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Research article

Interferon gamma inducible protein 16 (IFI16) expression is reduced in mantle cell lymphoma

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ABSTRACT

IFI16, member of the IFN-inducible PYHIN-200 gene family, modulates proliferation, survival and differentiation of different cell lineages. In particular, IFI16 expression, which is regulated during the differentiation of B cells, was recently studied in B-CLL as well. Here, we compared IFI16 expression in several lymphomas including Burkitt lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, marginal zone lymphoma and mantle cell lymphoma with respect to normal cell counterparts. We observed that IFI16 expression was significantly deregulated only in mantle cell lymphoma ($p < 0.05$). Notably, IFI16 was associated with the expression of genes involved in interferon response, cell cycle, cell death and proliferation and, interestingly, lipid and glucose metabolism, suggesting that IFI16 deregulation might be associated with relevant changes in cell biology. In our group of mantle cell lymphoma samples a correlation between patient survival and IFI16 expression was not detected even though mantle cell lymphoma prognosis is known to be associated with cell proliferation. Altogether, these results suggest a complex relationship between IFI16 expression and MCL which needs to be analyzed in further studies.

1. Introduction

IFI16 belongs to the IFN-inducible PYHIN-200 gene family [1, 2] encoding evolutionary related proteins that share a 200-amino acid signature motif (hematopoietic interferon-inducible nuclear, HIN). IFI16 displays two HIN domains separated by a spacer region the length of which is the result of an alternative mRNA splicing leading to the production of three IFI16 isoforms (A, the predominant isoform; B and C; **3**) detectable in several cell types [1]. IFI16 protein localizes both in nucleus and cytoplasm and it is able to either homodimerize or heterodimerize with different partners including BRCA1, TP53, ASC, RB and STING [3, 4, 5, 6, 7, 8]. It is noteworthy that IFI16 binds dsDNA sugar phosphate backbone through its positive charged residues in the HIN domain [9, 10]. Several reports indicated that IFI16 can be considered a DNA sensor involved both in the innate immune response, in particular against viral infections, including HSV-1, HCMV and HIV-1 [11, 12, 13, 14, 15, 16, 17] and in the activation of the interferon gene pathway. Indeed, IFI16 is required for the cGAMP-induced activation of STING, promoting its phosphorylation and translocation [18]. IFI16 and cGAS are involved in the activation of the innate immune response to infections by DNA viruses [6, 16]. The biological role of IFI16 in the cellular biology has been demonstrated by several studies showing that IFI16 regulates

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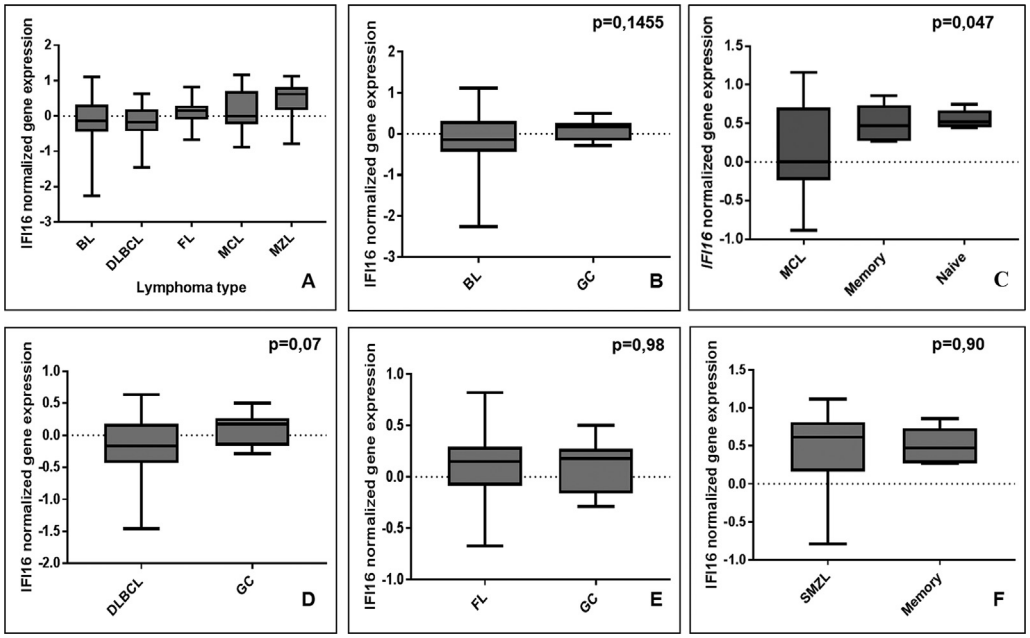

