SPANDIDOS PUBLICATIONS

Low SMC1A protein expression predicts poor survival in acute myeloid leukemia

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Abstract. Age is a strong adverse prognostic factor in acute myeloid leukemia. Little is known about the biology of acute myeloid leukemia in elderly patients. The aim of this study was to identify genes with age-dependent changes of expression in leukemic blasts and their relevance for the patient prognosis. Gene expression profiling was carried out by mRNA microarray analysis from blasts of 67 adult acute myeloid leukemia patients of different age (range, 17-80 years). Among the genes that correlated with age, PRPF4 and SMC1A were selected for protein expression studies on a tissue array containing bone marrow histologies of 135 patients with newly diagnosed AML of different ages. A significant correlation between mRNA expression levels and patient age was shown by 131 genes. Increasing age was associated with significantly decreased mRNA levels of SMC1A. On the protein level, expression of SMC1A was low or absent in 74 out of 116 acute myeloid leukemia specimens. Importantly, patients with low protein expression levels of SMC1A experienced significantly shortened event free (2.6 months versus 10.3 months, p=0.003) and overall survival (10.4 months versus 22.6 months, p=0.015). The SMC1A protein expression level remained a significant prognostic factor for event free survival (p=0.014) with a borderline significance for overall survival (p=0.066) in a multivariate analysis. SMC1A protein expression might play a role in the determination of the prognosis and might have possible implications in therapy decision in patients with acute myeloid leukemia.

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Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous disease. Several prognostic factors have been identified such as age, sex, lactate dehydrogenase serum levels, recurrent structural (i.e. balanced translocations, inversions, deletions, insertions) or numerical (monosomy, trisomy) cytogenetic abnormalities (1), genetic mutations (i.e. mutational status of FLT3, NPM1, C/EBP α, N-Ras, MLL, WT1) (2,3), or changes in mRNA (i.e. ERG, BAALC, MN1, EVI1) (4-7) or protein (P-glycoprotein, lung-resistance protein) (8,9) expression levels.

However, the identification of prognostic subgroups is a theme of growing complexity, for example a poor prognosis subgroup defined by c-kit mutations (10) or a loss of chromosome Y (11) can be demonstrated in the 'good' prognosis group of core binding factor leukemias in younger patients <60 years of age. These data argue for more detailed studies of risk factors in elderly patients with AML. However, most risk factors so far have been defined in patients aged <60 years.

Despite advances in the therapy of AML over the last decades, the prognosis of AML in patients >60 years of age is still dismal (12). Adverse prognostic factors such as an adverse cytogenetic profile (12) or the expression of P-glycoprotein occur more frequently in AML blasts of elderly patients (13,14). Recently, we demonstrated age as the most important independent risk factor for the prognosis among 2734 patients with AML in the multicenter German AML Co-operative Group (AMLCG) study AMLCG1999, and this remained highly significant as an independent risk factor in a multivariate analysis (15,16). Many studies have demonstrated a decrease of good-risk cytogenetics with increasing age, suggesting that AML in elderly patients differs biologically from AML in younger patients (17).

However, the relevant biological mechanisms of the poor prognosis of AML in older patients are unknown.

Therefore, the aim of this study was to detect age-dependent changes in AML by a genome-wide gene expression profile. We were able to identify a variety of genes with age-dependent changes in mRNA expression, and in addition we identified a new prognostic protein marker, SMC1A, in acute myeloid leukemia.

Materials and methods

Patient samples. For mRNA expression analysis, EDTA-anticoagulated bone marrow aspirates were obtained at diagnosis from 67 adult AML patients of different age (range, 17-80 years). For tissue microarray preparation, bone marrow trephines were obtained from 135 patients with newly diagnosed AML. Bone marrow trephines were formalin-fixed and paraffin-embedded according to standard procedures. Written informed consent was obtained from all patients and the study was approved by the local Institutional Review Board (Ethikkomission der WWU Münster) and only patients treated at the University Hospital of Muenster were included in this study. The studies were in compliance with all applicable national and local ethics guidelines.

RNA isolation. AML blasts were isolated from EDTA-anticoagulated bone marrow aspirates at diagnosis by Ficoll-hypaque density centrifugation according to standard procedures. Total RNA was isolated using TRIzol®-Reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol.

mRNA expression arrays. Microarray expression analysis was performed using the ABI 1700 microarray system accoding to the manufacturer's protocol (18). Two micrograms of total RNA were reverse-transcribed and second-strand synthesis was performed. Digoxigenin (DIG)-UTP was included in the IVT-reaction to produce DIG-labeled cRNA that after fragmentation was hybridized to the Genome Survey Expression Arrays covering 31,700 oligonucleotide probes representing 29,098 individual genes (ABI). Detection was performed by a chemoluminescent reaction with high sensitivity. Initial analyses were performed with the 1700 chemiluminescent microarray analyser (ABI).

