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ABSTRACT

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. Its clinical course is typically indolent;
however, based on a series of pathobiological, clinical, genetic, and phenotypic parameters, patient survival varies from
less than 5 to more than 20 years. In this paper, we show for the first time that the expression of the interferon-inducible
DNA sensor IFI16, a member of the PYHIN protein family involved in proliferation inhibition and apoptosis regulation,
is associated with the clinical outcome in CLL. We studied 99 CLLs cases by immunohistochemistry and 10
CLLs cases by gene expression profiling. We found quite variable degrees of IFI16 expression among CLLs cases.
Noteworthy, we observed that a reduced IFI16 expression was associated with a very poor survival, but only in cases
with ZAP70/CD38 expression. Furthermore, we found that IFI16 expression was associated with a specific gene expression
signature. As IFI16 can be easily detected by immunohistochemistry or flow cytometry, it may become a part of
phenotypic screening in CLL patients if its prognostic role is confirmed in independent series.

Key words: IFI16; chronic lymphocytic leukemia; ZAP70; prognosis; gene expression; immunohistochemistry.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common leukemia type in adults in Western countries (1). Rarely, the disease lacks the typical spread of leukemia, presenting with evident nodal localization, and it is classified as a small lymphocytic lymphoma (SLL) (1). Clinically, CLL is indolent, with a median overall survival (OS) exceeding 15 years (2). However, based on clinical (e.g., stage and doubling time), phenotypic, and genetic features, it can be completely indolent or quite aggressive.

Specifically, the overall expression of CD38, ZAP70, LAIR1, and CD49d biomarkers, though not univocally, indicates an activation of the leukemic B cell and it is related to a worse outcome (3–5). In addition, immunohistological analyses have demonstrated that the identification of abundant proliferating centers is associated to a worse behavior (6). Similarly, the absence of somatic hypermutations in the immunoglobulin heavy chain genes (IGH) or recognition of specific stereotypes indicates an aggressive disease (1, 3, 4). Genetically, few chromosomal imbalances that can be detected by a FISH analysis, including del(17p), del(11q), +12, and del(13q), are associated with a more or less aggressive clinical behavior (7). Recently, nextgeneration sequencing has allowed the identification of a series of lesions affecting *TP53*, *BIRC3*, *SF3B1*, *MYD88*, and *NOTCH1* that significantly influence patient prognosis (8). Expression levels of specific miRNA have also been associated with clinical outcome, although these results have occasionally been controversial (9–11). Notably, the integration of FISH and molecular data has been clinically meaningful in defining subgroups with significant differences in overall survival (8). However, despite such evidence, most patients are not currently evaluated at diagnosis with a complete molecular and genetic make-up. Patients sometimes undergo FISH and extensive gene sequencing at disease progression if aged below 60–65 years old (12). Conversely, most patients are studied at diagnosis using flow cytometry (CD38/ZAP70) and IGH@ sequencing only.

Recently, our group indicated that IFI16 is regulated during B-cell differentiation (13). Its levels are particularly elevated in memory B cells.

In addition, IFI16 expression has been shown to

have an intriguing relationship with some major transcription factors, such as BCL6, NF-jB, STAT3, and STAT5, involved in central processes of the B-cell biology (13,14). IFI16 is a member of the PYHIN family of proteins and is involved in immune response, cell viability, differentiation, proliferation, senescence, and restriction of virus replication (15–17). Specifically, IFI16 plays a central role in innate immunity, especially during viral infections. IFI16 is a DNA sensor that regulates IFN expression and inflammasome activation by negatively affecting the viability of infected cells (18–21). In addition to its role in innate immunity, several observations have indicated that IFI16 is a transcriptional regulator through heterodimerization with other transcription factors, which suggest different roles of IFI16 depending on the cell type (15). In different cell models, IFI16 has been demonstrated to either activate apoptosis or inhibit proliferation by interacting with other cell cycle and survival gatekeepers including p53 and Rb, and in addition, it is able to restrict virus replication (21–25). This antitumor activity is not absolute but is well-described in different cell models (16, 26, 27). Together, these studies suggest that an alteration in IFI16 activity and/or expression could play an important role in Bcell proliferation. Hence, we investigated IFI16 expression in CLL to uncover its potential pathogenetic/ prognostic role in this study.

MATERIALS AND METHODS

Case series

A total of 99 lymph node samples obtained from CLL patients diagnosed according to NCI criteria were included in this study. Lymph node biopsies in these patients were performed in the presence of progressive disease requiring treatment, with adenopathies ≥3 cm (6). Patients were referred to the Hematopathology Unit of S. Orsola-Malpighi Hospital, Bologna University, Bologna, Italy, for a histological diagnosis between 2002 and 2008. The only selection criterion was the availability of a sufficient amount of formalin-fixed paraffin-embedded (FFPE) tissue. The main clinical-pathological features of these patients are summarized in Table 1. The cases showing confluent proliferation centers (PCs) were classified as “PC-rich”. Additionally, 10 CLL cases were studied, previously generated by our group (28).

