13);[16–18] *CREBBP* in t(10;16)(q22;p13)[19] or t(11;16)(q23;p13),[20] respectively.

Despite the rarity of this AML subtype, it might qualify as a distinct classifiable entity due to its specific clinical, cytomorphologic and genetic profile. We thus intended to further characterize this rare AML subtype and describe a new cohort of 13 cases of AML with t(8;16) by a multimodal diagnostic approach. All cases were analyzed by a combination of cytomorphology, immunophenotyping, cytogenetics and molecular diagnostics. Additionally, whole-genome microarray analyses were performed and AML with t(8;16) gene expression patterns were compared to both AML cases with normal karyotype, including the French–American–British (FAB) subtypes M1 and M4/M5 with strong myeloperoxidase (MPO) or non-specific esterase (NSE) expression, and to distinct AML subtypes with balanced recurrent chromosomal aberrations according to the WHO classification.

Materials and methods

Study design

This study focused on 13 adult AML patients with t(8;16)(p11;p13). Details on diagnostic characteristics and

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clinical history are shown in Table 1. All patients gave their informed consent for participation after having been advised of the purpose and investigational nature of the study. The study design adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board.

For comparative microarray analyses of this rare subtype we included in addition 170 patients with other specific FAB subtypes, such as FAB M1 ($n=70$), FAB M4 ($n=69$), FAB M5a ($n=10$) and FAB M5b ($n=21$), which all showed a normal karyotype. This cohort was further completed by inclusion of 230 patients with the following balanced chromosomal translocations according to the first categorical hierarchy of the WHO classification: t(15;17)/*PML-RARA* ($n=58$), t(8;21)/*RUNX1-RUNX1T1* ($n=56$), inv(16)/*CBFB-MYH11* ($n=56$) and t(11q23)/*MLL*-rearrangements ($n=60$).

Diagnosis was performed from bone marrow samples in all patients between 1999 and 2008 by an individual combination of cytomorphology, cytogenetics, fluorescence *in situ* hybridization (FISH), immunophenotyping and molecular genetics as published elsewhere.[21] Cytomorphologic classification followed the FAB classification of AML.[22,23] AML with t(8;16) bone marrow smears were reviewed by two independent cytologists (TH and HL).

At the time of diagnosis, mononuclear cells from the bone marrow aspirate were purified by Ficoll density centrifugation and 5×10^6 cells were lysed (RLT lysis buffer; Qiagen, Hilden, Germany). For subsequent molecular analyses these stabilized lysates were frozen and stored at $-80\,^{\circ}$C. RT–PCR was performed as described by Borrow *et al.*[24] and by Schmidt *et al.*[25] Samples that failed to amplify a *MYST3-CREBBP* product where further screened according to primers as published by Murati *et al.*[26] The microarray sample preparation assay was performed as previously reported.[4,5,27]

Microarray data analysis

Gene expression data were processed according to the manufacturer's recommendations. After quality control, HG-U133A/B and HG-U133 Plus 2.0 data series were normalized separately using the robust multiarray average normalization algorithm as implemented in the R-package affy version 1.18.0.[28] To assess differential gene expression an empirical Bayes approach as proposed by Smyth was used.[29] This method borrows information from the ensemble of genes which can assist in inference about each gene individually. The genewise tests are based on moderated *t*-statistics in which posterior standard deviations are used in place of ordinary standard deviations. The process yields shrinkage of the genewise sample variances toward a common value, resulting in more stable inference compared to ordinary *t*-tests when the number of arrays is small. The resulting *P* values were adjusted for control of the false discovery rate according to Benjamini and Hochberg's method.[30] Analyses were performed in R with the limma package version 2.14.1.

For binary classification of AML with t(8;16) and non-t(8;16) specimens support vector machines (SVMs) were used (R-package *e1071* version 1.5-18; default parameters; linear kernel).[31] As the comparison of expression values between training (HG-U133A/B) and test data (HG-U133 Plus 2.0) revealed slight differences in probe set wise means and standard deviations within the two cohorts, each probe set in each dataset was centered and scaled to unit standard deviation before the SVM was trained and tested. Of note, similar effects have been observed by Metzeler *et al.*[32] and are probably due to different microarray platforms and separate normalization.

To visualize similarity of gene expression patterns, we applied hierarchical clustering and principal component analyses. Transformed gene expression data were analyzed using the GeneMaths XT Version 2.1 (Applied Maths, St-Martens-Latem, Belgium) and Partek Genomics Suite Version 6.4 (Partek Inc., St Louis, MO, USA). Biological networks were generated using Ingenuity Pathways Analysis Version 6.5 (Ingenuity Systems, Redwood City, CA, USA), a web-based application that generates networks by use of differentially expressed genes from expression array data analyses.[33]

Results

Incidence, history and prognosis

We here present a cohort of AML cases with t(8;16)(p11;p13). This translocation was rare with only 13 (0.2%) of 6124 cases of AML diagnosed from our overall cohort of patients over recent years (Table 1). The distribution of gender was 9 women and 4 men. AML with t(8;16) was found more frequently in therapy-related AML (t-AML) than in *de novo* AML (7/438 t-AML and 6/5686 *de novo*; $P=0.00001$). In the t-AML group 4/7 patients had a history of breast cancer. One case each was following T-cell non-Hodgkin's lymphoma or B-cell non-Hodgkin's lymphoma, and one case was following chronic myelomonocytic leukemia. As shown in Figure 1, prognosis of AML with

Table 1 Characteristics of the 13 patients of AML with t(8;16)