Fig. 1. IFI16 gene expression in lymphoma subtypes. Five NHL tumors were compared with their normal counterparts in terms of IFI16 expression, among which only MCL showed altered expression of IFI16.

proliferation, differentiation, apoptosis/pyroptosis, senescence and inflammation [19, 20, 21, 22, 23, 24, 25, 26]. More specifically, IFI16 has been shown to down-regulate cell proliferation and to elicit apoptosis in several cell types [21, 23, 26]. Consistently, IFI16 negatively regulates the cell cycle through binding and functional modulation of several molecules involved in the cell cycle regulation such as TP53, RB, and p21 [21, 25, 26]. The relationship between IFI16 and differentiation was pointed out during the differentiation of CD34 + hematopoietic stem cells to lymphoid and monocytic lineages [1, 27]. Furthermore, recent studies have shown that IFI16 expression is modulated in B-cell differentiation [28]. In particular, IFI16 expression is related to B-cell differentiation stages and it is significantly downregulated during both the transition from naive B-cell subsets to proliferating GC cells and the differentiation of GC-cells to plasma cells, whereas IFI16 expression

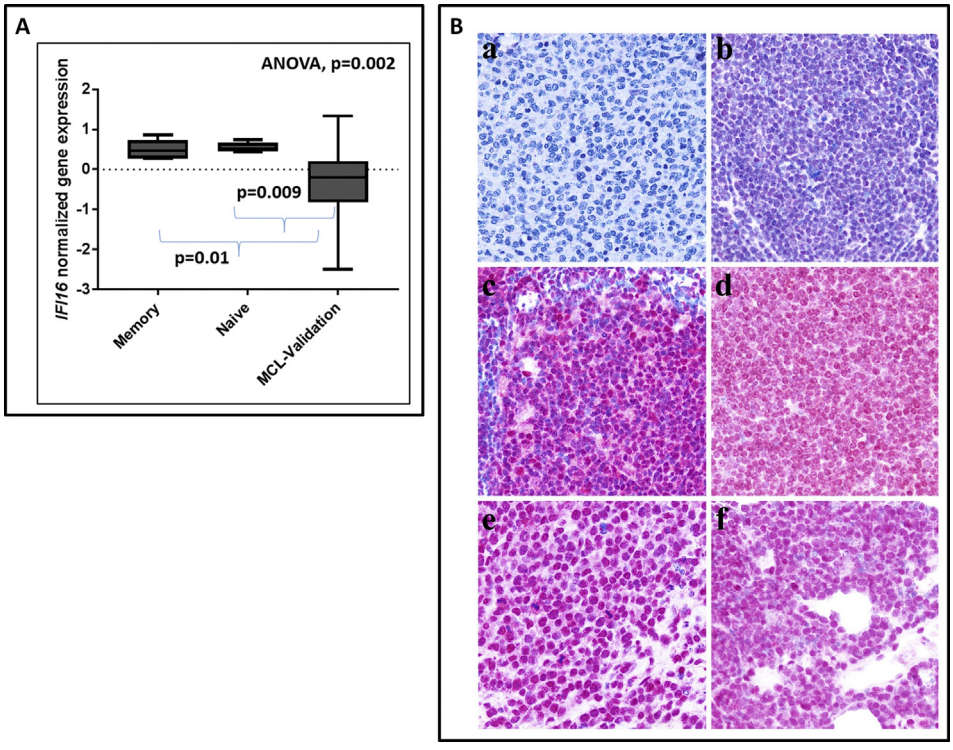

Fig. 2. IFI16 expression in validation cohorts. **A)** IFI16 gene expression in MCL. **B)** IFI16 protein expression at immunohistochemistry: mantle cell lymphoma a) negative and b) weak positive for IFI16 expression; c) follicular lymphoma, d) marginal zone lymphoma, e) diffuse large B-cell lymphoma and f) Burkitt lymphoma showing strong expression for IFI16.

