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High expression of *IGFBP2* is associated with chemoresistance in adult acute myeloid leukemia

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Abstract

Insulin-like growth factor (IGF) signaling plays an important role in many tumors and overexpression of *IGF Binding Protein (IGFBP) 2* has been associated with adverse outcome in childhood leukemia. Here, we evaluated *IGFBP2* mRNA expression and its prognostic implications in 99 adult acute myeloid leukemia (AML) patients by quantitative real-time RT-PCR. High *IGFBP2* was associated with a high incidence of primary resistant disease (*IGFBP2* high 65%, *IGFBP2* low 32%; *P*=0.02) and was independently predictive for therapy resistance [OR 3.6 (95% CI 1.2–11); *P*=0.02] in multivariate analyses. Gene-expression profiling revealed an up-regulation of genes implicated in leukemogenesis (*MYB*, *MEIS1*, *HOXB3*, *HOXA9*) and genes associated with adverse outcome (*ERG*, *WT1*) in patients with high *IGFBP2* expression. Thus, our data suggest a role of IGFBP2 and IGF signaling in chemoresistance of AML. Patients with high *IGFBP2* expression might benefit from molecular therapies targeting the IGF pathway.

Keywords

IGFBP2; acute myeloid leukemia; chemoresistance

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Introduction

Clinical outcome of patients with acute myeloid leukemia (AML) remains poor with a long-term survival of 30–50% in younger patients [1]. The identification of aberrant signaling pathways in AML has elucidated mechanisms of disease pathogenesis and led to the development of new targeted therapies. Further characterization of potentially druggable and prognostically relevant signaling pathways might improve risk-assessment and therapeutic strategies for AML patients.

Alterations of the insulin-like growth factor (IGF) system have been demonstrated in different hematological and solid malignancies and provide a potential target for novel anticancer therapies. The IGF pathway is important for regulating proliferation, differentiation, and apoptosis in normal tissues and tumor cells [2]. In AML, the two ligands IGF-I and IGF-II are known to stimulate proliferation of leukemic blasts [3]. Furthermore, autocrine expression of IGF-I has been detected in AML cells *in vitro* [4]. Moreover, inhibition of the IGF axis by IGF-IR kinase inhibitors or anti-IGF-receptor antibodies revealed substantial antiproliferative effects in leukemic cells [4–6].

The functional activity of IGF-I and IGF-II is regulated by seven high-affinity IGF binding proteins (IGFBP1–7). They act as carrier proteins and modulate bioavailability and half-life of the IGFs. In addition, *in vitro* and *in vivo* findings suggest that IGFBPs promote cell growth independently of IGFs [7]. IGFBP2, one of the major IGFBPs, is aberrantly expressed in a variety of solid tumors, lymphomas, and in patients with acute lymphoblastic leukemia (ALL) and AML [8–10]. Although the functional role of IGFBP2 in acute leukemias and other types of cancer is still unknown, overexpression of *IGFBP2* has been associated with higher tumor grade, tumorigenicity [8], and therapy resistance [11] in different human malignancies. In childhood AML, high expression of *IGFBP2* mRNA was related to inferior event-free survival (EFS) and was independently predictive for a higher relapse rate in small studies of 13 and 35 patients, respectively [10, 12]. In addition, high IGFBP2 serum protein levels have been associated with a high risk of relapse after hematopoietic stem cell transplantation (SCT) in childhood AML [13]. In children with ALL, high IGFBP2 serum protein levels were associated with chemotherapy resistance, a high relapse rate, and inferior EFS [14].

The prognostic significance of *IGFBP2* in children with acute leukemias prompted us to evaluate *IGFBP2* mRNA expression and its prognostic implications in adult AML. We determined *IGFBP2* mRNA expression in 99 adult patients with newly diagnosed AML in the context of established clinical and molecular risk factors. In addition, we analyzed gene-expression profiles (GEP) in an independent cohort of 61 adult cytogenetically normal (CN)-AML patients to gain insights into the functional role of IGFBP2 in leukemia.