No.	*Gender*	*Age*	*Diagnosis (FAB)*	*History*	*Pox (%)*	*Est (%)*	*Erythrophagocytosis*	*Blast (%)*
1	Female	40	AML M5a	*de novo* AML	90	80	—	N.A.
2	Female	63	AML M4/M5	*de novo* AML	83	100	1/500	N.A.
3	Male	58	t-AML M4	t-AML (following T-NHL)	100	100	5/500	N.A.
4	Male	77	AML M4/M5a	*de novo* AML	72	86	1/300	90
5	Female	52	t-AML M5a/M4a	t-AML (following breast cancer)	100	89	—	99
6	Male	64	t-AML M4	t-AML (following CMML)	30	84	2/100	90
7	Female	39	t-AML M5a	t-AML (following breast cancer)	84	95	—	90
8	Male	31	AML M5a	*de novo* AML	N.A.	95	N.A.	95
9	Female	56	t-AML M0	t-AML (following breast cancer)	85	—	Yes	N.A.
10	Female	63	AML M5a	*de novo* AML	95	90	—	76
11	Female	83	t-AML M4	t-AML (following B-NHL)	85	65	1/100	68.5
12	Female	53	t-AML M4	t-AML (following breast cancer)	80	40	1/200	76.5
13	Female	49	AML M4	*de novo* AML	95	60	—	30

Abbreviations: AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; Est, esterase activity; FAB, French–American–British; Pox, peroxidase activity; N.A., not available; t-AML, therapy-related AML; T-NHL, T-cell non-Hodgkin's lymphoma.

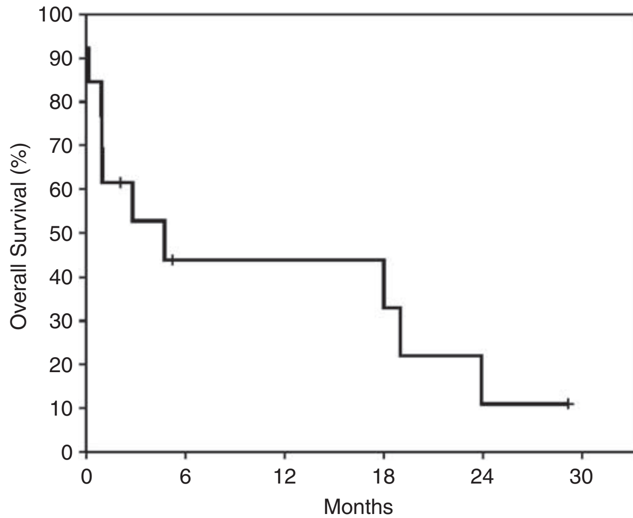

Figure 1 Survival analysis of acute myeloid leukemia (AML) with t(8;16) cases. Data is shown for 13 patients of AML with t(8;16). Tick marks represent patients whose data were censored at the last time they were known to be alive.

t(8;16) was poor with median overall survival of 4.7 months. Five patients deceased within the first month after diagnosis.

Cytomorphology, cytogenetics, RT–PCR and immunophenotype

All cases were evaluated by May–Gruenwald–Giemsa staining on bone marrow and blood smears. Cytomorphology showed blasts in ⩾30% of all cells without differentiation (range 30–99%; median: 90%). As shown in Table 1, in all 13 cases the positivity for MPO on bone marrow smears was ⩾30% (range 30–100%; median: 85%). Intriguingly, in parallel also ⩾40% (range 40–100%; median: 88%) of blast cells stained strongly positive for NSE, suggesting that AML with t(8;16) arise from a very early stem cell with both myeloid and monoblastic differentiation potential. Therefore, AML with t(8;16) cases can not be classified according to standard FAB categories.

In many cases suspicion of the t(8;16) arises already from cytomorphology, because the blasts are characterized by the striking phenomenon of erythrophagocytosis.[34–36] Also in our cohort erythrophagocytosis was frequently present (7/13 cases). Figure 2 is illustrating the cytomorphology of a typical AML with t(8;16) sample.

Chromosome banding analyses were performed in all cases and showed an isolated t(8;16)(p11;p13) in 6/13 cases (Supplementary Table 1); 7/13 patients demonstrated additional abnormalities, with 4 cases showing single additional aberrations and 3 cases having two or more additional aberrations, respectively. Molecular analyses detected the *MYST3-CREBBP* fusion transcript in all cases tested (12/12).

Multiparameter flow cytometric analysis of the immunophenotypes of leukemic cells was performed in seven cases. As has been previously reported,[9] all cases examined in our series expressed the myeloid markers MPO, CD33, CD13, CD65 and CD15, but lacked the expression of the progenitor cell markers CD34, CD117 and CD133 (Supplementary Table 2). Furthermore, these cases displayed an expression pattern of the monocytic antigens CD14, CD64, CD11b as well as a coexpression of CD56 and CD4. Differing from most other AML cases, the present series featured a strong side-scatter signal in all cases (Supplementary Figure 1).

Exploratory gene expression microarray analysis

Gene expression signatures were analyzed in seven cases using Affymetrix microarrays and two different kinds of analyses were performed. In both analyses we separated between a training cohort and an independent test cohort (Supplementary Tables 3, 4). Differentially expressed genes were identified in the cohort analyzed with HG-U133A/B microarrays. The signatures then were validated in the cohort analyzed with HG-U133 Plus 2.0 microarrays.