Preparation of tissue microarrays. Formalin-fixed and paraffin-embedded tissue of 135 patients with newly diagnosed AML were used for the production of a tissue microarray as previously described (19). A diagnostic Giemsa-stained section served as control to enable the definition of areas with the highest amount of blast cells. Two punches were arrayed per sample. The spot diameter was 0.6 mm, and the distance between the spots was 1 mm.

Array immunohistochemistry and quantification. Tissue sections (4-μm) were mounted on SuperFrost/Plus slides and dewaxed in xylene. The sections were autoclaved in 10 mM citrate buffer pH 6.0 (10 min, 120˚C). After washing in PBS, sections were incubated with the primary antibodies (PRPF4, Santa Cruz Biotechnology, T-24, dilution 1:100; SMC1A, Atlas Antibodies, dilution 1:50). Detection was performed by the use of a 3,3'-diaminobenzidine detection kit (ER Ventana), and with the APAAP using a monoclonal APAAP complex (1:100 in RPM-1640, 60 min in room temperature; Dako). Quantification of immunohistochemical staining in AML blasts by a pathologist was performed according to conventional standards (Dako score).

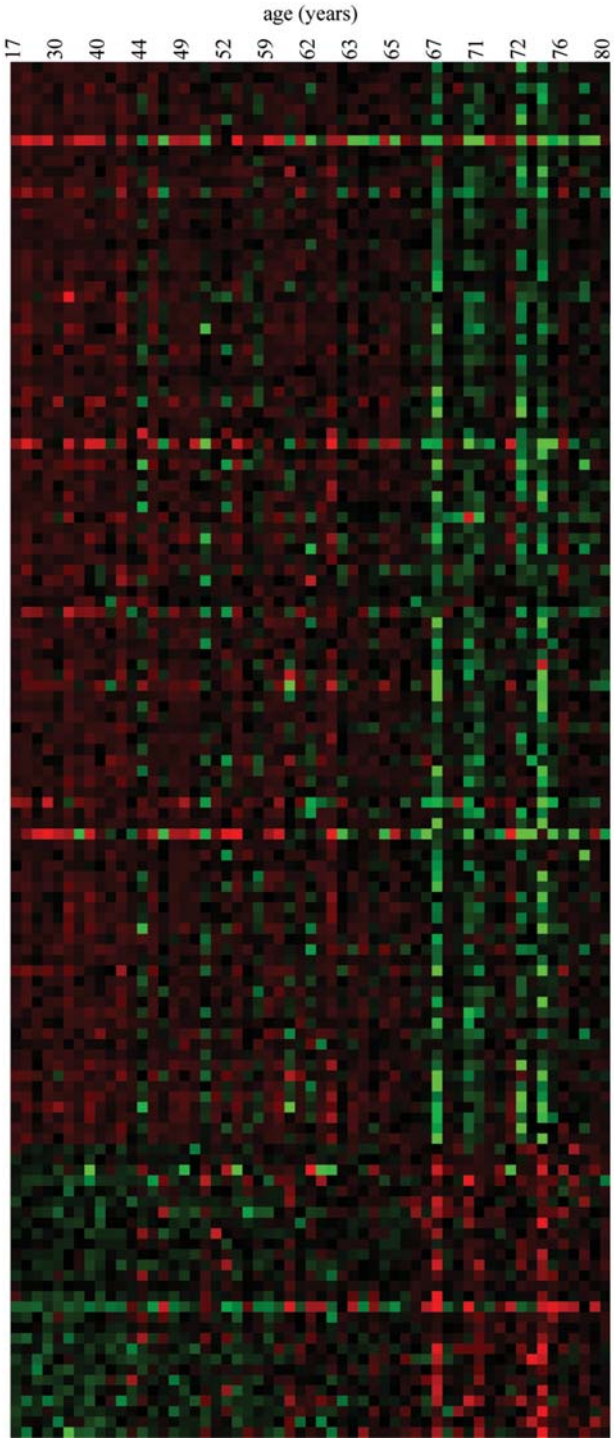

Figure 1. Heatmap of mRNA expression levels with regard to patient age. Genes correlating with age were identified and analyzed in a hierarchical cluster analysis. Patients are grouped on the heatmap according to age. Green, expression level lower than the median for this gene; red, expression level higher than the median for this gene. The gene names can be identified in Table I, where the genes are displayed the same order as in the Figure.