Material and methods

Patients

We analyzed 99 adult patients with newly diagnosed AML admitted to the Charité Berlin (Germany) between 2003 and 2010. Only patients that were eligible for clinical trials using

intensive induction chemotherapy regimens were included; thus, our patient cohort is not fully representative of all AML patients. Bone marrow (BM) samples were selected from consecutive patients that had sufficient material available. The median blast percentage in the BM specimens was 80%, with no significant difference between the *IGFBP2* expression groups. In addition, *IGFBP2* was determined in peripheral blood samples (PB; n=16), BM mesenchymal stromal cells (BMSC; n=9), and serum samples (n=23) in patients of the same cohort.

Patients with AML FAB M3 or M3v were excluded from this study. Patient's median age was 58 years (range 19–80). Cytogenetic risk groups were assigned as defined by the European LeukemiaNet (ELN) classification: favourable: t(8;21)(q22;q22)/*RUNX1-RUNX1T1*; inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB-MYH11*; mutated *NPM1* without *FLT3*-ITD (normal karyotype); mutated *CEBPA* (normal karyotype), intermediate-I: *FLT3*-ITD (normal karyotype) or wildtype *NPM1* without *FLT3*-ITD (normal karyotype), intermediate-II: t(9;11)(p22;q23)/*MLLT3-MLL*; cytogenetic aberrations not classified as favourable or adverse, adverse: inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/*RPN1-EVI1*; t(6;9)(p23;q34)/*DEK-NUP214*; t(v;11)(v;q23)/*MLL* rearranged; −5 or del(5q); −7; abnl(17p); complex karyotype [15].

Patients received standard cytarabine/anthracycline-based intensive induction chemotherapies according to the following multicenter study protocols: AML 2003 Study Alliance Leukemia (SAL) protocol (n=54), AML 2004 East German Study Group Hematology and Oncology (OSHO) #069 (n=27), and AML SORAML SAL (n=18). Consolidation therapies included high dose cytarabine-based therapies and allogeneic SCT. For details please refer to the particular study protocol available in the European Leukemia Trial Registry [16].

Diagnostic analyses were performed on fresh pretreatment BM and PB samples. Morphologic and cytogenetic analyses were done in the institutional laboratories using standard procedures. *FLT3*-ITD mutations [17], *NPM1* mutations [18], and *BAALC* expression [19] were determined as previously described. BMSC were generated as previously reported [20].

Healthy donors were used as controls (BM: n=8; PB: n=6; BMSC: n=9; serum: n=3). Informed consent was obtained from all patients and donors according to the Declaration of Helsinki. The studies were approved by the ethics board of the Universities of Dresden and Leipzig (Germany).

RNA isolation and synthesis of complementary DNA (cDNA)

BM and PB mononuclear cells from diagnostic AML samples and healthy controls were obtained by density-gradient centrifugation (Ficoll-PaquePlus; Amersham Biosciences, Uppsala, Sweden) and total RNA isolation was carried out using Trizol reagent (Invitrogen, Karlsruhe, Germany) following the manufacturer's instructions. Synthesis of cDNA was performed using 500ng of total RNA, AMV-reverse transcriptase, RNase inhibitor and oligo dT20-primer at 50°C for 60 min (Roche, Mannheim, Germany).

Real-time RT-PCR

IGFBP2 expression was determined by multiplex quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) on the Rotorgene RG-3000 real time cycler (Corbett Research, Wasserburg, Germany). The housekeeping gene *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) was coamplified to obtain relative expression levels. Primers for *GAPDH* and *IGFBP2* were as follows: *GAPDH* probe 5′-HEX-ATGCCATCACTGCCACCCAGAAGAC-BHQ1, *GAPDH* forward 5′-GGTATCGTGGAAGGACTCATGAC, *GAPDH* reverse 5′-ATGCCAGTGAGCTTCCCGTTCAG; *IGFBP2* probe 5′FAM-CCTGCCAGGACTCCCTGCCAAC-TAMRA, *IGFBP2* forward 5′-CATCACCTTGGCCTGGAG, *IGFBP2* reverse 5′-GGATGTGCAGGGAGTAGAGG. *IGFBP2* expression was calculated with the mean of the cycle number difference of the two replicates (ΔC_T = *GAPDH-IGFBP2*), expressed as $2^{\mu(\Delta CT)}$. PCR assays were performed in duplicate and each assay included positive and negative controls. RNA from the cell line BE-13 was used to calibrate between runs. If amplification of *GAPDH* did not reach the threshold within 30 cycles, samples were excluded.