Immunohistochemistry and fluorescence in situ hybridization (FISH) on tissue microarrays

A Giemsa-stained slide was prepared from each paraffin

block containing representative tumor regions marked on every slide. Tissue cylinders with a diameter of 1.0 mm were punched from the marked areas on each block and placed in a recipient paraffin block using a precision instrument as previously described (6, 10). Punches were performed on areas of monotonous small lymphocytes and on areas with proliferating centers, when present. Tissue microarrays (TMAs) were prepared for immunohistochemistry and fluorescence in situ hybridization FISH studies. by immunohistochemistry (IHC) on TMAs from the 99 cases (in duplicate cores). From each recipient block, 1.5-lm-thick sections were cut and tested with anti-ZAP70 (Upstate, Millipore, Billerica, MA, USA, clone 2F3.2: dilution 1:80; (29,30)), anti-CD38 (Novocastra, Menarini Diagnostics, Grassina, Italy, clone SPC32: dilution 1:80), and anti-IFI16 mouse monoclonal antibodies (Sigma, Milan Italy, dilution 1:100). Briefly, paraffin-embedded sections were dewaxed and submitted to antigen retrieval by heating in Dako PTLink (DakoCytomation, Glostrup, Denmark; code PT100/PT101) in an EnVision Flex Target Retrieval Solution High pH (DakoCytomation; code K8004) at 92 °C for 5 min. Sections were incubated at room temperature with fetal calf serum (10 min) and then with the specific primary antibody (for 30 min). Each evaluation was performed by at least two expert hematopathologists blinded to the study. Scores were compared and consensus agreement was reached at the microscope in all cases.

Immunohistochemistry stainings were scored based on the percentage of positive neoplastic cells (visual count performed by two hematopathologists) as follows: 0 = no positive cells; 1 = 1–20%; 2 = 21–40%; 3 = 41–60%; 4 = 61–80%; and 5 = 81–100%. Micrographs were obtained using an Olympus BX61 microscope equipped with an Olympus DP-70 digital camera, and image acquisition, evaluation, and color balance were performed using Cell^F software. The probes used in fluorescence in situ hybridization FISH study, as well as preparation of the slides, hybridization, and cut-off for positivity FISH signal screening are detailed in our previous report (6).

Gene expression analyses

We analyzed the previously generated gene expression profile (GEP) data reported by our group on 10 CLL cases (28) not included within the 99 studied by IHC. All data were obtained using Affymetrix HG-U133 2.0 plus microarrays (Affymetrix, Inc. http://www.affymetrix.com/support/index.affx) available at http://www.ncbi.nlm.nih.gov/projects/geo/(28). For further technical details and patient characteristics, see references (28).

We focused our analysis on IFI16 expression. We identified

IFI16 expression using three different probe sets (206332_s_at; 208966_x_at; and 208965_s_at) in the HGU133 datasets and one (1456_s_at) in HG-U95. The mean values from the three probes were used to analyze HGU133 data. GEP analyses were performed using GeneSpring GX 12.0 Software (Agilent Technologies, Santa Clara, CA, USA; (28, 29, 31, 32)).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 20.0. ANOVA and unpaired T-tests were used. When a sample size was less than 10 cases in at least one group, a non-parametric (Mann–Whitney) test was used to analyze the GEP data to compare IFI16 expression in different subgroups (11). The limit of significance for all analyses was defined as $p < 0.05$. Two-sided tests were used in all calculations. Possible relationships between the immunohistochemical expression of IFI16 and other clinicopathological parameters were evaluated using a T-test or chi-square test for continuous and non-continuous variables, respectively (6, 10). The univariate association between individual clinical features and DSS was determined with the log-rank or Wilcoxon test, when appropriate. A multivariate analysis using the Cox proportional hazards regression model was performed to compare the factors studied in univariate analysis. The limit of significance for all analyses was defined as $p < 0.05$; two-sided tests were used in all calculations.

RESULTS

IFI16 is variably expressed in B-CLL

We tested IFI16 protein expression in the 99 lymph nodes from patients affected by CLL/SLL and the 5 lymph nodes characterized by florid reactive hyperplasia. We observed IFI16 expression in all the CLL and non-neoplastic hyperplastic samples. As expected, the latter had intense staining mainly localized in the mantle zone. This confirmed our data from previous studies (13). In the CLL cases, nuclear staining was detected in 10–100% of the cells. The overall staining was intense. Only a few cases had a weaker reaction that was not related to other features, including the percentage of positive cells. In cases with a prominent proliferating center (PC), we observed a gradient of IFI16 expression, which was higher within the PC (Fig. 1). However, no significant correlation between the abundance of PC and IFI16 expression was recorded. The expression pattern (mainly nuclear) corresponded to the one, previously reported in human lymphocytes (13). Overall, TMAs resulted adequate for a proper

evaluation and full section re-evaluation was not needed.

IFI16 lower protein levels are associated with unfavorable outcome

We investigated whether IFI16 protein expression was correlated with any specific clinic-pathological parameter. No significant correlation was observed, with the exception of del(13q) that was associated with a significantly lower IFI16 expression (Table 2; Fig. 2). Despite the significance at T-test, as IFI16 protein expression values in terms of percentage of positive cells at immunohistochemistry (IHC) appeared quite overlapping in the two groups (with and without del13p, respectively) (Fig. 2), we also tested by chi-square whether cases without del13p had more frequently higher (i.e., more than 50% of positive cells at IHC) IFI16 expression. Indeed, also this correlation turned out to be statistically significant ($p = 0.02$).

We then tested the possible impact of the main clinic-pathological parameters on the clinical outcome. Following the univariate analysis, only the presence of del(13q) and del(17p) and a low IFI16 expression correlated with an inferior OS (Table 3; Fig. 3).