First, we compared the gene expression patterns of seven cases of AML with t(8;16) to AML cases with normal karyotype representing the AML FAB subtypes M1 with strong MPO expression and M4/M5 with strong NSE expression. As shown in Figure 3a, using Affymetrix HG-U133A/B microarrays 4 cases of AML with t(8;16) were compared to AML FAB subtype M1 ($n=46$) and 66 patients with various monocytic subtypes (M4: $n=41$; M5a: $n=9$; M5b: $n=16$). The principal component analysis (PCA) showed a continuum of cases for the defined FAB subtypes whereas AML with t(8;16) clustered separately, underlining their distinctiveness, as compared to classical FAB characteristics. Still, gene expression patterns seemed more strongly influenced by monocytic than by myeloid characteristics as the cluster of cases of AML with t(8;16) was found in close proximity to the FAB M5a/M5b grouping. This result was confirmed when an independent cohort of samples was analyzed. As given in Figure 3b, the discovered signature from HG-U133A/B microarrays was validated using testing data obtained from an independent cohort of patients analyzed with HG-U133 Plus 2.0 microarrays. Again, a similar clustering was observed for an independent series of 3 cases of AML with t(8;16) and the AML FAB subtypes M1 ($n=24$) and 34 patients with various monocytic subtypes (M4: $n=28$; M5a/b: $n=6$).

Secondly, AML with t(8;16) cases were compared to the four reciprocal rearrangements of the first hierarchy of the WHO classification of AML: 43 cases of t(15;17)/*PML-RARA*, 40 cases of t(8;21)/*RUNX1-RUNX1T1*, 49 cases of inv(16)/*CBFB-MYH11* and 50 cases of t(11q23)/*MLL* gene rearrangements (Figure 3c). In this analysis, using HG-U133A/B gene expression patterns, AML with t(8;16) samples were repeatedly grouped in the vicinity of the t(11q23)/*MLL* cases and partly intercalated with the t(11q23)/*MLL* group. Also, this dominant signature was confirmed when an independent cohort of samples was analyzed. As given in Figure 3d, the discovered signature from HG-U133A/B microarrays was validated using data obtained from an independent cohort of patients analyzed with HG-U133 Plus 2.0 microarrays. A similar clustering was observed for an independent series of 3 cases of AML with t(8;16), repeatedly intercalating with the group of t(11q23)/*MLL* ($n=10$), but being clearly distinct from other AML subtypes with balanced chromosomal aberrations (inv(16), $n=7$; t(15;17), $n=15$; t(8;21), $n=16$).

However, in a subsequent direct pairwise comparison AML with t(8;16) cases could also be clearly discriminated from t(11q23)/*MLL* by a unique signature (Figure 3e). This was also seen for the independent testing series from the HG-U133 Plus 2.0 cohort (Figure 3f). The genes differentially expressed included candidates such as *SGSM*, a small G protein-signaling modulator, or the proposed tumor suppressor *HINT1*, a histidine triad nucleotide-binding protein, with lower expression in AML with t(8;16). The histone methyltransferase *SETD8*, *SETD1B*, another component of a histone methyltransferase complex,

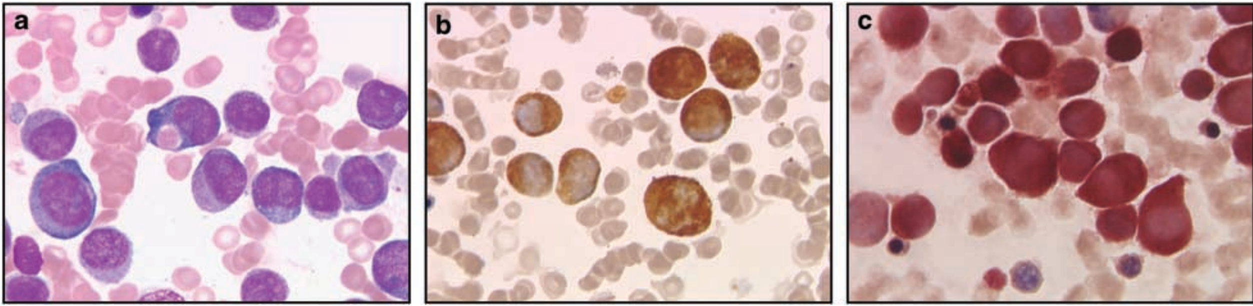

Figure 2 Cytomorphology of a typical case of acute myeloid leukemia (AML) with t(8;16). Bone marrow smears are displayed according to (**a**) May–Gruenwald–Giemsa (MGG) staining. (**b**) Myeloperoxidase (MPO) staining. (**c**) Non-specific esterase (NSE) staining.

RAS oncogene family member *RAB33A*, the oncogene *PIM1* kinase, or *GPR124*, a G protein-coupled receptor, were observed to be higher expressed in AML with t(8;16), respectively, as compared to AML with t(11q23)/*MLL*. An additional hierarchical clustering heatmap is given in the Supplementary Material (Supplementary Figure 2). A full list of differentially expressed genes for each comparison is also available online.

In summary, in direct comparison to other FAB subtypes or categorized WHO entities with chromosomal aberrations, dominant and unique gene expression patterns were confirmed for AML with t(8;16), underlining the molecular distinctiveness of this rare AML entity.

Similarity of AML with t(8;16) and AML with t(11q23)/MLL

In the next analysis, the similarity of AML with t(8;16) and AML with t(11q23)/*MLL* was further investigated. As shown in the hierarchical clustering analysis in Figure 4, both types of AML shared common blocks of genes with similar expression patterns as compared to other AML subtypes. An enlarged section of dendrogram no. 1 highlights three exemplary series of genes that were observed to be consistently lower expressed and included candidates such as *CCND2*, *CBX2*, *CD99*, *MLLT11*, *NCOA7*, *RRAGD*, *PDGFC* and *SMYD2* (Figure 5).

As enlarged from the common groups derived from dendrogram no. 2, genes with higher expression in both AML with t(8;16) and AML with t(11q23)/*MLL* included markers such as *HOXA3, HOXA4, HOXA5, HOXA6, HOXA7, HOXA9, HOXA10*, *PBX3, MEIS1 and HNMT* (Figure 5). In summary, a similar deregulated expression of HOXA cluster member genes was observed leading to the hypothesis of shared pathways for these two types of AML with poor prognosis.