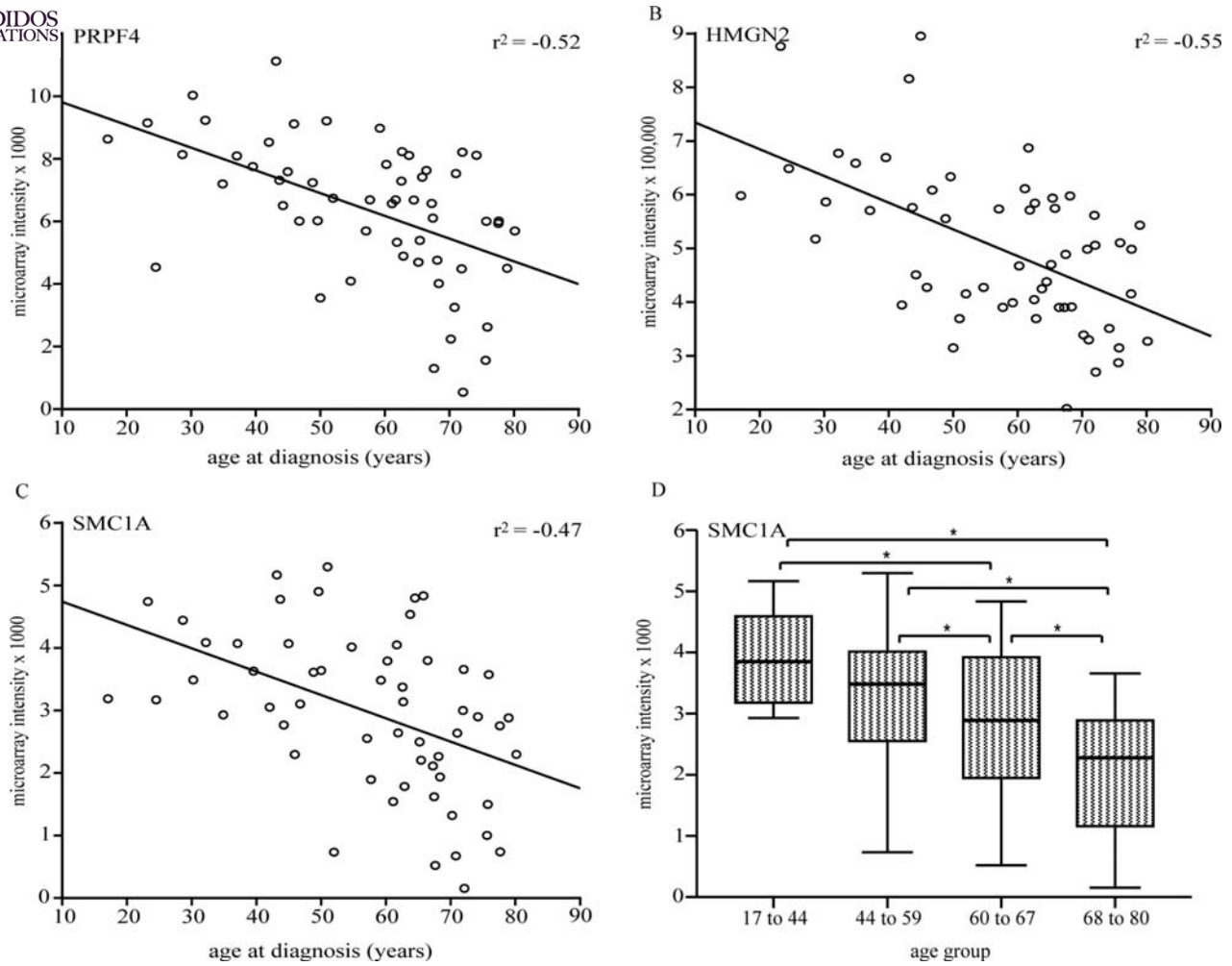

Figure 2. Age-dependent mRNA expression changes in AML for three representative genes. (A) PRPF4, (B) HMGN2, (C and D) SMC1A. *p<0.05.

Statistical analysis. Microarray data were analyzed by BRB Array tools. A correlation analysis was performed between expression and age at a significance level of p<0.001. Statistical analysis of protein expression was performed using the Statistical Package for the social Sciences (SPSS), version SPSS V17.0 for Windows. Survival analysis was performed by Kaplan-Meier analysis with a log-rank test for significance and a significance level of α=0.05. Statistical analysis of clinical parameters were performed with either Wilcoxon-test or χ^2-test for homogeneity where applicable, both with a significance level of α=0.05. Multivariate analysis was performed by multivariate Cox regression with a significance level of α=0.05.

Results

Identification of age-dependent gene expression by microarray expression analysis. To start to unravel the molecular basis for the prognostic effect of age in AML we performed microarray expression analyses in AML patients. In the mRNA microarray analysis, 131 genes showed a significant correlation between the expression level and the patient age at the time of diagnosis; 103 displayed a negative and only 28 a positive correlation (Fig. 1; Table I). Three examples of age dependent genes are shown (Fig. 2). HMGN2 (high-mobility group nucleosomal binding domain 2), PRPF4 (pre-mRNA processing factor 4 homolog) and SMC1A (structural maintenance of chromosomes 1A) microarray expression results negatively correlated with the patient age at diagnosis.

PRPF4 and SMC1A protein expression analysis and correlation with mRNA expression. Association of mRNA level changes with clinical parameters might be an indication of regulatory phenomenon rather than a direct evidence for biologically relevant genes. We therefore used an AML tissue microarray to identify whether the altered mRNA expression levels were associated with patient survival based on protein expression data. PRPF4 and SMC1A were further analyzed by immunohistochemistry due to its known function during mitosis and due to antibody availability. Assessment of the PRPF4 and the SMC1A protein expression was sufficient in 115 and 116 out of 135 patient samples, respectively. For the remaining 20 and 19 samples, an insufficient number of AML blasts were spotted on the tissue microarray. One representative picture of a negative (expression level 0) and a positive (expression level 3) expression of SMC1A is presented (Fig. 3).