When the CLLs were divided into two groups, based on IFI16 expression (i.e., scored at immunohistochemistry 0–2 vs 3–5, IFI16low vs IFI16high, respectively), the group with lower IFI16 expression was characterized by a significantly shorter mean OS (55.6 vs 95.4 months, 95% confidence interval 78.9–111.7 vs 27.7–83.6). A median OS, in the IFI16high group was not reached. However, the median OS in the IFI16low group was 46 months (Fig. 3). By contrast, in the multivariate analysis, only the presence of genetic lesions affecting chromosomes 14p and 17p were associated with a shorter OS.

Finally, because IFI16 expression can correlate with physiological lymphocyte activation, we investigated whether IFI16 expression might be related to CLL cell activation, as well. For this purpose, each CLL case was considered “activated” if either CD38 or ZAP70 was expressed. Based on this criterion, we divided the cases into four groups according to IFI16 and ZAP70/CD38 protein expression (Fig. 3; Table 4) to test a possible prognostic impact. The four groups were, in fact, significantly different in OS ($p = 0.004$). Specifically, patients

with high ZAP70/CD38 but low IFI16 expression (group 3) had worse outcomes (median 6.5 months, 95% confidence interval 4.4–8.6 months). Those with higher IFI16, both with lower (group 2) or negative (group 1) ZAP70/CD38 expression had the best outcomes (median not reached; mean 86.7 and 97.7 months, respectively; 95% confidence interval 69–104.4 and 76.3–119.2 months, respectively). The CLLs characterized by IFI16 low and no ZAP70/CD38 expression had an intermediate clinical profile (median survival 46 months; 95% confidence interval 0–93.4 months; Fig. 5).

IFI16 low expression is associated with a peculiar gene expression profile

To better understand the pathobiological significance of IFI16 expression in CLL, we compared the GEP of CLLs with higher (>50% percentile) and lower (< 50% percentile) IFI16 gene expression. In a supervised analysis (T-test, $p < 0.05$), we identified 271 probe sets, which corresponds to 226 unique genes differentially expressed between the two groups (Fig. 4; raw data available upon request). Specifically, 163 were up-regulated and 63 were down-regulated in the more aggressive group (i.e., with lower IFI16 expression).

In the IFI16low CLL group, we observed up-regulation of JUN and JUNB, two transcription factors often up-regulated in malignant phenotypes, and CXCR4, a chemokine receptor that regulates bone marrow adhesion of neoplastic elements which possibly contributes to chemoresistance in CLL (33). Down-regulation of IKZF3, a tumor suppressor previously found to be altered in lymphoid leukemias and other cancers (34) was also observed. When we investigated pathways and cellular programs possibly overrepresented among the differentially expressed genes, we found several biological processes and cancer-related molecular signatures/pathways (Fig. 4; Tables 5-6). We noted a significant enrichment in the expression of genes involved in DNA repair, RNA processing and transcription, and response to stress and DNA damage. We also found that the expression of genes usually involved in the EGFR, VEGF, MTOR, and PDGFR signaling, and genes, associated with RB1, RBL2, and KRAS deregulation, were enriched.

DISCUSSION

Chronic lymphocytic leukemia is typically indolent. However, patient survival varies from less than 5 to

more than 20 years depending on the biological characteristics of the disease (8). Accordingly, treatments have to be tailored based on patients' specific features. These treatments range from watch-and-wait to chemo-immunotherapy, signaling inhibitors, and stem cell transplantation (35–37). The main biological parameters that influence CLL aggressiveness are genetic (such as immunoglobulin somatic hypermutations/ISHM or somatic lesions detectable by FISH or DNA sequencing) and functional (usually detected by flow cytometry and indicative of cellular activation). Although a complete genetic characterization is probably the optimal approach to CLL prognostication, for practical reasons, mainly timing and cost, only phenotypic characterization is used in elderly people. Genetic characterization is used more often in patients presenting with symptoms at a younger age. Recently, the importance of the phenotypic profiling has been highlighted by the identification of progranulin, a molecule overexpressed in CD38+/ZAP70+ cases with negative prognostic relevance (38).

In this study, we explored the possible relation between IFI16 expression and prognosis. IFI16 is an interferon-related molecule that regulates cellular activation, signaling, and eventually, cell proliferation and apoptosis (15–17, 21, 39, 40). In B cells, IFI16 expression consistently parallels maturation and differentiation of the germinal center toward memory and plasma cells (13, 41). For the first time, we found that IFI16 expression might be related to CLL clinical outcomes. Specifically, reduced IFI16 corresponded to a worse clinical scenario when molecules such as ZAP70 and CD38 were expressed (median survival 6.5 months). As IFI16 expression is retained in the normal cellular counterparts of CLL (13), a reduced expression can be used as a pathological feature. Because of the known functions of IFI16, it is conceivable that the lack of IFI16, which is associated with a reduced antiproliferative effect, might be particularly severe in cells constitutively receiving an activation/proliferation signaling. Therefore, it should be noted that the prognostic role of ZAP70 and CD38 expression has not been confirmed. Based on the present results, we can assume that the balance between IFI16 expression and activation signaling (mediated by ZAP70/CD38) may represent a more reliable parameter. A maintained IFI16 expression is capable of at least partially compensating for the negative influence of ZAP70 and CD38.