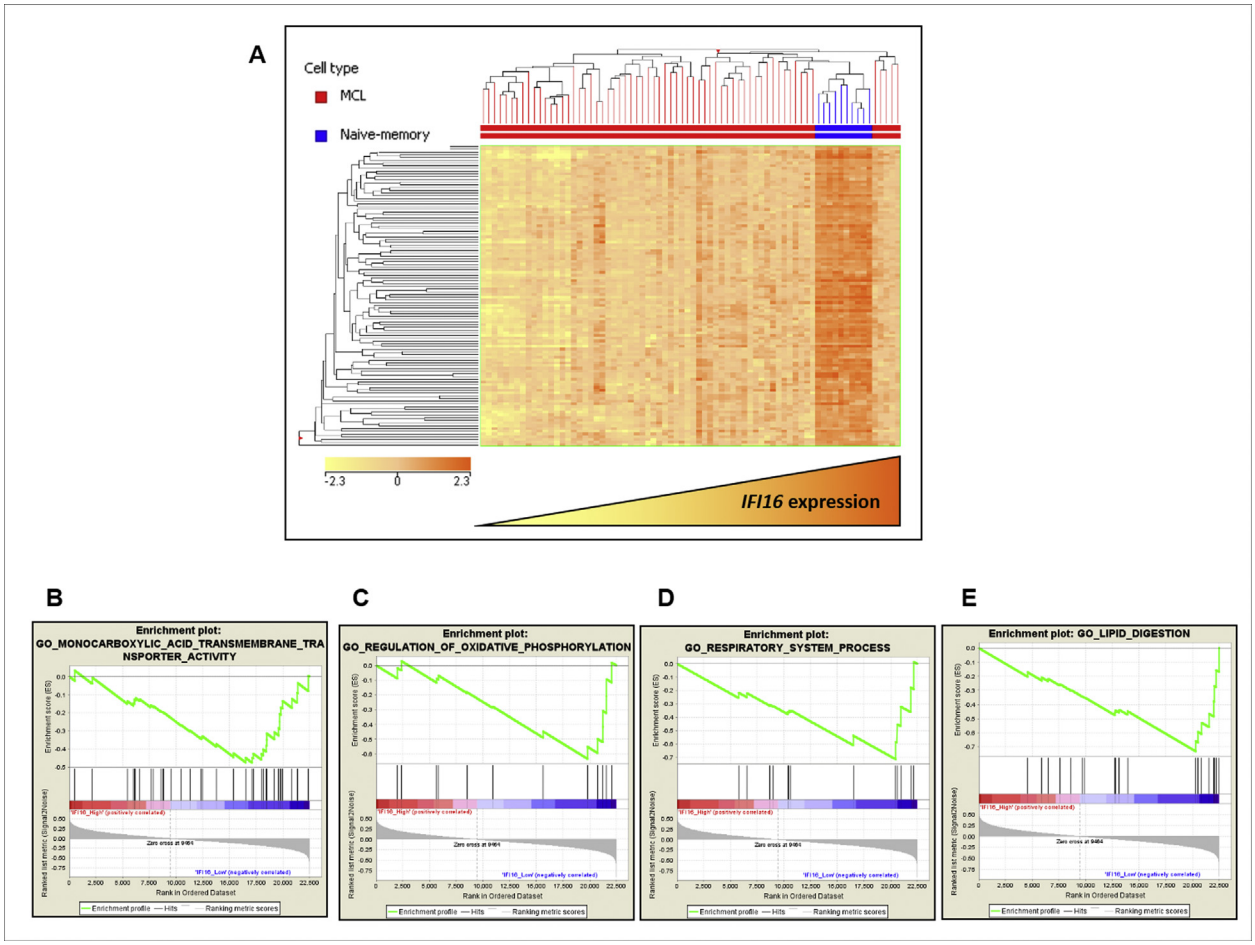

Fig. 3. Association of IFI16 expression with other genes and specific cellular functions. In A, Hierarchical clustering of MCL and normal B cells (Naive and memory) based on the expression of genes the expression of which is significantly related to that of IFI16 (Pearson correlation >0.5) is depicted. In Panel B–E, GSEA indicative of metabolic reprogramming in cases with reduced IFI16 expression is presented.

increases during the shift from GC cells to memory cells [28]. IFI16 expression is believed to have an intriguing relationship with key transcription factors, including BCL6, NF-κB, STAT3, and STAT5, involved in central processes of the B-cell biology [28, 29]. Interestingly, gene expression profiling analysis showed that IFI16 expression was inversely related to the expression of the transcription factor BCL6, a master regulator of GC constitution [28, 29]. IFI16 was also studied as prognostic biomarkers in chronic lymphocytic leukemia (CLL) patients. Interestingly, reduced IFI16 expression was linked to a worse clinical outcome when ZAP70 and CD38 molecules were expressed. Probably, the lack of IFI16, associated with an abridged antiproliferative effect, might be particularly severe in cells constitutively receiving an activation/proliferation signaling. Therefore, the balance between IFI16 expression and activation signaling (mediated by ZAP70/CD38) may be a more reliable prognostic parameter [30].

Table 1