Protein levels of either PRPF4 or SMC1A protein detected by immunohistochemistry on tissue microarrays did not

Table I. Age-dependent changes in mRNA expression levels.

Unique id	Correlation coefficient	Description	Clone	GeneBank accession no.	Gene symbol
109690	-0.553	PRP4 pre-mRNA processing factor 4 homolog (yeast)	86685231	NM_004697	PRPF4
146792	-0.542	High-mobility group nucleosomal binding domain 2		NM_005517	HMGN2
110983	-0.531	Chromosome 19 open reading frame 28	3474869	NM_174983	C19orf28
197989	-0.523	Mitochondrial ribosomal protein L18	160857025	NM_014161	MRPL18
101755	-0.52		107999084		
165131	-0.513	SMC1 structural maintenance of chromosomes 1-like 1 (yeast)	57232625	NM_006306	SMC1L1
236541	-0.511			AL391416	
126222	-0.51	Hypothetical protein FLJ32942	54501444	AK057504	FLJ32942
212663	-0.508		111502434		
173693	-0.506	HSPC244	58495163	NM_016499	MGC:13379
197251	-0.505	Solute carrier family 1 (neutral amino acid transporter), member 5	44082394	NM_005628	SLC1A5
109395	-0.505		57264512		
169499	-0.503	Anillin, actin binding protein (scraps homolog, *Drosophila*)	36413980	NM_018685	ANLN
128737	-0.503				
135002	-0.502	High-mobility group nucleosomal 2 binding domain	5599960	NM_005517	HMGN2
183246	-0.501	Heterogeneous nuclear ribonucleoprotein C (C1/C2)	1538809	NM_031314	HNRPC
139758	-0.5		52964773		
185984	-0.498	KIAA0261	82193861	NM_015045	KIAA0261
140552	-0.498		45407426		
106136	-0.497		3569763		
143238	-0.491	CSE1 chromosome segregation 1-like (yeast)	44367400	NM_001316	CSE1L
153495	-0.49		44104207		
134014	-0.488	Sialyltransferase 6 (N-acetyllacosaminide α 2,3-sialyltransferase)	42455024	NM_174972	SIAT6
188782	-0.485		79209516		
130790	-0.485		13757670		
143413	-0.485	DUF729 domain containing 1	137292608	NM_138419	DUFD1
125707	-0.48		79836088		
101962	-0.474		134948938		
125645	-0.473		80451333		
155269	-0.471	ATP synthase, H^+ transporting, mitochondrial F1 complex, ß polypeptide	56683667	NM_001686	ATP5B
140313	-0.47	High-mobility group nucleosomal 2 binding domain	7664137	NM_005517	HMGN2
100130	-0.469		25910808	Z83840	
191627	-0.469	Nuclear receptor coactivator 4	42865708	NM_005437	NCOA4
208076	-0.465	Transportin 3	123390730	NM_012470	TNPO3
191104	-0.465	High-mobility group nucleosomal binding domain 2	25195509	NM_005517	HMGN2
211546	-0.464	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), ß polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)	76400747	NM_000918	P4HB

 Continued.