ELISA assay

Serum IGFBP2 was measured by ELISA using a microplate precoated with anti-human IGFBP2 antibodies (RayBiotech Inc., Norcross, GA, USA) following the manufacturer's instructions. Recombinant IGFBP2 at final concentrations from 0.16–10 ng/ml was used for calibration. Extinction was measured at 450 nm using a multiplate ELISA reader (Dynatech International, Chantilly, VA, USA).

Oligonucleotide microarray analyses

An independent cohort of 61 BM samples from adult patients aged less than 60 years with newly diagnosed, untreated CN-AML confirmed by central morphologic and karyotype reviews enrolled on the Cancer and Leukemia Group B (CALGB) treatment protocol 9621 [21] was used to generate a microarray-based *IGFBP2*-associated GEP. RNA samples were analyzed using Affymetrix U133 plus 2.0 GeneChips (Affymetrix, Santa Clara, CA, USA) as previously reported [21].

Data were pre-processed using GCOS software (Affymetrix, Santa Clara, CA, USA) and further analyzed with GeneSpring software version 4.2 (Silicon Genetics, Redwood City, CA, USA). Probe sets were included in the comparisons if they were called present by the Affymetrix data analyses in at least 75% of samples. *IGFBP2* expression was represented by the probe set 202718_at and samples were divided into quartiles (q1–q4) according to *IGFBP2* expression levels. The low *IGFBP2* GEP group included samples of the lowest quartile q1 and the high *IGFBP2* GEP group samples of q4 to assure a specific gene-expression signature that sufficiently discriminated between low and high *IGFBP2* expressers. Probe sets were defined as differentially expressed if they exhibited at least a 2-fold change in expression. Statistical significance was calculated by the nonparametric Mann-Whitney U test (P value ≤ 0.001).

Statistical analyses

IGFBP2 mRNA expression levels of the 99 BM samples were grouped into quartiles (Q1–4) and divided into low *IGFBP2* [Q1–3: (n=74)] and high *IGFBP2* [Q4: (n=25)]. Q4 was chosen as cut point as the difference in CR rates was most relevant between Q1–3 and Q4 (P=0.01), compared to Q1 vs. Q2–4 (P=0.48), and Q1–2 vs. Q3–4 (P=0.32). Thus, after correction for searching 3 different cutpoint comparisons, the difference between Q1–3 and Q4 was still of statistical significance. *IGFBP2* expression ranged between 0–1.26 with the following median expression levels for each quartile: 0.003 (Q1), 0.04 (Q2), 0.11 (Q3), 0.33 (Q4). Q1, Q2, Q3, and Q4 are capitalized to distinguish the data from microarray based expression (q1–q4).

Complete remission (CR) was defined by a granulocyte count of at least 1.5/nl, a platelet count of at least 100/nl, the absence of PB blasts, a BM cellularity of at least 20% with maturation of all cell lines and less than 5% blasts, and the absence of extramedullary leukemia. Primary resistant disease was defined as persistence of PB blasts or more than 5% blasts in BM after completion of induction therapy. Relapse was defined as reappearance of PB blasts, more than 5% blasts in BM, or reappearance/development of extramedullary leukemia after achievement of CR. Overall survival (OS) was measured from the beginning of therapy until date of death or last follow-up. Relapse-free survival (RFS) was determined from the date of first CR until relapse. Patients without reported relapse were censored on the date of last follow-up. Survival curves were calculated by the Kaplan-Meier-method with the log-rank comparing differences between survival curves. The median follow-up for all living patients was 33.2 months (range 1.2–74). Patients who received SCT were censored at the time of transplantation for survival analyses.

Clinical features across groups were compared using the χ^2 or a two-sided Fisher's exact test for categorical data and the nonparametric Mann-Whitney U test for continuous variables. A P value ≤ 0.05 (two-sided) was considered significant. Multivariate analyses were performed using logistic regression with stepwise forward selection including the following variables in the full model: *IGFBP2* expression [high (Q4) vs. low (Q1–3)], leukocyte count (1/nl increase), age (10 year increase), LDH (> 350 U/l vs. ≤ 350 U/l), type of AML (de novo AML vs. secondary (s) AML), cytogenetic risk groups (favourable, intermediate-I, intermediate-II, adverse). Secondary AML was defined as treatment-related AML or AML following myelodysplastic syndrome (MDS). SPSS software package (version 18.0 for Windows; SPSS Inc., Chicago, IL, USA) was used for calculations.