Molecular network analysis

Genes with a specific expression profile in the AML cases with t(8;16) were further examined in pathway analyses. First, t(8;16) specific genes were identified by comparing t(8;16) cases in one-versus-all comparisons versus other FAB subtypes and also versus other WHO entities. This resulted in a list of 177 differentially expressed probe sets with specific expression for AML with t(8;16) for the HG-U133A/B cohort and 161 differentially expressed probe sets with specific expression for AML with t(8;16) for the HG-U133 Plus 2.0 cohort. Secondly, we compared the overlap of the gene lists from the two different chip types and this resulted in a common list of 55 differentially expressed probe sets with highly specific overexpression in AML cases with t(8;16) (Supplementary Table 5). A detailed annotation of the three gene lists is available online.

The 55 t(8;16) specific probe sets were then analyzed with Ingenuity's pathway analysis application. In total, 47 probe sets were successfully mapped to annotated genes in the pathway application. Figure 6 shows the two top-scoring networks with 9 and 10 of the AML with t(8;16) specific genes included, respectively.

In network 1, nine genes from the AML with t(8;16) candidate list were mapped around *MYC* and *TP53*, and included the candidates *ALDH4A1*, *BMP8B*, *CLPB*, *HNF4G*, *PERP*, *PHF10*, *POU4F2*, *SERPINI2* and *TRHDE*. TP53 is well known to play a critical role as gatekeeper in cell division and apoptosis. The second network, which centered on AKT and RAS, controls cell response to mitogens. In this network, 10 genes from the AML with t(8;16) candidate list included *AGT*, *CAMSAP1L1*, *CHCHD7*, *HOXA9*, *ITSN1*, *PRL*, *PRLR*, *RYR2*, *SCGB3A1* and *SMAD6*. Both networks are implicated in oncogenesis and the observed disregulation in AML with t(8;16) as compared to other AML subtypes may explain some of the unique cytomorphological, cytogenetic, molecular and prognostic features of this group. Overall, in the AML with t(8;16) signature 7 genes out of the 55 probe sets were associated with transcriptional regulation.

Classification analysis

We next assessed the predictive strength of the gene expression signature for AML with t(8;16) by using a classification algorithm. First, SVMs were used to predict AML with t(8;16) in the context of other AML FAB subtypes within the HG-U133A/B dataset. As observed by leave-one-out cross-validation, all samples were correctly classified. Secondly, another SVM algorithm was trained using the gene expression signature as described above, that is, 177 AML with t(8;16) specific probe sets (Supplementary Table 5), restricted to training data from the HG-U133A/B microarray cohort. This SVM was subsequently applied to classify the AML with t(8;16) and FAB samples from the independent HG-U133 Plus 2.0 test dataset. Again, correct predictions were observed for the AML with t(8;16) specimens in the testing cohort. This successful classification was observed not only for both FAB-type but also for the corresponding WHO-type signatures. Therefore, a correct classification of all cases of AML with t(8;16) was observed both across the different patient cohorts and the two available microarray designs in this study.

Discussion

In AML, distinct subtypes can clearly be specified by applying cytomorphology, cytogenetics and molecular genetic methods. Particularly, this is the case for AML M4eo with