Unique id	Correlation coefficient	Description	Clone	GeneBank accession no.	Gene symbol
166551	-0.464		1248647	X55989	
100220	-0.463				
199549	-0.462	CDC91 cell division cycle 91-like 1 (*S. cerevisiae*)	29897423	NM_080476	CDC91L1
234027	-0.462		13859989		
138129	-0.461	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	95858416	NM_001084	PLOD3
137614	-0.461	Hypothetical protein MGC3234	935474	NM_023947	MGC3234
141237	-0.461	Protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), ß isoform	108749256	NM_181699	PPP2R1B
103896	-0.461	Zinc finger protein 239	40055428	NM_005674	ZNF239
131916	-0.458		70596609		
107045	-0.457	Chromosome 10 open reading frame 69	95647794	NM_006459	C10orf69
179303	-0.456	Polymerase (DNA directed), α	28834934	NM_016937	POLA
235868	-0.456	Glyceraldehyde-3-phosphate dehydrogenase	8260480	NM_002046	GAPD
197341	-0.453	Polo-like kinase 1 (*Drosophila*)	22468349	NM_005030	PLK1
198244	-0.453	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 2	141828520	NM_013432	NFKBIL2
104988	-0.453	COP9 constitutive photomorphogenic homolog subunit 3 (Arabidopsis)	18089553	NM_003653	COPS3
212751	-0.452		26090623		
162809	-0.451	Ribokinase	27849146	NM_022128	RBKS
186772	-0.451	HSPC154 protein	68680276	NM_014177	HSPC154
202943	-0.451		151204786		
169279	-0.451	AT rich interactive domain 3A (BRIGHT-like)	861394	NM_005224	ARID3A
217900	-0.449	Tumor rejection antigen (gp96) 1		NM_003299	TRA1
129518	-0.449	Putative endoplasmic reticulum multispan transmembrane protein	53092105	NM_052859	RFT1
157917	-0.447	Serine/threonine kinase 6	51683782	NM_003600	STK6
208898	-0.447		1864863	NM_014867	
129633	-0.446			AK023204	
144711	-0.445	Mitochondrial ribosomal protein L27	44906512	NM_016504	MRPL27
105423	-0.445	H2A histone family, member Z	98166661	NM_002106	H2AFZ
176188	-0.444	Golgi autoantigen, golgin subfamily a, 2	101669854	NM_004486	GOLGA2
236939	-0.442		81080616		
111979	-0.441	DKFZP434I116 protein	91686315	NM_015496	DKFZP434I116
163631	-0.441		73658572		
174829	-0.441	Phosphatidylinositol-4-phosphate 5-kinase, type I, α	124286312	NM_003557	PIP5K1A
151175	-0.441		45121345		
150309	-0.441	Chromosome 10 open reading frame 7	12167391	NM_006023	C10orf7
104729	-0.44	Similar to common salivary protein 1	3080178	NM_145252	LOC124220
123864	-0.44	Hypothetical protein H17	123279476	NM_017547	H17
160632	-0.44	Signal recognition particle 68 kDa	70625229	NM_014230	SRP68
134187	-0.438	Membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)	57188481	NM_006138	MS4A3
211285	-0.438		65806540		
174651	-0.438	Karyopherin α 1 (importin α 5)	120553552	NM_002264	KPNA1
223004	-0.438	Zinc finger protein 561	9615731	NM_152289	ZNF561
209604	-0.438		178067648		

Table I. Continued.

Unique id	Correlation coefficient	Description	Clone	GeneBank accession no.	Gene symbol
200565	-0.437	F-box protein 22	53122127	NM_012170	FBXO22
110781	-0.436	ATPase type 13A	19660081	NM_020410	ATP13A
178526	-0.436	Thioredoxin domain containing 7 (protein disulfide isomerase)	10838664	NM_005742	TXNDC7
157593	-0.436	Lectin, mannose-binding 2	171722402	NM_006816	LMAN2
118987	-0.436	NADH dehydrogenase (ubiquinone) 1 α subcomplex, assembly factor 1	18447520	NM_016013	NDUFAF1
197221	-0.436	Methylene tetrahydrofolate dehydrogenase (NAD^+ dependent), methenyltetrahydrofolate cyclohydrolase	74256918	NM_006636	MTHFD2
116667	-0.435	Calpain small subunit 2	40114805	NM_032330	CAPNS2
110562	-0.435	Processing of precursor 4, ribonuclease P/MRP subunit (*S. cerevisiae*)	26795014	NM_006627	POP4
115589	-0.434	MCM4 minichromosome maintenance deficient 4 (*S. cerevisiae*)	44858253	NM_005914	MCM4
192701	-0.434	Ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	30476899	NM_020629	RET
148733	-0.434			BC020307	
135285	-0.433	Integral membrane protein 1; CHK1 checkpoint homolog (S. pombe)	122623025	NM_001274	ITM1; CHEK1
186340	-0.433	DKFZP566O084 protein	21447444	NM_015510	DKFZp566O084
210481	-0.433	Golgi associated, γ adaptin ear containing, ARF binding protein 2	22252488	NM_138640	GGA2
229412	-0.432		40788561		
199189	-0.432		136896507		
236093	-0.431	Eukaryotic translation initiation factor 4A, isoform 1	7502345	NM_001416	EIF4A1
175324	-0.43	Glyceraldehyde-3-phosphate dehydrogenase	8260480	NM_002046	GAPD
160577	-0.43	Kinesin family member 23	46595415	NM_138555	KIF23
212857	-0.43	Bifunctional apoptosis regulator	14903442	NM_016561	BFAR
213392	-0.429		118332014		
186923	-0.429	3'-phosphoadenosine 5'-phosphosulfate synthase 1	105829640	NM_005443	PAPSS1
184872	-0.429	Dystonia 1, torsion (autosomal dominant; torsin A)	103225965	NM_000113	DYT1
105868	-0.428	Translocase of outer mitochondrial membrane 34	40279403	NM_006809	TOMM34
143257	-0.428	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase	19301250	NM_005216	DDOST
111860	0.431	Solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 2	136759020	NM_031947	SLC25A2
212706	0.433		38909003	BC039000	
133654	0.434	Tumor protein p73	2823529	NM_005427	TP73
108888	0.435	Kinesin family member 21A	38491505	NM_017641	KIF21A
148924	0.436	Hypothetical protein MGC10500	72687482	NM_031477	MGC10500
194902	0.438	Chemokine (C-C motif) ligand 22	41892711	NM_002990	CCL22
145792	0.442		51330963	BC041467	
234743	0.443	chr3 synaptotagmin	136578332	NM_031913	CHR3SYT
212352	0.444		246294	AX657520	
116672	0.445	Cryptochrome 2 (photolyase-like)	46016602	NM_021117	CRY2

Continued.