Results

Aberrant expression of *IGFBP2* in AML

IGFBP2 mRNA expression was analyzed in pretreatment BM samples of 99 adult patients with newly diagnosed AML. In addition, *IGFBP2* expression was determined in 16 PB samples and 9 BMSC samples, and IGFBP2 protein levels were analyzed in 23 serum samples of patients in this same cohort.

AML patients showed significantly higher *IGFBP2* mRNA expression in BM (median: 0.07 vs. 0.04; P=0.037) and PB (median: 0.0002 vs. <0.00001; P=0.04) compared to healthy

controls (Figure 1A). Moreover, IGFBP2 serum protein levels were significantly higher in AML patients compared to controls (median: 608 ng/ml vs. 180 ng/ml; *P*=0.002; Figure 1B). BM *IGFBP2* mRNA expression was positively correlated with IGFBP2 serum protein levels (r=0.6; *P*=0.007). In contrast, BM and PB *IGFBP2* mRNA expression levels were not significantly correlated (r=0.5; *P*=0.08), probably due to a high variation of the peripheral blast counts. BMSC showed about a 6-fold higher expression of *IGFBP2* compared to hematopoietic cells, with no significant difference between BMSC derived from AML patients and healthy donors (median: 0.39 vs. 0.43; *P*=0.86; Figure 1A). Thus, elevated IGFBP2 serum protein levels found in AML patients might result from the high expression of *IGFBP2* in leukemic blasts. Of note, the comparison of AML patients and healthy controls is potentially biased by differences in age (patients: mean 56 years; controls: mean 40 years) and different cell compositions in the BM samples (presumably more lymphocytes, monocytes, plasma cells, and differentiated myeloid cells in the control samples).

IGFBP2 expression with respect to clinical and molecular characteristics

Patients were divided into *IGFBP2* high (Q4) and *IGFBP2* low (Q1–3) expression groups according to *IGFBP2* BM mRNA expression levels as described in the statistical section. Low *IGFBP2* expression was associated with FAB M4/M5 subtypes (*P*=0.008; Table 1). No other significant correlations with clinical or genetic characteristics were found between the *IGFBP2* expression groups (Table 1). Furthermore, there was no significant difference regarding therapy regimens applied in high and low *IGFBP2* expressers (Table 1).

IGFBP2 expression and outcome in AML patients

High *IGFBP2* expression was significantly associated with a higher incidence of primary resistant disease (*IGFBP2* high 65%, *IGFBP2* low 32%; *P*=0.02) and with a lower CR rate (*IGFBP2* high 28%, *IGFBP2* low 59%; *P*=0.01; Table 2). In multivariate analyses, high *IGFBP2* expression was independently predictive for resistant disease [OR 3.6 (95% CI 1.2–11); *P*=0.02] and achievement of CR [OR 0.3 (95% CI 0.09–0.8); *P*=0.01; Table 3]. The only other significant variable was presence of sAML. There were no significant differences regarding deaths in induction therapy (*P*=0.52; Table 2), deaths in CR (*P*=1.0), relapse rate (*P*=0.66), RFS (*P*=0.67; data not shown), or OS (*P*=0.53; Table 2) between the *IGFBP2* expression groups. No significant differences were observed in subgroup analyses stratifying for age and treatment protocols. Similar results regarding OS and RFS were obtained when patients who received allogeneic SCT were not censored at the time of transplantation (data not shown).

IGFBP2-associated GEP in CN-AML

To gain insights into the underlying biological pathways of *IGFBP2* and its potential role in drug resistance, a microarray-based GEP analysis using an independent cohort of 61 patients with CN-AML treated on the CALGB protocol 9621 [21] was performed. Samples were grouped into high and low *IGFBP2* (q4 vs. q1) according to the microarray expression data as described in the statistical section. The median *IGFBP2* probe set expression was 60-fold higher in q4 as compared to q1. Between the two *IGFBP2* expression groups, 576

differentially expressed probe sets corresponding to 415 genes and hypothetical proteins were identified (Figure 2). Among these, 181 were up-regulated and 234 were down-regulated (Table S1).