Descriptive statistics for *IFI16* gene expression in lymphoma subtypes.

	Count	Mean	Std. Dev	Std. Err.
Burkitt lymphoma	46	-0.146	0.617	0.091
Diffuse large B-cell lymphoma/NOS	46	-0.19	0.448	0.066
Follicular lymphoma	40	0.104	0.306	0.048
Mantle cell lymphoma (training set)	22	0.123	0.595	0.127
Mantle cell lymphoma (validation set)	64	-0.375	0.766	0.096
Marginal zone lymphoma	24	0.472	0.456	0.093
Germinal center B-cells	10	0.102	0.251	0.079
Memory B-cells	5	0.498	0.242	0.108
Naïve B-cells	5	0.544	0.074	0.033

In this study, we aimed to assess the expression of IFI16 at gene and protein levels in a large series of B-cell derived non-Hodgkin lymphomas (NHLs), aiming to assess IFI16 expression as a possible prognostic marker in these NHLs.

2. Materials and methods

2.1. Case series

We studied the expression of *IFI16* gene in a discovery cohort of 198 cases for which gene expression profiles (GEPs) were previously generated (GSE12195) [31]. This data is available on GEO Profiles database (Gene Expression Omnibus of the National Center for Biotechnology Information-NCBI) which stores gene expression profiles derived from

Table 2
Immunohistochemical evaluation of IFI16 protein expression.

Lymphoma type	Number of cases	Positive cases (>20% cells) (%)	Staining intensity grade
Burkitt lymphoma	11	8 (72.7)	Strong
Diffuse large B-cell lymphoma	26	15 (57.7)	Strong
Follicular lymphoma	24	8 (33.3)	Strong/Weak*
Mantle cell lymphoma	32	19 (59.4)	Weak
Marginal zone lymphoma	13	4 (30.8)	Strong

* 4 cases regular; 4 cases reduced.