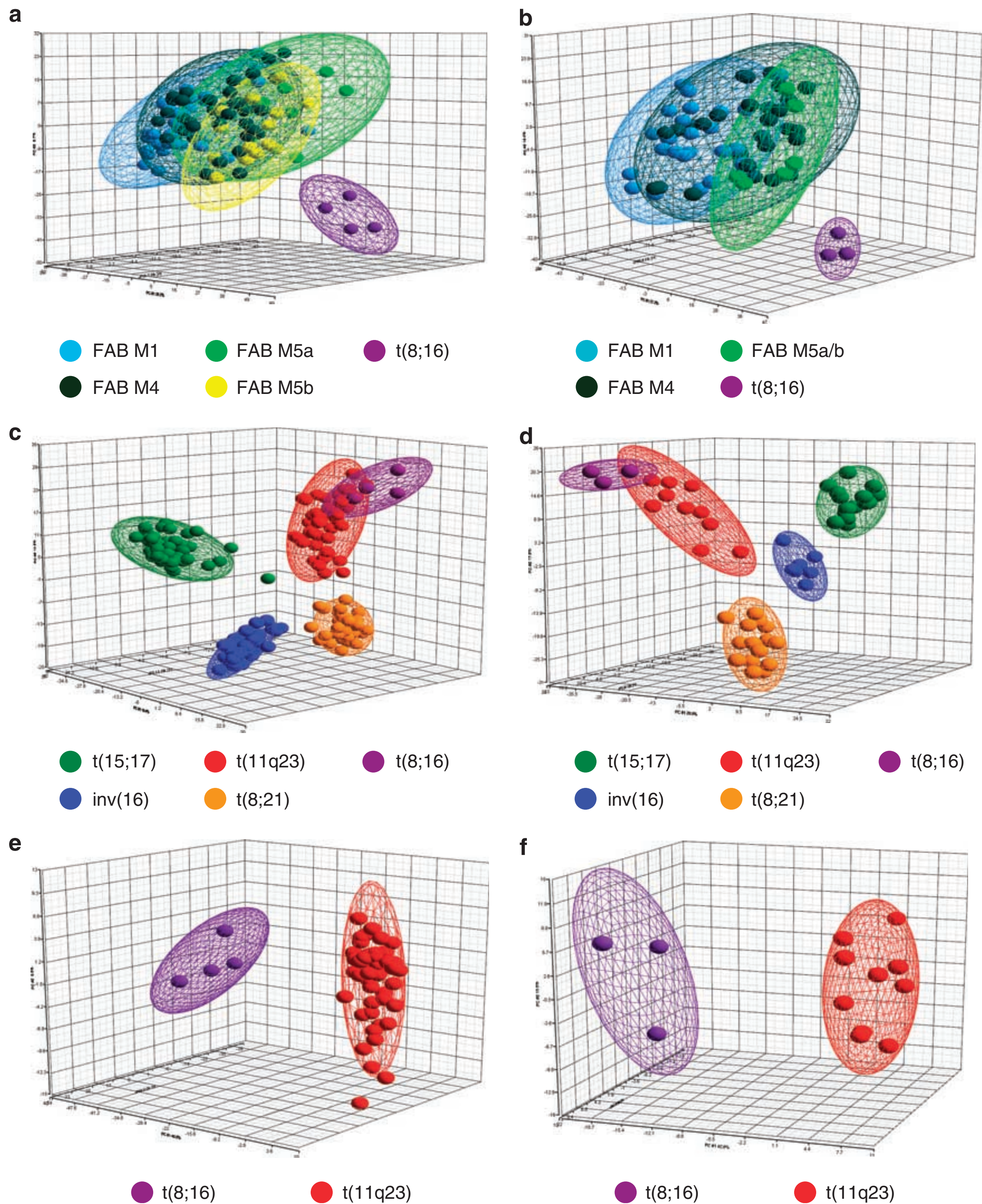

Figure 3 Principal Component Analysis (PCA). The leukemia samples are plotted in a three-dimensional space using the three principal components (PC) capturing most of the variance in the original dataset. Each patient sample is represented by a single color-coded sphere. (**a**) French–American–British (FAB)-subtype analysis using the discovery cohort from HG-U133A/B arrays. The genes used for analysis were selected from five one-versus-all (OVA) comparisons for FAB M1, M4, M5a, M5b and acute myeloid leukemia (AML) with t(8;16), respectively. From each pairwise analysis the top 300 differentially expressed genes were combined, resulting in 1351 unique probe sets. (**b**) FAB-type analysis using the validation cohort (HG-U133 Plus 2.0 microarrays). The analysis is based on the 1351 probe sets from the discovery cohort. (**c**) WHO-subtype analysis using the discovery cohort from HG-U133A/B arrays. The genes used for analysis were selected from five OVA comparisons for t(11q23)/*MLL*, inv(16), t(15;17), t(8;21), and AML with t(8;16), respectively. From each pairwise analysis the top 300 differentially expressed genes were combined, resulting in 1449 unique probe sets. (**d**) WHO subtype analysis using the validation cohort (HG-U133 Plus 2.0 microarrays). The analysis is based on the 1449 probe sets from the discovery cohort. (**e**) Pairwise analysis between t(11q23)/*MLL* and AML with t(8;16) using the discovery cohort from HG-U133A/B arrays and selecting the top 300 differentially expressed genes. (**f**) Pairwise analysis between t(11q23)/*MLL* and AML with t(8;16) using the validation cohort (HG-U133 Plus 2.0 microarrays). The analysis is based on the 300 probe sets from the discovery cohort. For all analyses corresponding supporting material is available online.

inv(16)/*CBFB-MYH11*, acute promyelocytic leukemia (APL) with t(15;17)/*PML-RARA*, as well as AML with t(8;21)/*RUNX1-RUNX1T1* which also show specific patterns with respect to the immunophenotype, for example, the negativity for HLA-DR in APL, and molecular gene expression.[5,37–40] This correlation is less striking for AML with 11q23/*MLL* rearrangements.