Unique id	Correlation coefficient	Description	Clone	GeneBank accession no.	Gene symbol
103227	0.446	Cytosolic acetyl-CoA hydrolase	76530115	NM_130767	CACH-1
228159	0.449		23232740	BX647251	
175816	0.452	Hypothetical protein FLJ25070; amylase, α 2B; pancreatic; amylase, α 2A; pancreatic; amylase, α 1A; salivary	102260708	NM_000699	FLJ25070; AMY2B; AMY2A; AMY1A
115606	0.453		52351877	BC057822	
231470	0.454		8763111	BC038195	
124737	0.46	PRKC, apoptosis, WT1, regulator	79645370	NM_002583	PAWR
208153	0.465	Hypothetical protein FLJ25070; amylase, α 2B; pancreatic; amylase, α 2A; pancreatic; amylase, α 1A; salivary	102260708	NM_017619	FLJ25070; AMY2B; AMY2A; AMY1A
123083	0.466	Chromosome 16 open reading frame 7	74837974	NM_004913	C16orf7
141785	0.468	Interleukin 11 receptor, α	34582169	NM_004512	IL11RA
199475	0.475		133220558	BC058028	
179061	0.476	Fetuin B	184794512	NM_014375	FETUB
164097	0.48	Hypothetical protein MGC34732	82875751	NM_173556	MGC34732
209982	0.485	Hypothetical protein MGC34728	124693182	NM_152533	MGC34728
137266	0.498	Protease inhibitor 16	38471077	NM_153370	PI16
194177	0.503	Hypothetical protein FLJ21159	153598959	NM_024826	FLJ21159
166432	0.503			BC020998	
160157	0.524		58498648		
116936	0.554		86943902	NM_014883	

Changes in mRNA expression were measured by microarray spot intensity. Genes are displayed in the same order as in Fig. 1. Sequences of the oligonucleotides spotted on the array can be obtained by the corresponding author.

correlate with age (data not shown). Both mRNA and protein expression data were available for 19 patients each for PRPF4 and SMC1A. In this subgroup, also no close correlation was observed between PRPF4 mRNA and protein expression or between SMC1A mRNA and protein expression (data not shown). These findings suggest that protein levels of the PRPF4 and SMC1A protein are regulated on several levels with mRNA expression levels being only one of them.

Loss of SMC1A protein expression indicates a poor prognosis in AML. Immunohistochemistry for SMC1A showed only weak or absent expression in 74 patients. Moderate to high expression was observed in 42 patients. Similar to mRNA levels, patients with low SMC1A expression were slightly older (median 62 years) than patients with moderate to high expression (median 59 years). However, in contrast to the mRNA expression data, this difference was not statistically significant (Table II). No correlation was observed between SMC1A protein expression and the presence of a complex-aberrant karyotype or between SMC1A protein expression and a normal karyotype versus the presence of any chromosomal abnormalities (data not shown).

A moderate to high SMC1A protein expression (expression level >1+) was associated with a significantly improved event free survival as well as overall survival (Fig. 4). Median overall survival was 22.6 versus 10.4 months (p=0.015) and median event free survival was 10.3 versus 2.6 months (p=0.003) for SMC1A moderate or high expression versus SMC1A low or absent expression, respective.

The patient characteristics of the 116 patients with low or undetectable (≤1$^+$) versus moderate or high (>1$^+$) SMC1A protein expression did not reveal significant differences regarding sex, age, FAB type, cytogenetic risk group, or an intensive versus a non-intensive treatment strategy (Table II). For this patient cohort, younger age at diagnosis (<60 vs. ≥60 years), female sex, *de novo* versus secondary leukemia, and low lactate dehydrogenase (LDH) serum level at diagnosis (≤700 vs. >700 U/l) correlated with a significant better EFS in a univariate analysis in addition to moderate or high SMC1A protein level, whereas the cytogenetic risk group (low, intermediate or high) or white blood cell count at diagnosis (≤20,000 vs. >20,000/μl) did not show a significant correlation with EFS. Out of this risk factors age, LDH serum level and SMC1A protein expression remained significant