Table 3
Genes whose expression is significantly related to that of *IFI16* (Pearson correlation >0.5).

Gene Symbol	Entrez Gene	Pearson correlation	Validated expression in MCL
ACTR2	10097	0,669208933	
ANP32E	81611	0,627841533	
ARF6	382	0,641927687	
BANK1	55024	0,67225964	
BAZ1A	11177	0,630230747	
BBIP1	92482	0,605706487	
BCL11A	53335	0,569828467	Liu H et al.,2006
BCLAF1	9774	0,68433418	
BLOC1S2	282991	0,6491966	
CD22	933	0,618896733	Jovanovic D et al., 2014
CDC42	998	0,576680253	
CEP350	9857	0,617435543	
CNOT6L	246175	0,58337405	
COLCA1	399948	0,616912253	
CTSS	1520	0,638810313	Magunacelaya NM et al.,2004
DAPP1	27071	0,635009267	
DDI2	84301	0,571980767	
DEGS1	8560	0,574676047	
DICER1	23405	0,592437383	
DNAJA2	10294	0,648920167	
EIF3M	10480	0,560212283	
EIF4G3	8672	0,573264233	
ERBB2IP	55914	0,70120698	
FGD2	221472	0,607602133	Norihiko Kawamata et al., 2005
FGD2	221472	0,586247077	Norihiko Kawamata et al., 2005
GNAI3	2773	0,595270897	
GPR18	2841	0,542490437	Henson SE, 2011 and Zhu et al., 2001
GVINP1	387751	0,6061212	
HHEX	3087	0,61690237	Nagel S et al., 2018
HHEX	3087	0,559629767	Nagel S et al., 2018
HNRNPU	3192	0,679190433	
IAH1	285148	0,682675547	
IFNGR1	3459	0,610652857	
IL10RB	3588	0,61914352	
KIAA0922	23240	0,610119713	
KIAA1551	55196	0,620209807	
KRAS	3845	0,601086023	
LNPEP	4012	0,633301233	
MGAT2	4247	0,564773553	
MOB1A	55233	0,565978067	
NCOA3	8202	0,66112306	
NCOA3	8202	0,631385923	
NIFK	84365	0,628177233	
NIPBL	25836	0,682132577	
NUDT4/// NUDT4P1/// NUDT4P2	11163/// 170688/// 440672	0,55685546	
OPHN1	4983	0,576196457	
P2RY10	27334	0,647883497	
P2RY10	27334	0,606733317	
PDIA6	10130	0,549549553	
PGGT1B	5229	0,6341306	
PPP1CB	5500	0,618264833	Velusamy et al.,2013
PRKD3	23683	0,562186867	
PRPS2	5634	0,680414633	
PRRC2C	23215	0,66378871	
PRRC2C	23215	0,6341602	
PRRC2C	23215	0,625491777	
PTP4A2	8073	0,60902381	
PTPRC	5788	0,644289767	Carulli G et al., 2008
RAPGEF6	51735	0,578388253	
RASEF	158158	0,60727635	
RHOQ	23433	0,699054647	
RHOQ	23433	0,630388733	
RHOQ	23433	0,573807233	
RIOK3	8780	0,603100097	
RPL38	6169	0,553390113	
SAMD9	54809	0,54420831	
SCAF11	9169	0,577746523	
SEC22B	9554	0,699726023	
SLC25A16	8034	0,6782821	
SNX2	6643	0,610563997	
SNX5	27131	0,66154758	
SP110	3431	0,61964704	
SP110	3431	0,608826357	
SRSF10	10772	0,651625337	
ST8SIA4	7903	0,587273833	
STK4	6789	0,63018144	
SUMO4	387082	0,64389485	
TMED8	283578	0,660115983	
TOR1AIP1	26092	0,617741593	
TRAK2	66008	0,628364817	
TRIM38	10475	0,6677971	
TRIM38	10475	0,635720103	
UBXN4	23190	0,6740565	
UBXN7	26043	0,5930396	
WSB1	26118	0,633321007	
WTAP	9589	0,676307537	
YWHAZ	7534	0,61512527	
ZC3H7B	23264	0,53514501	
ZNF106	64397	0,612370733	
ZNF207	7756	0,586681463	
ZNF430	80264	0,631612967	
ZNF652	22834	0,61678393	
ZNF665	79788	0,6342984	