Up-regulated genes in patients with high *IGFBP2* expression included genes that were previously shown to be up-regulated in high-risk leukemic subgroups including *FLT3*-ITD AML (*MEIS1*, *HOXB3*, *WT1*) [22], *MLL*-rearranged leukemias (*HOXA9*, *MYB*, *MEIS1*) [23–25], and *BCR-ABL* ALL (*CTNND1*) [26]. Moreover, the transcription factor *ERG*, an adverse prognostic factor in acute leukemias [21, 27], was found to be up-regulated in the high *IGFBP2* group. Differentially down-regulated genes in patients with high *IGFBP2* expression included putative tumor suppressor genes (*CEBPD*, *G0S2*, *KLF4*, *KLF11*) [28–31], and the growth regulation and DNA repair gene *GADD45B*. Among the down-regulated genes we further identified *RGS2* that has been associated with a favourable outcome in AML [32]. This *IGFBP2*-derived GEP signature indicates a more aggressive, resistant leukemic phenotype associated with high *IGFBP2* expression.

Discussion

The identification of prognostic factors and aberrant signaling pathways in AML is important for the development of new molecular therapies and might improve risk-adapted therapeutic strategies for AML patients. Here, we have demonstrated aberrant expression of *IGFBP2* in adult AML and identified overexpression of *IGFBP2* as a novel marker for chemoresistance in these patients. High *IGFBP2* expression independently predicted primary therapy resistance and achievement of CR in adult AML patients. The risk for primary resistant disease was about 4-fold higher in patients with high *IGFBP2* expression compared to low *IGFBP2* expressers.

High *IGFBP2* expression has already been associated with inferior EFS and a higher relapse rate in children with AML in small studies with less than 40 patients [10, 12]. In adult AML, high levels of IGFBP2 serum protein have been related to an inferior response to induction therapy in a study of 22 patients [33]. Here, we have for the first time evaluated the prognostic significance of *IGFBP2* mRNA expression in adult AML. Our results strengthen the association of *IGFBP2* expression on chemotherapy resistance in acute leukemias.

The role of *IGFBP2* in cancer cells is still poorly understood. *In vitro* studies have demonstrated both inhibitory and stimulatory effects of IGFBP2 protein on cell growth in different tumor cells [8]. The impact of IGFBP2 on leukemic cells has not been evaluated yet. Elmlinger *et al*. found that secretion of IGFBP2 protein is induced by autocrine expression of IGF-II in leukemic T-cells [34]. It has been postulated that IGFBP2 may provide a storage pool of IGFs in the BM microenvironment resulting in a growth advantage of leukemic cells [9]. Thus, elevated *IGFBP2* expression in leukemic blasts could either be an epiphenomenon of an activated IGF pathway or might be directly involved in a pathogenetic role of aberrant IGF signaling in acute leukemias.

To elucidate the biological function of *IGFBP2* in AML, we analyzed an *IGFBP2*-associated GEP in an independent cohort of CN-AML. Patients with high *IGFBP2*

expression revealed an up-regulation of *MYB*, *MEIS1*, *HOXB3*, and *HOXA9*, genes that are known to collaborate in leukemic transformation [24, 35, 36], while putative tumor suppressor genes (*CEBPD*, *G0S2*, *KLF4*, *KLF11*) [28–31] were down-regulated. Interestingly, one of the markedly up-regulated genes in the high *IGFBP2* group was *WT1*, a gene considered as a downstream target of IGF-I [37]. Overexpression and mutations of the *WT1* gene have been associated with adverse outcome in AML and ALL patients [38–40]. *WT1* was shown to be highly expressed in chemoresistant leukemic stem cells [41] and *WT1* mutations have been associated with resistant disease in CN-AML [42]. Surprisingly, we did not find up-regulation of other IGF target genes on the mRNA expression level; this however does not exclude that activation of downstream targets occurred on the protein level not captures by GEP.

High *IGFBP2* expression was also associated with up-regulation of *ERG*, an independent adverse prognostic factor both in CN-AML [21] and T-ALL [27]. The two Krüppel-like transcription factor family members *KLF4* and *KLF11* were among the down-regulated genes in the high *IGFBP2* group. *KLF4* has been reported to induce cell-cycle arrest and apoptosis in leukemic cells [30] and was found hypermethylated in adult T-cell leukemia [43]. *KLF11*, also a negative regulator of cell growth [31], was shown to be frequently epigenetically inactivated in patients with MDS [44]. Among the down-regulated genes we further found *RGS2*, previously reported to be down-regulated in B-precursor ALL patients with high *BAALC* expression and a high incidence of resistant disease [45].