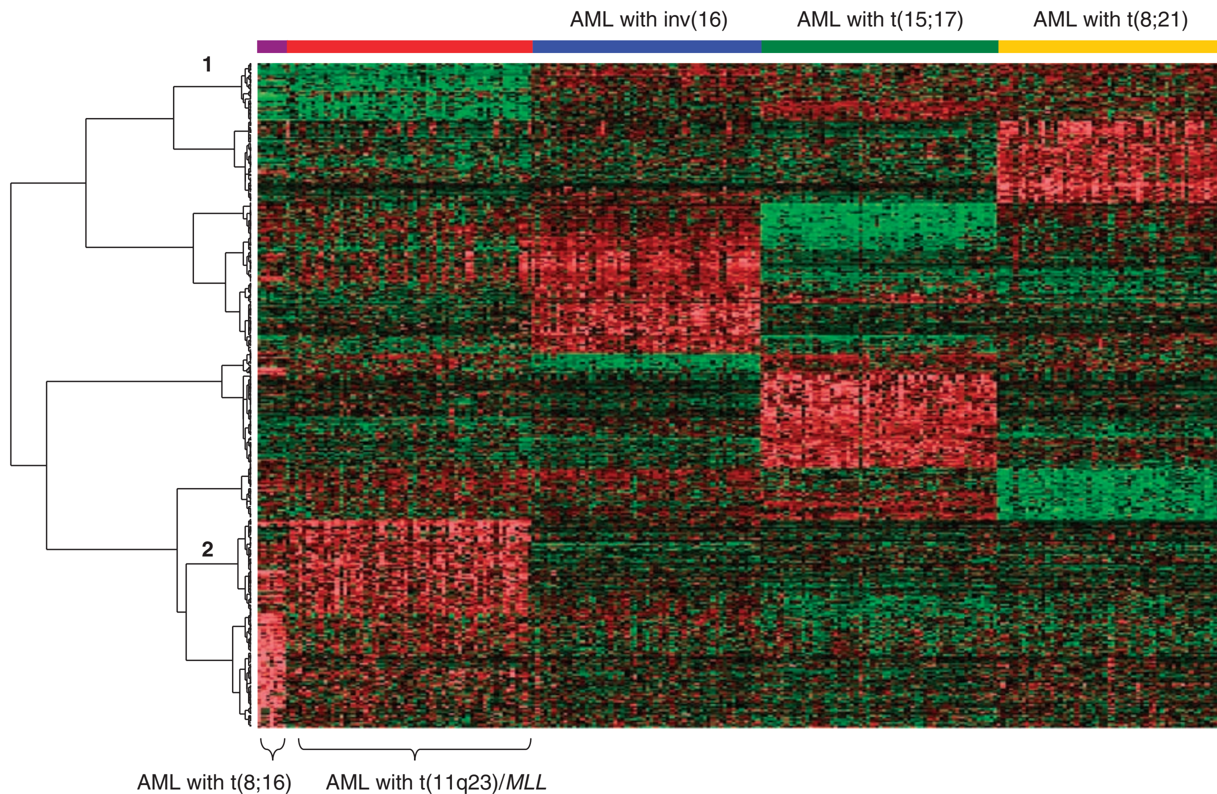

Figure 4 Hierarchical clustering analysis. In the hierarchical clustering analysis 237 acute myeloid leukemia (AML) cases are displayed, that is, visualizing both the HG-U133A/B and HG-U133 Plus 2.0 cohorts. The genes used for the cluster are based on the discovery cohort only (HG-U133A/B) and were selected from five one-versus-all comparison for each of the AML subtypes AML with t(8;16), t(11q23)/*MLL*, inv(16), t(15;17) and t(8;21). From each pairwise analysis the top 300 differentially expressed genes were combined, resulting in 1449 unique probe sets (given in rows). The similarity was computed by Euclidean distance, and then Ward's method was used to cluster the gene expression profiles based on these measures. The normalized expression value for each gene is coded by color (standard deviation from mean). Red cells indicate high expression and green cells indicate low expression. The coloring of the leukemia groups is identical to Figure 3. Supporting material is available online.

However, this subtype is closely associated to monocytic differentiation in cytomorphology, is characterized by expression of 7.1 antigen in multiparameter flow cytometry, and also shows unique gene expression signatures.[41,42] Thus far, these four entities are combined into the first hierarchical step in the WHO classification as so-called 'AML with recurrent balanced translocations'.[8]

This hierarchy of the WHO classification of AML might be amended by further addition of other reciprocal translocations, such as the rare t(8;16)(p11;p13), if unique clinical, morphologic and genetic features were established. We thus focused on a detailed characterization of a novel cohort with t(8;16)(p11;p13) by a multimodal diagnostic approach, which was supported by microarray-based gene expression profiling. We were able to add 13 new cases to the so far less than 100 reported cases in the Mitelman database (http://cgap.nci.nih.gov/Chromosomes/Mitelman).[9] Also in our series of AML, the t(8;16) was observed to be very rare, that is, 0.2% of all cases in this study, compared to 0.4% according to Mitelman *et al.*,[43] but its incidence in therapy-related AML was confirmed to be significantly higher, that is, 1.6% in this study. This is in line with data that described for many therapy-induced cases of the t(8;16) subtype the association to previous therapy with alkylating drugs in combination with topoisomerase-II-inhibitors, for example, anthracyclines or epipodophyllotoxins. The observed interval from the primary malignancy to therapy-induced AML with t(8;16) is short without preleukemic phase in accordance with therapy induction of AML with other reciprocal translocations.[44,45] With respect to the gender distribution, female patients represented 69% in our series (9/13 patients) which is supporting data from a previous study where 67% of cases were women.[9]

We further could confirm the poor prognosis reported previously,[34,35,44–46] as patients from our series demonstrated a median survival of 4.7 months only. For some part this negative outcome might be explained by the high incidence of t-AML cases in cases of t(8;16) with 54% in our cohort.[47] However, outcome was even inferior than in AML with t(11q23)/*MLL* rearrangements, which is also associated with t-AML, but shows a median survival of 9 months.[48] This indicates a high-risk grading of patients with t(8;16) from a therapeutic point of view. One of the few patients with longer follow-up within our cohort had received allogeneic transplantation, which might improve outcome in single cases. However, the short survival in many reported cases may not even allow the preparation for this treatment option.

The above-mentioned association of the t(8;16) and the t(11q23)/*MLL* AML subtypes to previous chemo/radiotherapy and to an inferior prognosis suggests also parallels in the leukemogenic pathways. Intriguingly, cytomorphologic findings support the hypothesis of such vicinity: due to the morphologic appearance of blasts and due to the strong NSE staining AML with t(8;16) is mostly diagnosed as myelomonocytic or even monocytic leukemia mimicking FAB M4 or M5a. The latter is