curated GEO DataSets. Specifically, we analyzed 46 Burkitt lymphoma (BL), 46 diffuse large B-cell lymphoma (DLBCL), 40 follicular lymphoma (FL), 24 marginal zone lymphoma (MZL), and 22 mantle cell lymphoma (MCL) cases. Furthermore, 20 samples representative of normal B-cell subsets (10 germinal center, GC, 5 naïve, and 5 memory) were also analyzed. In addition, we studied a validation cohort of cases including 86 MCL cases for which GEP were available at the NCBI GEO database (Accession Numbers: GSE21452 and GSE16455) as well as 106 cases for which formalin fixed paraffin embedded (FFPE) tissue blocks were retrieved at the Hematopathology Unit of S. Orsola-Malpighi Hospital, Bologna University, Bologna, Italy, and used for histopatological examination. The latter cases included 11 BL, 26 DLBCL, 24 FL, 13 MZL, and 32 MCL. All diagnoses were made according to the World Health Organization (WHO) classification.

2.2. Immunohistochemistry 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 [28, 30]. Punches were performed on areas mainly represented by neoplastic cells based on morphological and immunophenotypic evaluation. Tissue microarrays (TMAs) were then prepared for immunohistochemistry.

We studied IFI16 expression by immunohistochemistry (IHC) on TMAs including 32 cases (in duplicate cores). From each recipient block, 1.5 μm-thick sections were cut and tested with anti-IFI16 mouse monoclonal antibody (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.

Table 4
Gene set enrichment analysis of genes related to *IFI16*.

Gene set category	Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
Hallmark	ALLOGRAFT REJECTION	200	6	0,03	1,25E-06	3,12E-05
	INTERFERON GAMMA RESPONSE	200	6	0,03	1,25E-06	3,12E-05
	MITOTIC SPINDLE	200	4	0,02	3,86E-04	6,43E-03
	PROTEIN SECRETION	96	3	0,0312	6,00E-04	7,50E-03
Gene Ontology Biological Process	CELLULAR MACROMOLECULE LOCALIZATION	1234	15	0,0122	2,25E-09	1,33E-05
	PROTEIN LOCALIZATION	1805	17	0,0094	7,54E-09	2,23E-05
	ESTABLISHMENT OF LOCALIZATION IN CELL	1676	16	0,0095	1,85E-08	3,65E-05
	CELL SUBSTRATE JUNCTION	398	9	0,0226	2,70E-08	3,99E-05
	CYTOSKELETON ORGANIZATION	838	11	0,0131	1,78E-07	2,11E-04
	CELL PROJECTION ORGANIZATION	902	11	0,0122	3,68E-07	3,63E-04
	RIBONUCLEOTIDE BINDING	1860	15	0,0081	4,78E-07	4,04E-04
	ESTABLISHMENT OF PROTEIN LOCALIZATION	1423	13	0,0091	7,75E-07	5,63E-04
	EARLY ENDOSOME	301	7	0,0233	8,56E-07	5,63E-04
	POSTTRANSCRIPTIONAL REGULATION OF GENE EXPRESSION	448	8	0,0179	9,90E-07	5,86E-04

Immunohistochemistry staining was 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%. A Semi-quantitative scoring system was used to assess staining intensity and graded as "strong", "weak", and "absent" [32].

Micrographs were obtained using an Olympus BX61 microscope equipped with an Olympus DP-70 digital camera. Image acquisition, evaluation and color balance were performed using by CellF software [28, 30].