Hence, the *IGFBP2*-derived GEP signature marks a chemoresistant and proliferative subgroup of CN-AML patients less than 60 years of age. This data supports the clinical finding of a resistant leukemic phenotype associated with high *IGFBP2* expression in our more heterogeneous AML patient cohort that differs in cytogenetic features and age. Our results are further highlighted by the overlap of the *IGFBP2*-associated GEP with expression profiles derived from other high-risk subgroups like *FLT3*-ITD, *MLL*-rearrangement, *BCR-ABL*, or leukemias with high *BAALC* expression. The differentially expressed candidate genes found in patients with high *IGFBP2* expression might provide a basis for further investigating the functional role of *IGFBP2* in acute leukemias.

These high-risk patients characterized by high *IGFBP2* expression should be considered for new molecular drugs, particularly drugs targeting the IGF pathway [6], to overcome primary therapy resistance. In recent years, different prognostic markers that could potentially direct new therapeutic strategies have been identified in AML, like *OGG1* [46], *CXCR4* [47], *ANG-2* [48], or heat shock proteins [49]. With the increasing number of molecular markers, prospective studies are needed to investigate the independent prognostic significance of each new marker in relation to other molecular markers and known risk factors and to evaluate the predictive value of these markers for specific therapies.

In conclusion, this is the first study evaluating *IGFBP2* expression in adult AML with respect to clinical features, therapy response, and gene-expression profiling. Our data support a role of IGFBP2 and IGF signaling in chemoresistance of AML. Determination of *IGFBP2* expression might help to allocate AML patients with an unfavourable chemotherapy response to alternative first line treatment protocols. The prognostic

significance of *IGFBP2* in adult AML will have to be further evaluated in larger prospective trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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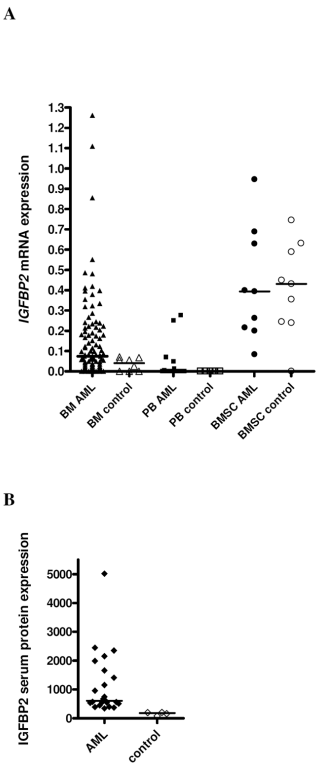

Figure 1. *IGFBP2* expression in AML patients and healthy controls
(A) mRNA expression ($2^{\mu(\Delta CT)}$). (B) Serum protein levels (ng/ml).

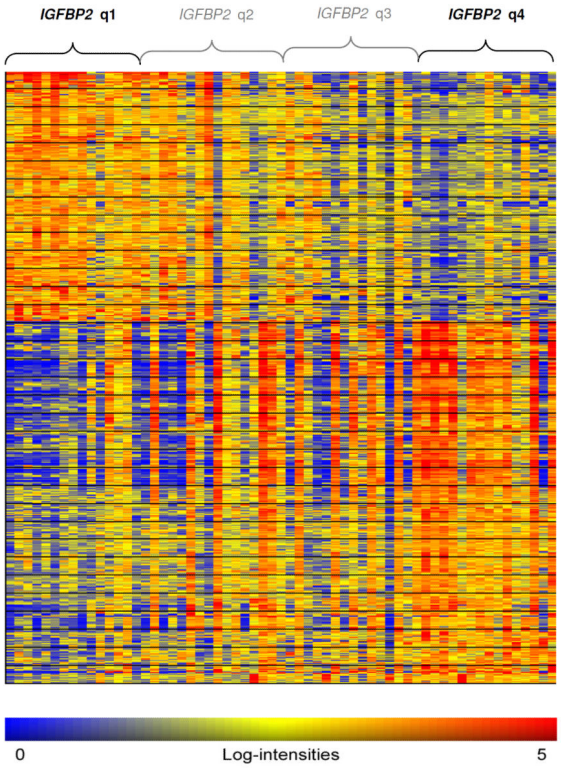

Figure 2. Heat map of differentially expressed probe sets between high and low*IGFBP2* expression groups

Columns represent samples ordered by *IGFBP2* expression and rows represent genes ordered by hierarchical cluster analysis. Colors indicate relative expression of each gene with respect to the median expression level (red above, yellow equal, blue below the median value).