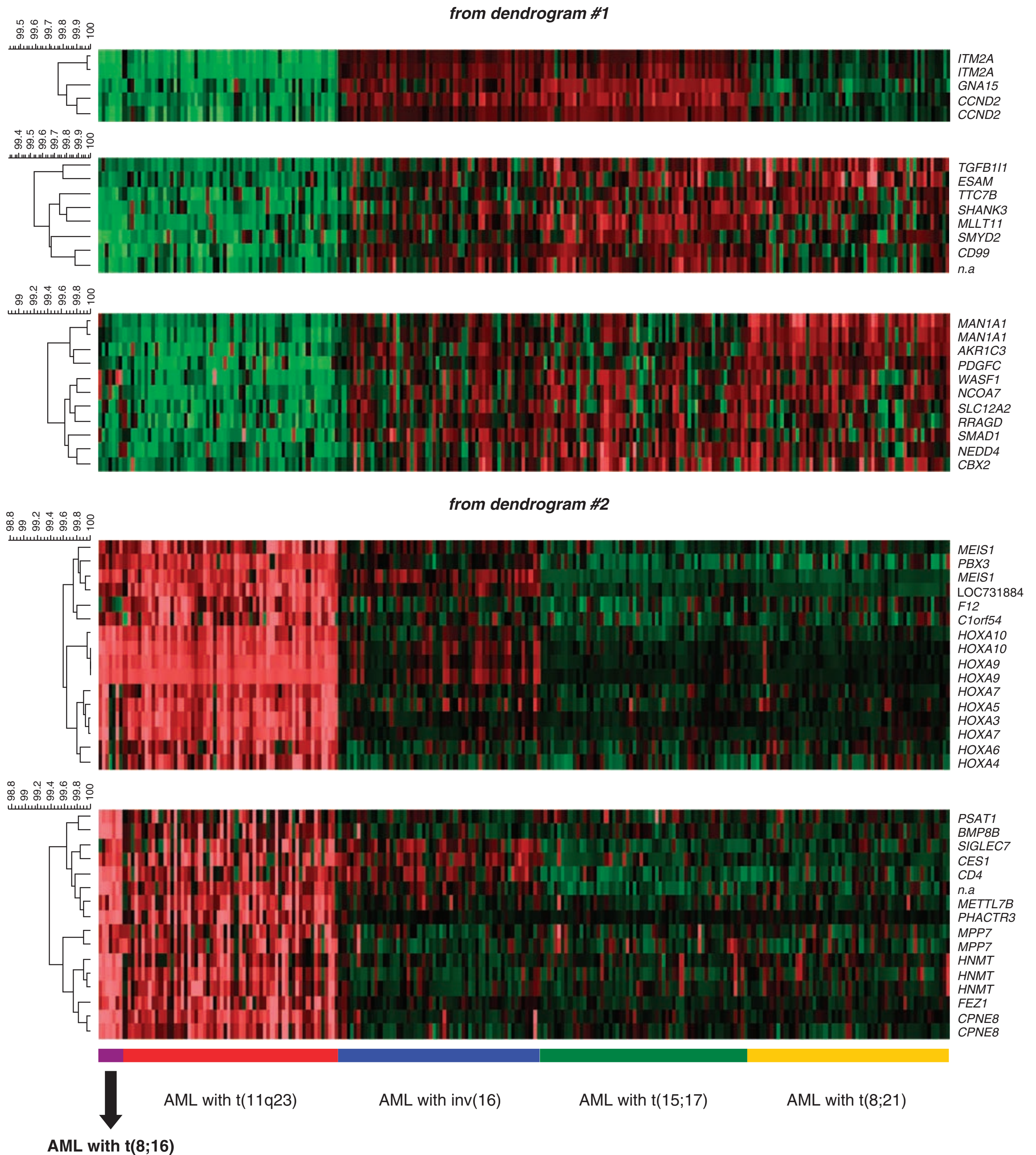

Figure 5 Shared gene expression signatures between acute myeloid leukemia (AML) with t(8;16) and AML with t(11q23)/*MLL*. Three sections from dendrogram 1 from Figure 4 are enlarged and visualized genes with a lower expression in both AML subtypes as compared to AML with t(8;21), AML with t(15;17) or AML with inv(16), respectively (green cells). Another two sections from dendrogram 2 focused on genes with consistent higher expression in both AML subtypes (red cells). Supporting material is available online.

highly associated to t(11q23)/*MLL* rearrangements in AML.[49,50] However, the t(8;16) shows positive MPO staining in parallel to positive NSE in nearly all cases,[36] which separates it from all other AML including the monocytic subtypes. This combination of features of FAB M1, M4 and M5a subtypes in AML with t(8;16) renders classification according to FAB or WHO criteria impossible. Secondly, it suggests that the blasts in t(8;16) might origin in a very immature stem cell bearing monocytic as well as myelocytic characteristics which in part might explain the frequent resistance to standard chemotherapy regimens.