The major limitation of this study is the lack of

a comprehensive genetic characterization of the disease, including all novel markers, because DNA was not available for such studies. Certainly, the genetic background might affect IFI16 expression. Regardless, ISHM, TP53 mutations, and FISH could be performed as a good basic evaluation. On the other hand, testing the prognostic value of IFI16 in an independent, possibly prospective, series of cases is warranted. Furthermore, it should be noted that the distribution of cytogenetic aberrations was quite untypical. In particular, the incidence of del(13q) was lower, while, on the contrary, the incidence of del(17p) and especially, del(11q) and del(14q) were higher than expected. However, as previously discussed (6), the only selection bias was represented by the availability of tissue.
Finally, to simplify pathological assessment and increase reproducibility of immunohistochemical assays, an automated digital analyses would be possibly applied.
In conclusion, we identified IFI16 as a possible prognostic marker in CLL. It might be clinically useful, especially in patients receiving a phenotypic rather than complete molecular characterization for any reason.

CONFLICT OF INTEREST

The authors have no conflicting financial interests to declare.

PPP and DG designed the study, performed analyses, and wrote the manuscript. CA, SR, MR, and MC performed IHC and FISH analyses. MCR, IB, GM, CT, ED, CS, and CP performed data analysis. OP collected surgical samples. AC was responsible for patient care and clinical data collection. SL and DZ contributed to conceiving the study and the paper drafting and writing. This work was supported by BolognAIL, AIRC, RFO (Prof. Piccaluga), FIRB Futura 2011 RBFR12D1CB (Prof. Piccaluga), and Fondi Dipartimentali (Gibellini).

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Table 1

Patients	99
Age (median, range), years	67.5 (28–84)
Gender (M/F)	66/33
FISH	
11q	22/66 (33.3%)
+12p	15/74 (20.3%)
13q	26/84 (31%)
14q	21/72 (29.2%)
17p	12/84 (14.3%)
Disease stage	
0	4
1	21
2	11
3	5
4	5
TP53 mutations	0/99
PC-rich	39/99 (39.4%)
CD38	34/99 (34%)
ZAP70	26/96 (26%)
ZAP70 and/or CD38	44/99 (44%)
IFI16	
High (IHC score 1–2)	57/99 (57.6%)
Low (IHC score 3–5)	42/99 (42.4%)

PC-rich, proliferating centers rich, IHC, immunohistochemistry.