Table 1

Clinical and molecular characteristics of AML patients according to *IGFBP2* expression groups

Characteristic	***IGFBP2* low n = 74**	***IGFBP2* high n = 25**	***P***
Age [years]			0.82
Median	58	57	
Range	23–80	19–79	
Sex [%]			0.35
Male	56	68	
Leukocyte count [× 10^9/L]			0.22
Median	7	31	
Range	0.5–368	0.5–385	
LDH >350 U/l [%]	57	60	0.82
sAML [%]	32	20	0.31
BAALC expression, high [%]†	49	53	1.0
FAB Classification [no. (%)], n=89	66	23	0.04
M0	5 (8)	0 (0)	
M1	7 (11)	5 (22)	
M2	8 (12)	7 (30)	
M4eo	4 (6)	2 (9)	
M4	19 (29)	4 (17)	
M5	20 (30)	1 (4)	
M6	3 (5)	3 (13)	
M7	0 (0)	1 (4)	
FAB M4/M5	39 (59)	5 (22)	0.008
Genetic risk groups‡ [no. (%)], n=91	68	23	0.90
Favourable	8 (12)	4 (17)	0.49
Intermediate-I	29 (43)	10 (44)	1.0
Intermediate-II	11 (16)	3 (13)	1.0
Adverse	20 (29)	6 (26)	1.0
Therapy [no. (%)]			
AML 2003 SAL	40 (54)	14 (56)	1.0
AML SORAML SAL	13 (18)	5 (20)	0.77
AML 2004 OSHO #069	21 (28)	6 (24)	0.80
Allogeneic SCT	30 (41)	10 (40)	1.0
In CR1	13 (43)	1 (10)	0.07

FAB: French-American-British, SCT: stem cell transplantation, CR: complete remission.

† According to median expression level; n=62.

‡ According to ELN classification.

Table 2

Clinical outcome according to *IGFBP2* mRNA expression

Clinical Outcome	*IGFBP2* low	*IGFBP2* high	*P*
Resistant disease[†]			
Resistant/total [no. (%)]	20/63 (32)	13/20 (65)	0.02
Complete remission rate[‡]			
CR/total [no. (%)]	43/73 (59)	7/25 (28)	0.01
Death in induction therapy[‡]			
Death/total [no. (%)]	10/73 (14)	5/25 (20)	0.52
Relapse rate[$]			
Relapse/total [no. (%)]	12/25 (48)	2/6 (33)	0.66
Median remission duration[¶]			
[years (95% CI)]	2.8 (1.9–3.8)	3.0 (1.4–4.7)	0.67
Overall survival[¶¶]			0.53
Alive at 2 years [% (95% CI)]	57 (39–75)	48 (18–78)	
Median survival [years (95% CI)]	3.3 (2.5–4.2)	2.8 (1.6–4.1)	

CR: complete remission, CI: confidence interval.

[†]N=83. One patient who did not complete induction therapy and 15 patients who died during induction therapy were excluded.

[‡]N=98. One patient who did not complete induction therapy was excluded.

[$]N=31. Patients who received SCT in first CR were excluded.

[¶]N=49. Patients who received SCT in first CR were censored at the time of transplantation.

[¶¶]N=98. One patient with development of second neoplasia was excluded. Patients who received SCT in first CR were censored at the time of transplantation.

Table 3

Multivariate analysis of *IGFBP2* mRNA expression for response to induction therapy

Variable[†]	OR	95% CI	*P*
Resistant disease			
IGFBP2 high vs. low	3.6	1.2–11	0.02
sAML	3.1	1.1–8.8	0.04
CR			
IGFBP2 high vs. low	0.3	0.09–0.8	0.01
sAML	0.3	0.09–0.7	0.01

OR: odds ratio, CI: confidence interval, CR: complete remission.

[†]Variables considered for model inclusion are described in the statistical section. The final model is shown.