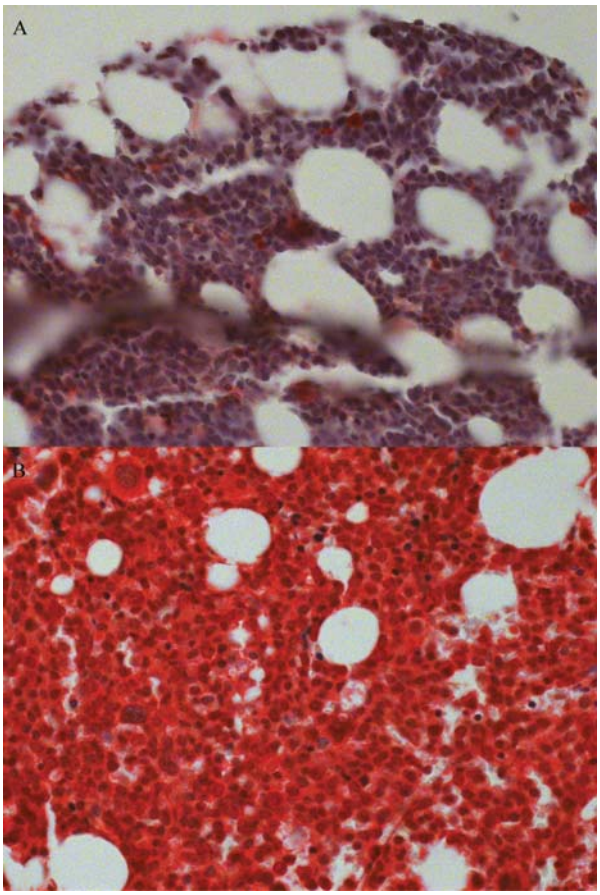

Figure 3. Immunohistochemistry staining of SMC1A on an AML tissue microarray. A tissue microarray containing AML bone marrow and control specimens was utilized to analyze protein expression of SMC1A. Images were acquired using a Zeiss Axio Imager M1 at a magnification x400. Examples for positive and negative expression are depicted. (A) Negative staining (Dako score 0): <10% of the blast cells are positive for SMC1A. (B) Strong positive staining (Dako score 3+). The majority of blasts show a strong cytoplasmatic signal for SMC1A.

Table II. Clinical characteristics of the patients analyzed for SMC1A protein expression.

SMC expression level	Absent/ low	Moderate/ high	P-value
No. of patients (n)	74	42	
Age at diagnosis (years), median (range)	62 (18-85)	59 (21-82)	0.78
Gender			0.90
Male, n (%)	44 (59)	22 (52)	
Female, n (%)	30 (41)	20 (48)	
FAB type			0.93
M0, n (%)	0 (0)	1 (2)	
M1, n (%)	8 (11)	6 (14)	
M2, n (%)	13 (18)	11 (26)	
M3, n (%)	2 (3)	0 (0)	
M4, n (%)	17 (23)	11 (26)	
M5, n (%)	27 (37)	9 (21)	
M6, n (%)	6 (8)	3 (7)	
M7, n (%)	0 (0)	1 (2)	
Not determined	1	0	
Cytogenetic risk[a]			1.00
Low, n (%)	5 (8)	2 (5)	
Intermediate, n (%)	36 (61)	23 (62)	
High, n (%)	18 (31)	12 (32)	
Missing	15	5	
Treatment			0.42
Intensive, n (%)	71 (96)	37 (88)	
Palliative/supportive care, n (%)	3 (4)	5 (12)	

[a]Cytogenetic risk is defined as: low, t(8;21), t(16;16) or inv(16); intermediate, normal karyotype or all other abnormalities not classified into low or high risk group; high, inv(3), t(3;3), -5, del(5q), -7, del(7q), 11q23-aberrations, complex karyotype defined by 3 or more chromosomal aberrations.

in a multivariate Cox-regression analysis (Table III). For overall survival, younger age, female gender and low LDH serum levels showed a significant positive correlation in addition to moderate or high SMC1A protein expression in a univariate analysis. Only gender remained significant in a multivariate analysis, with borderline significance (p=0.066, two-tailed) for SMC1A protein expression (Table III).

Since only two out of 115 evaluable samples showed a moderate PRPF4 expression, no further analysis of PRPF4 expression with clinical parameters was performed.

Discussion

In this study, we identified age-related changes in gene expression of AML blasts. In our analysis, 131 genes showed a significant correlation of the mRNA expression level with the patient age at diagnosis. For none of these genes has an age-dependent expression been described previously in AML. Interestingly, at the high stringency level of analysis that we utilized, we could not detect the previously described age-dependent increase of mRNA expression of the gene ABCB1 (MDR1) (13,14).

Since AML of elderly patients display an increased incidence of chromosomal imbalances, we hypothesized that age-dependent differences in genes involved in spindle formation and chromosome segregation might be relevant. We therefore selected the two genes PRPF4 and SMC1A for further analysis of the protein expression as determined by immunohistochemistry of tissue microarrays.

PRPF4 is a kinetochore component necessary for a functional spindle assembly checkpoint (20). The spindle assembly checkpoint prevents the initiation of the anaphase by halting cells in M2 phase in the presence of chromosomes not attached to spindle apparatus microtubules (21). PRPF4 depletion by siRNA leads to chromosomal segregation defects and aneuploidy (20). However, the detection of PRPF4 protein expression was not informative in our study.