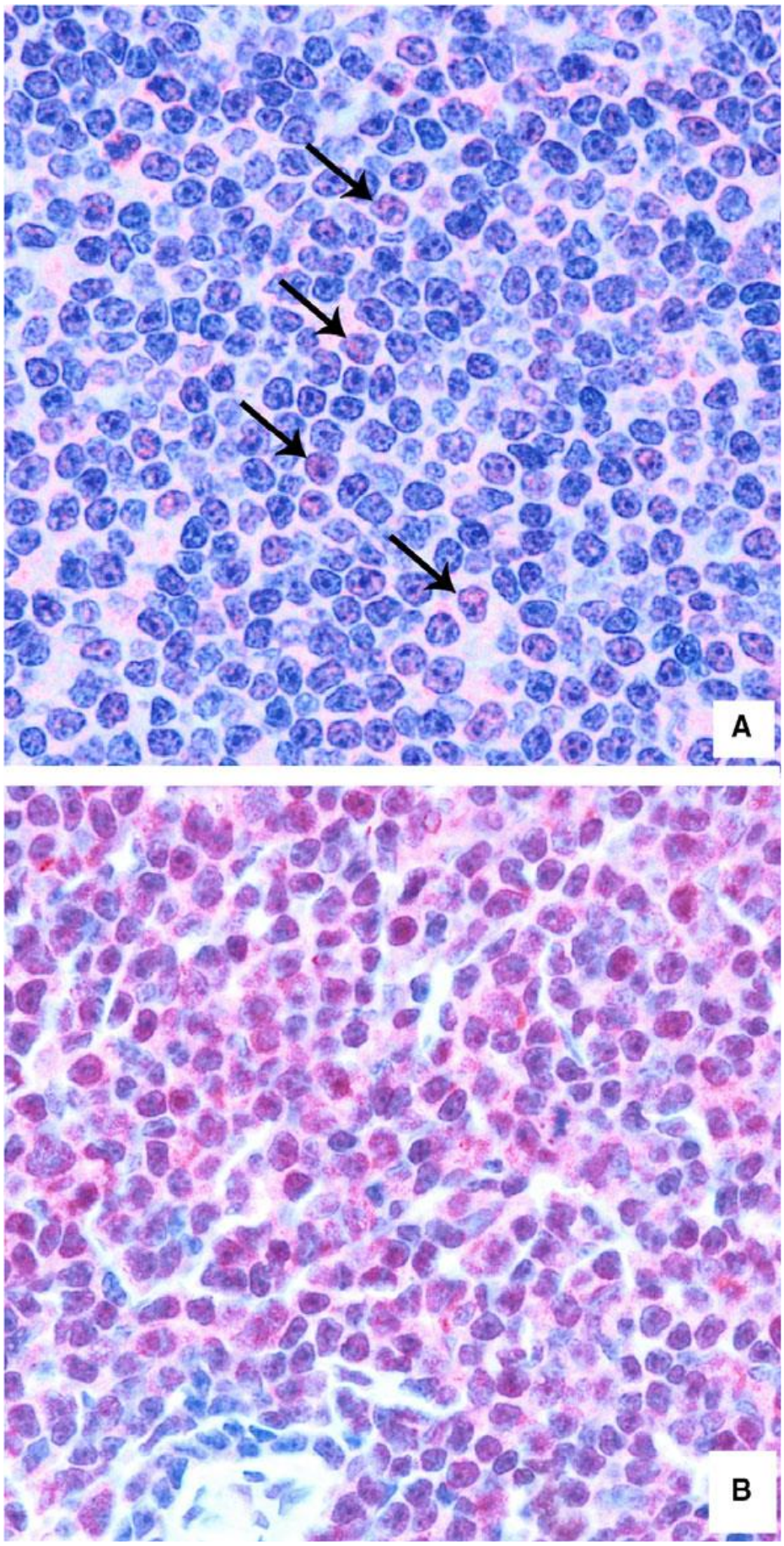
A
B

Fig. 1. IFI16 protein expression. IFI16 expression in CLL lymph node biopsies: examples of cases with low expression
(partial expression below cut-off of 50% of the cells;
examples of weak positive cells are highlighted by black
arrows) (A) and high expression (B).

Table 2. Correlation between protein IFI16 expression and the main clinico-pathological parameters
Parameter Test p-value for possible correlation

Parameter	Test	p-value for possible correlation
ZAP70	Chi-square	0.028
CD38	Chi-square	0.299
ZAP70 and or CD38	Chi-square	0.02
WBC at diagnosis	T-test	0.7
del(11q)	Chi-square	1
+12p	Chi-square	0.76
del(13q)	Chi-square	0.015
del(14q)	Chi-square	0.79
del(17q)	Chi-square	0.35
TP53 mutations	Chi-square	1
B2	T-test	0.85
Stage	Chi-square	0.86
Age	T-Test	0.55
PC-rich	Chi-square	0.2

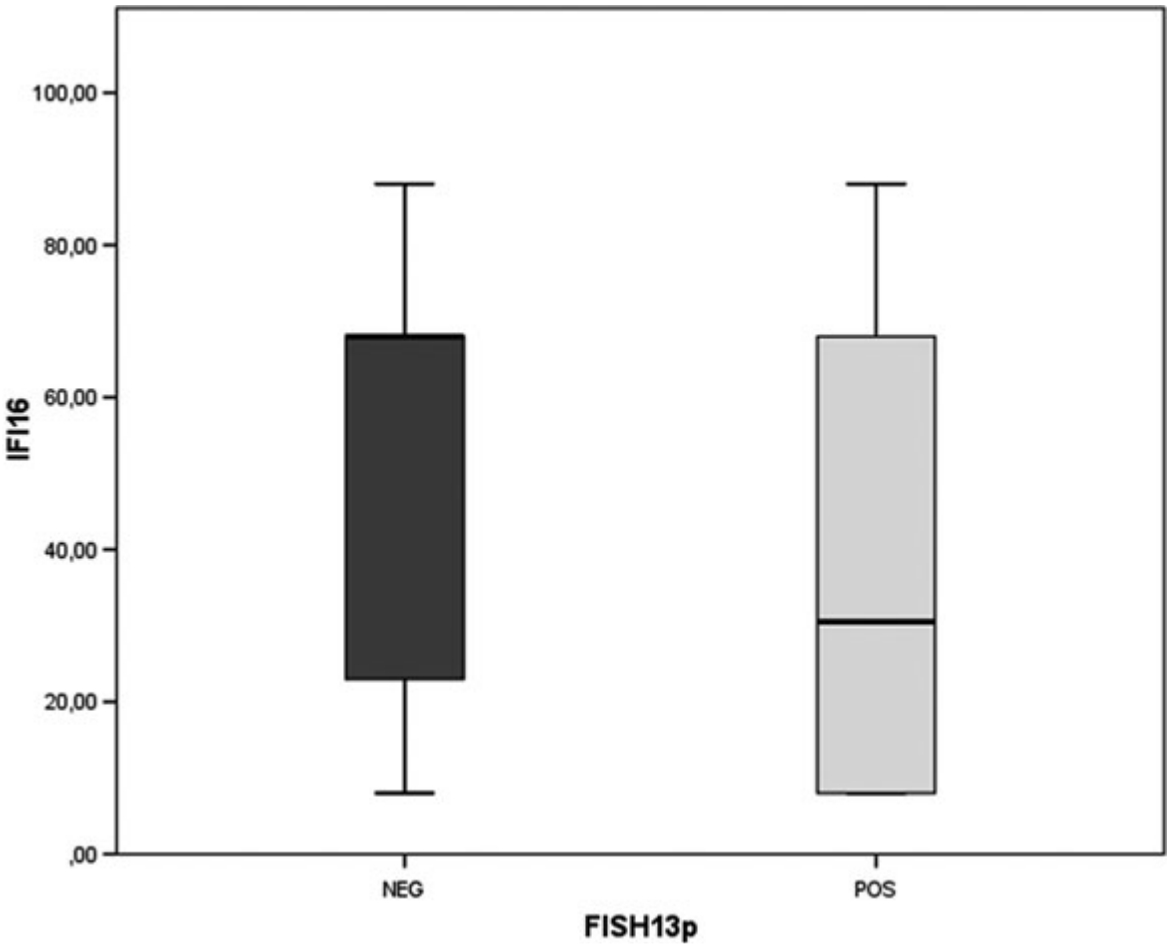

Fig. 2. Differential expression of IFI16 protein in cases carrying or not 13q abnormalities as detected by FISH analysis. Two-tailed T-test was used for comparison.