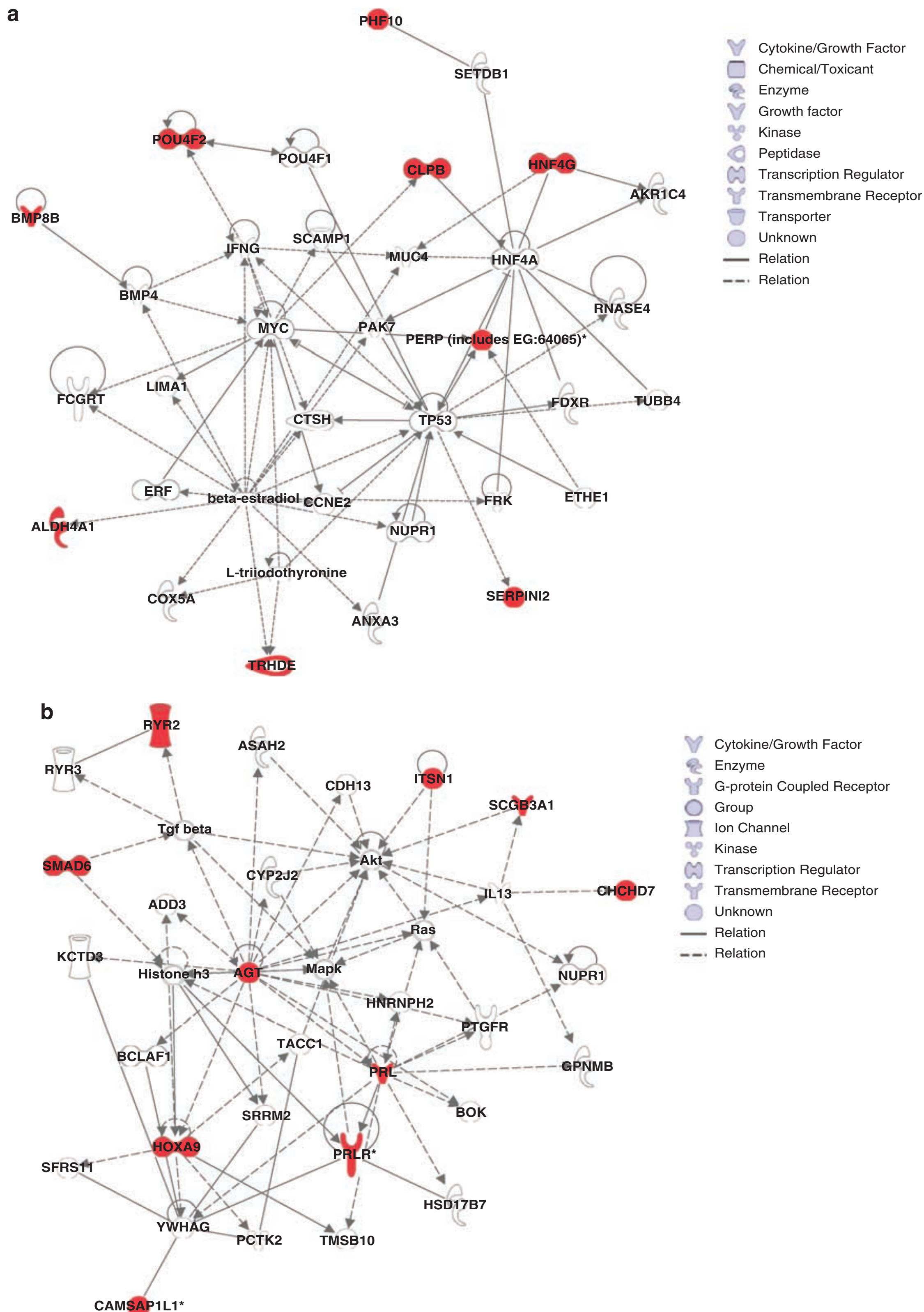

Figure 6 Molecular pathway analysis of genes distinguishing acute myeloid leukemia (AML) with t(8;16) leukemias from other AML subtypes. The network is graphically displayed with genes as nodes and the biological relationships between the nodes as edges. Red intensities correspond to an observed higher expression in AML with t(8;16), that is, upregulated fold change. Focus genes for the Ingenuity application were included in the original text format file derived from the list of 55 overlapping differentially expressed probe sets with specific expression in AML with t(8;16). Non-focus genes were derived from queries for interactions between focus genes and all other gene objects stored in the Ingenuity knowledge database. (**a**) Network 1 mapped around *MYC* and *TP53*. (**b**) Network 2 centered on *AKT* and *RAS*.

We also add new data to the finding of *MOZ* involvement in AML and erythrophagocytosis. In our cohort, erythrophagocytosis of the blasts was observed in 7/13 cases. Interestingly, erythrophagocytosis so far not only was observed in AML with t(8;16), but also in the rare inversion inv(8)(p11q13), which fuses the *MOZ* and *NCOA2* (nuclear receptor coactivator 2, alias *TIF2*) genes.[16] Thus, this phenomenon seems to be a frequent event for *MOZ* involvement in AML.

With respect to the transcriptional program active in AML with t(8;16) we observed striking similarities of t(8;16) cases and AML with t(11q23)/*MLL* rearrangements. Hierarchical clustering and PCA revealed a common signature of the HOX cluster genes overexpressed in both subtypes. This is in line with recent data from Camos *et al.*[51] and Murati *et al.*,[52] who detected overexpression of *HOXA9*, *HOXA10* and their cofactor *MEIS1* in their series of t(8;16). We also confirm the overexpression of *PRL*, *CHD3*, *CPEB2*, *NR2F6* and *RET* genes in AML with t(8;16) and decreased expression of the *CCND*2 and *HINT1* genes.[51] Additionally, we were able to clearly discriminate cases with t(8;16) from cases with t(11q23)/*MLL* rearrangements by a direct pairwise comparison underlining that indeed a unique t(8;16) specific signature exists and can be defined by gene expression profiling.

Overall, dominant functions for genes with higher expression in AML with t(8;16) were observed to be involved in DNA binding processes and transcription factor activity (supporting online information for gene lists N177 and N161, respectively).

Amongst those, *CHD3* (chromodomain helicase DNA-binding protein 3, alias Mi-2a), in particular, seems to be an interesting target gene for further research. CHD3 proteins have been reported to be ATP-dependent chromatin remodelers that contribute to repression of developmentally regulated genes in both animal and plant systems.[53] Recently, in a two-hybrid screening CHD3 was identified as an interaction partner for human c-Myb and gain of the MYB locus was found as recurrent abnormality in AML with t(8;16) cases using array comparative genome hybridization technology.[54] Thus, one can speculate that aberrant expression of *CHD3* would perturb the MYB pathway and therefore may contribute to a maturation block in monocyte-macrophage differentiation as reported previously.[55]

Using a classification analysis we were able to demonstrate that AML with t(8;16) harbors underlying gene expression signatures that are robust enough to also serve as a potential classifier to predict new cases of AML with t(8;16). The successful classification in all cases of AML with t(8;16) was not only observed for both FAB-type and WHO-type signatures, but also correctly classified the cases across the different patient cohorts and microarray designs.

In conclusion, our results suggest that in all aspects as discussed above AML with t(8;16) is clearly separated from the so-called favorable translocations t(8;21), t(15;17) and inv(16). The latter correlate with a favorable prognosis and occur much more often in *de novo* than in t-AML. Their morphologic features—AML FAB M1 and M2 in the t(8;21), APL in t(15;17) and AML FAB M4eo in inv(16)—show no parallels to AML with t(8;16). Moreover, we were able to demonstrate that AML cases with t(8;16) are characterized by distinct features with respect to cytomorphology, cytogenetics, RT–PCR and gene expression patterns, as well as by their association to t-AML and very poor prognosis.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)