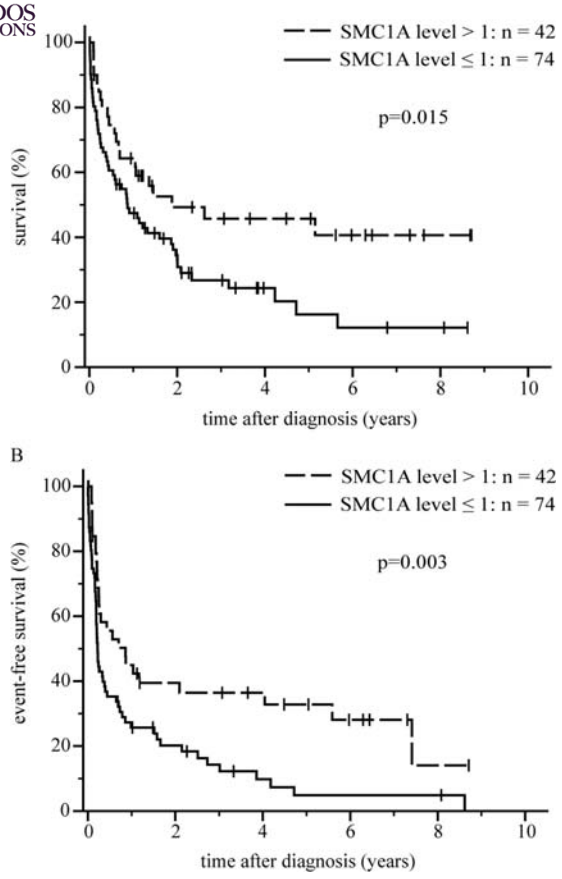

Figure 4. Association of SMC1A protein expression with event free and overall survival in AML patients. (A) Kaplan-Meier plot of the overall survival stratified by SMC1A protein expression. (B) Event free survival stratified by SMC1A protein expression.

On the other hand, low SMC1A protein levels were a strong predictor of impaired survival in AML. SMC1A belongs to the highly conserved structural maintenance of chromosome genes. The human SMC1A protein is part of the cohesin multiprotein complex which is required for sister chromatin cohesion during mitosis (22). SMC1A was found to be mutated in some colorectal cancers (23). In addition, one study demonstrated an upregulation of SMC1A mRNA in cervix cancer cells compared to normal cervix (24). No protein expression studies with SMC1A in cancer cells have been published to date. Recently, SMC1A was identified as an important gene for self-renewal in embryonic stem cells and a knock-down of SMC1A protein expression by siRNA results in an impaired self-renewal capacity of murine embryonic stem cells (25).

Hypothetically, a loss of SMC1A protein expression might lead to a chromosomal instability and aneuploidy due to the disruption of sister chromatin cohesion. However, no correlation was found between either SMC1A protein expression and the presence of a complex-aberrant karyotype or SMC1A protein expression and the presence of any cytogenetical abnormalities versus a normal karyotype in our study.

We did not observe a direct correlation between SMC1A mRNA and protein levels. The median age of patients with low SMC1A expression was higher than the age of patients with moderate to high SMC1A expression, but this difference was statistically not significant. These findings suggest complex regulation of SMC1A protein levels with mRNA levels being only one of them. It has been previously published that SMC1A mRNA expression detected by oligonucleotide microarray did not correlate with prognosis in a set of 285

Table III. Univariate (log-rank test) and multivariate Cox regression analysis.

	Event free survival p-value	Overall survival p-value
Univariate analysis		
Age (<60 vs. ≥60 years)	0.030	0.062
Gender	0.025	0.003
De novo versus secondary leukemia,	0.028	0.049
Cytogenetic risk (low, intermediate, high)	0.537	0.399
LDH serum level (<700 vs. ≥700 U/l)	0.004	0.031
White blood count (<20,000 vs. ≥20,000/μl)	0.227	0.253
SMC1A protein level (≤1 vs. >1)	0.003	0.015
Multivariate analysis		
Age (<60 vs. ≥60 years)	0.026	
Gender	0.103	0.010
De novo versus secondary leukemia	0.123	0.079
Cytogenetic risk (low, intermediate, high)		
LDH serum level (<700 vs. ≥700 U/l)	0.007	0.104
White blood count (<20,000 vs. ≥20,000/μl)		
SMC1A protein level (≤1 vs. >1)	0.014	0.066

Known risk factors and SMC1A protein level were analyzed with regard to event free and overall survival. Variables with significant correlation in the univariate analysis were included in the multivariate analysis.

AML samples (26). Regardless of the underlying mechanisms of regulation, the association of low SMC1A mRNA with aging and the poor prognosis of low SMC1A protein correspond well with each other.

mRNA expression level of certain genes have been identified as a prognostic marker in AML (4-6). However, given the lack of correlation with protein expression, few if any of the identified mRNAs might actually play a direct role in the mechanisms that lead to AML relapse. Thus, independent verification on the protein level is required before any conclusions on the biological relevance can be drawn. In addition, the expression of proteins in AML blasts at diagnosis, i.e. P-glycoprotein, is able to identify prognostic subgroups in AML. Tissue microarrays are a powerful tool to discover the protein expression level on a high-throughput level (27). Our approach was suitable to test a hypothesis regarding the protein expression level on a high number of samples. With this approach, we identified SMC1A protein expression as a potential new prognostic marker in AML which can be easily assessed on bone marrow biopsy samples obtained at diagnosis.

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