Table 3

Means and medians for survival time									p-value (Log-Rank (Mantel–Cox))
	Mean[2]				Median				
	Estimate	Std. Error	95% Confidence interval		Estimate	Std. Error	95% Confidence interval		
			Lower bound	Upper bound			Lower bound	Upper bound	
IFI16[1]									
High	95.363	8.372	78.953	111.773					0.003
Low	55.619	14.256	27.677	83.561	46.000	19.140	8.485	83.515	
Overall	88.570	10.581	67.832	109.308	87.000	12.357	62.780	111.220	
FISH14q									
NEG	86.282	6.376	73.785	98.780	104.000	14.513	75.554	132.446	0.011
POS	55.946	9.946	36.451	75.440	46.000	.721	44.588	47.412	
Overall	78.997	5.549	68.121	89.872	87.000	11.703	64.062	109.938	
FISH17p									
NEG	97.376	7.334	83.002	111.751	113.000	9.187	94.994	131.006	0.002
POS	51.939	6.922	38.372	65.506	45.000	6.606	32.053	57.947	
Overall	89.828	6.719	76.660	102.997	104.000	16.101	72.441	135.559	

[1]Assessed by IHC on tissue microarrays.
[2]Estimation is limited to the largest survival time if it is censored.

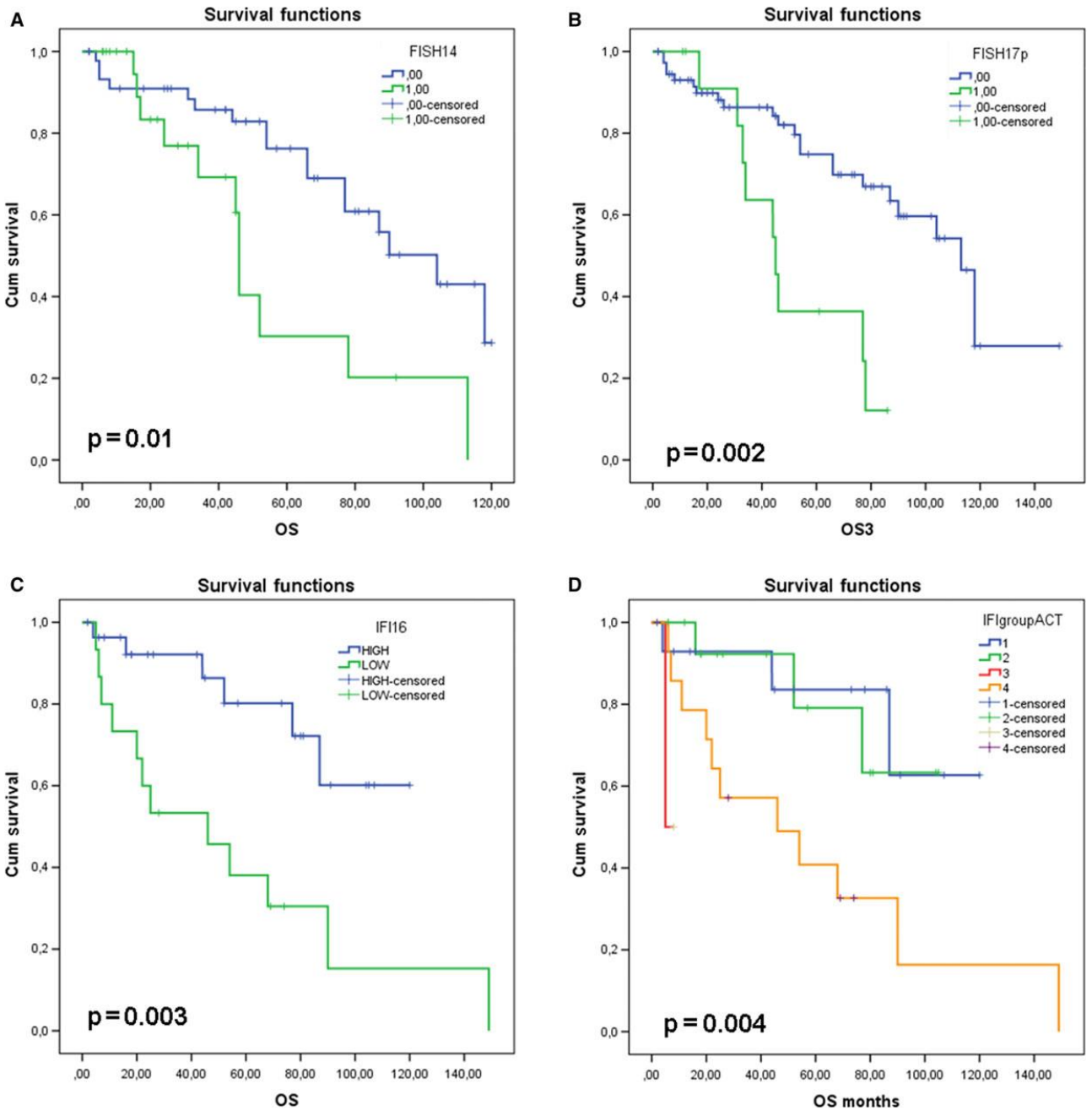

Fig. 3. Kaplan–Meier plots of CLL patients according to risk factors significantly associated with overall survival. The
outcome according to chromosome 14 abnormalities (A), chromosome 17 abnormalities (B), and IFI16 protein expression
(C) are shown. In panel D, four groups were designated based on the expression of IFI16, CD38, and ZAP70 (Group 1:
IFI16+ and ZAP70/CD38-; Group 2: IFI16+ and ZAP70/CD38+; Group 3: IFI16- and ZAP70/CD38+; Group 4: IFI16-
and ZAP70/CD38-).

Table 4

Group	Means and medians for survival time								p-value (Log-Rank (Mantel–Cox))
	Mean[1]				Median				
	Estimate	Std. Error	95% Confidence interval		Estimate	Std. Error	95% Confidence interval		
			Lower bound	Upper bound			Lower bound	Upper bound	
1	97.763	10.935	76.330	119.195					0.004
2	86.734	9.015	69.065	104.403					
3	6.500	1.061	4.421	8.579	5.000				
4	59.235	14.827	30.174	88.296	46.000	24.201	0.000	93.434	
Overall	90.435	10.453	69.946	110.923	87.000	9.600	68.183	105.817	

Group 1: IFI16 + and ZAP70/CD38- (N = 25 case);
Group 2: IFI16 + and ZAP70/CD38 + (N = 30 case);
Group 3: IFI16- and ZAP70/CD38 + (N = 12 case);
Group 4: IFI16- and ZAP70/CD38- (N = 28 case).

[1]Estimation is limited to the largest survival time if it is censored.

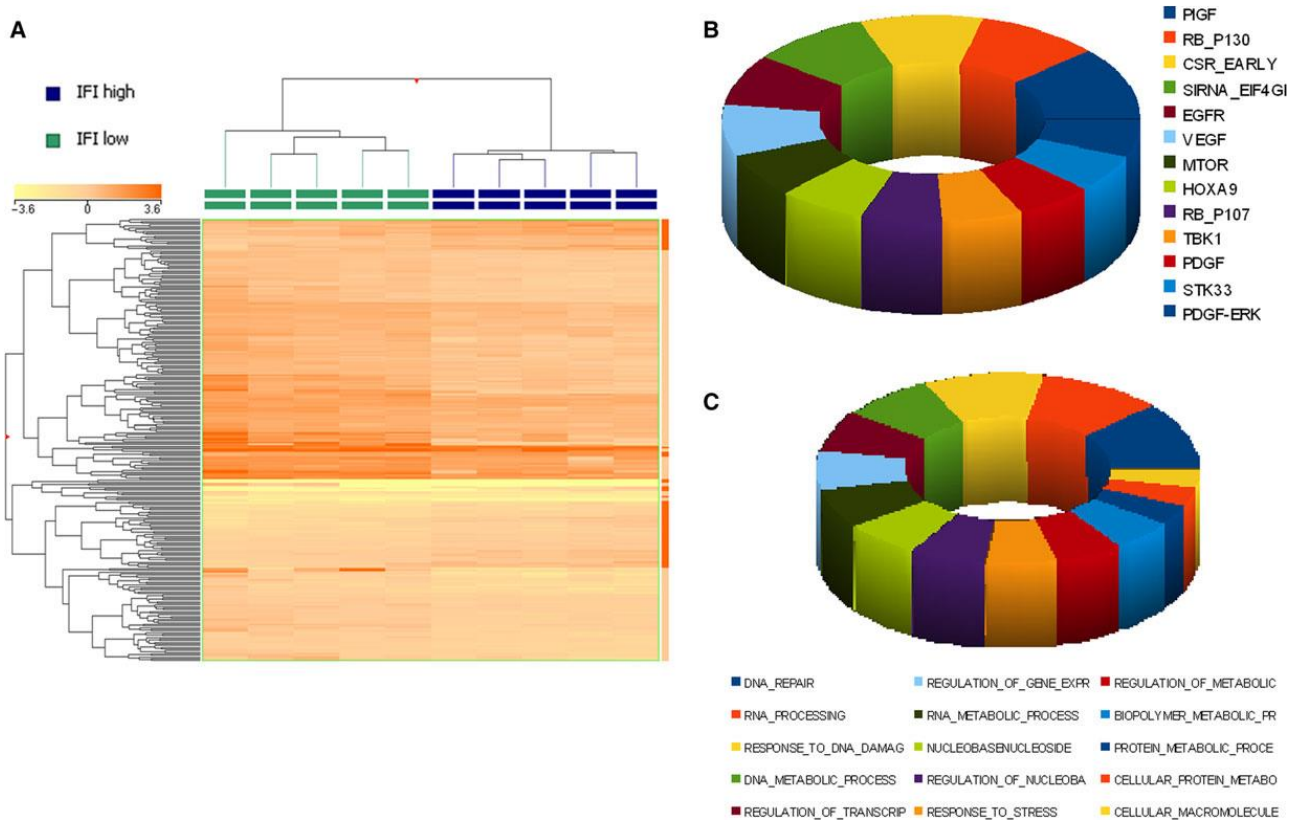

Fig. 4. Gene expression analysis of CLL according to IFI16 expression. Supervised analysis was performed in CLL cases
with high vs low IFI16 gene expression. Based on the expression of 226 genes (corresponding to 271 probe sets), cases with
higher or lower IFI16 levels were clearly discriminated (A). The dendrograms are generated using a hierarchical clustering
algorithm based on the average-linkage method. In the matrix, each column represents a sample and each row represents a
gene. The color scale bar shows the relative gene expression changes normalized by the standard deviation (0 is the mean
expression level of a given gene). The above-mentioned 271 genes turned out to be significantly enriched in relevant oncogenic

pathways (B) and GeneOntology Biological Processes (C).

Table 5

Gene Set Name	# Genes in Gene Set (K)	Description	# Genes in Overlap (k)	k/K	p-value	FDR q-value
PIGF UP.V1 UP	191	Genes up-regulated in HUVEC cells (endothelium) by treatment with PIGF [Gene ID = 5281]	10	0.0524	1.31E-08	2.48E-06
RB P130 DN.V1 DN	139	Genes down-regulated in primary keratinocytes from RB1 and RBL2 [Gene ID = 5925, 5934] skin-specific knockout mice.	6	0.0432	3.40E-05	1.11E-03
CSR EARLY UP.V1 UP	164	Genes up-regulated in early serum response of CRL 2091 cells (foreskin fibroblasts)	7	0.0427	8.05E-06	7.60E-04
SIRNA EIF4GI UP	95	Genes up-regulated in MCF10A cells vs knockdown of EIF4G1 [Gene ID = 1981] gene by RNAi	4	0.0421	7.98E-04	1.26E-02
EGFR UP.V1 UP	193	Genes up-regulated in MCF-7 cells (breast cancer) positive for ESR1 [Gene ID = 2099] and engineered to express ligand-activatable EGFR [Gene ID = 1956]	7	0.0363	2.31E-05	1.09E-03
VEGF A UP.V1 DN	193	Genes down-regulated in HUVEC cells (endothelium) by treatment with VEGFA [Gene ID = 7422]	7	0.0363	2.31E-05	1.09E-03
MTOR UP.N4.V1 DN	193	Genes up-regulated in CEM-C1 cells (T-CLL) by everolimus [PubChem = 6442177], an mTOR pathway inhibitor	6	0.0311	2.07E-04	4.03E-03
HOXA9 DN.V1 UP	194	Genes up-regulated in MOLM-14 cells (AML) with knockdown of HOXA9 [Gene ID = 3205] gene by RNAi vs controls	6	0.0309	2.13E-04	4.03E-03
RB P107 DN.V1 UP	140	Genes up-regulated in primary keratinocytes from RB1 and RBL1 [Gene ID = 5925, 5933] skin-specific knockout mice	4	0.0286	3.31E-03	4.46E-02
TBK1.DF DN	287	Genes down-regulated in epithelial lung cancer cell lines upon overexpression of an oncogenic form of KRAS [Gene ID = 3845] gene and knockdown of TBK1 [Gene ID = 29110] gene by RNAi	8	0.0279	3.97E-05	1.11E-03
PDGF UP.V1 UP	146	Genes up-regulated in SH-SY5Y cells (neuroblastoma) in response to PDGF [Gene ID=] stimulation	4	0.0274	3.84E-03	4.84E-02
STK33 UP	293	Genes up-regulated in NOMO-1 and SKM-1 cells (AML) after knockdown of STK33 [Gene ID = 65975] by RNAi	8	0.0273	4.59E-05	1.11E-03
PDGF ERK DN.V1 DN	149	Genes down-regulated in SH-SY5Y cells (neuroblastoma) in response to PDGF [Gene ID=] stimulation after pre-treatment with the ERK inhibitors U0126 and PD98059 [PubChem = 3006531, 4713]	4	0.0268	4.13E-03	4.88E-02

FDR, False discovery rate.

Table 6

Gene set name	# Genes in gene set (K)	Description	# Genes in overlap (k)	k/K	p-value	FDR q-value
DNA repair	125	Genes annotated by the GO term GO:0006281. The process of restoring DNA after damage. Genomes are subject to damage by chemical and physical agents in the environment (e.g., UV and ionizing radiations, chemical mutagens, fungal and bacterial toxins, etc.) and by free radicals or alkylating agents endogenously generated in metabolism. DNA is also damaged because of errors during its replication. A variety of different DNA repair pathways have been reported that include direct reversal, base excision repair, nucleotide excision repair, photoreactivation, bypass, double-strand break repair pathway, and mismatch repair pathway	7	0.056	1.33E-06	5.22E-05
RNA processing	173	Genes annotated by the GO term GO:0006396. Any process involved in the conversion of one or more primary RNA transcripts into one or more mature RNA molecules	9	0.052	7.50E-08	3.64E-06
Response to DNA damage stimulus	162	Genes annotated by the GO term GO:0006974. A change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating damage to its DNA from environmental insults or errors during metabolism	8	0.0494	5.99E-07	2.47E-05
DNA metabolic process	257	Genes annotated by the GO term GO:0006259. The chemical reactions and pathways involving DNA, deoxyribonucleic acid, one of the two main types of nucleic acid, consisting of a long, unbranched macromolecule formed from one, or more commonly, two, strands of linked deoxyribonucleotides	10	0.0389	2.12E-07	9.73E-06
Regulation of transcription	566	Genes annotated by the GO term GO:0045449. Any process that modulates the frequency, rate or extent of the synthesis of either RNA on a template of DNA or DNA on a template of RNA	19	0.0336	8.08E-12	9.52E-10
Regulation of gene expression	673	Genes annotated by the GO term GO:0010468. Any process that modulates the frequency, rate or extent of gene expression. Gene expression is the process in which a gene's coding sequence is converted into a mature gene product or products (proteins or RNA). This includes the production of an RNA transcript as well as any processing to produce a mature RNA product or an mRNA (for protein-coding genes) and the translation of that mRNA into protein. Some protein processing events may be included when they are required to form an active form of a product from an inactive precursor form	22	0.0327	2.81E-13	5.79E-11