2.3. Gene expression analyses

Gene expression analysis was carried out as previously reported [31, 33, 34, 35, 36, 37, 38]. Analysis of GEP data in terms of supervised and unsupervised analysis was achieved using GeneSpring GX 12 (Agilent Technologies, Santa Clara, CA, USA). All data were obtained using Affymetrix HG-U133 2.0 plus microarrays (Affymetrix, Inc. http://www.affymetrix.com/support/index.affx). Briefly, the expression value of each selected gene was normalized to have a zero mean value and unit standard deviation. The distance between two individual samples was determined by Pearson correlation using the normalized expression values. Unsupervised clustering was generated using a hierarchical algorithm based on the average-linkage method. To perform the supervised gene expression analysis, differentially expressed genes between different groups were identified using a two-tails Student t-test and adjusted Benjamini-Hochberg correction for false discovery rate, applying the following filtering criteria: p-value $\leq$0.05, and fold change $\geq$2.

Gene Set Enrichment Analysis (GSEA) was performed to better understand the potential biological significance of the identified molecular signatures. Briefly, GSEA (also known as *functional enrichment analysis*) is a method to identify classes of genes that are over-represented in a large set of genes (molecular or gene signature) and may have a functional association with disease phenotypes [39]. GSEA of the interested gene sets was performed in terms of Gene Ontology (GO) Biological Processes, Curated Gene Sets and Hallmark Genes using GSEA MsigDB (www.broadinstitute.org/gsea/msigdb) web-based analysis tool [39], setting the options to the default (displaying top 10 gene sets with FDR q-value below 0.05).

When 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 HG-U133 2.0 plus microarray. The median value from the three probes was used for the analysis.

2.4. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 20.0 and Prism (GraphPad softwares, USA). ANOVA and unpaired T-tests were employed. When a sample size was less than 10 cases in at least 1 group, a non-parametric (Mann-Whitney) test was used to analyze the GEP data to compare *IFI16* expression in different subgroups. Survival analyses were performed by Kaplan-Meier method. Two-sided tests were used in all calculations. The limit of significance for all analyses was defined as $p \leq 0.05$.

3. Results and discussion

3.1. IFI16 gene expression is reduced in mantle cell lymphoma

We studied *IFI16* gene expression in the cohort of lymphoma cases, including 46 BL, 46 DLBCL, 40 FL, 24 MZL, and 22 MCL cases (Fig. 1A). Particularly, we compared gene expression between each tumor type and the corresponding cellular counterpart (i.e. GC cells for BL, FL, and DLBCL and Naïve and/or Memory B-cells for MCL and MZL). Only MCL showed a significant deregulation when compared to the non-neoplastic components (0.123 vs. 0.525; standard error 0.127 vs. 0.059, p = 0.047; Fig. 1B–F, Table 1). It should be noted that MCL were compared to both naïve and memory B-cell compartments that represent the postulated counterparts of unmutated and mutated MCL cases, respectively. Since our cases were not distinct based on the immunoglobulin mutational status, naïve/memory subsets have comparable levels of *IFI16* [28], and *IFI16* is not differentially expressed in mutated vs. unmutated MCL cases [40], we were confident that this approach could be considered reliable.

Table 5
Gene differentially expressed in cases with higher (75° percentile) vs. lower (25° percentile) *IFI16* gene expression (T-test, $p < 0.05$; fold change >2).

Probe Set ID	Gene Symbol	Corrected pvalue	FC (absolute)	Regulation in Low
229128_s_at	*ANP32E*	0,002372331	2,6861453	Down
209642_at	*BUB1*	0,012277976	2,1282046	Down
231862_at	*CBX5*	0,001654886	2,0196693	Down
208711_s_at	*CCND1*	0,004513383	2,067266	Down
205789_at	*CD1D*	0,004146486	2,0740635	Down
224428_s_at	*CDCA7*	0,003771309	2,0563602	Down
219375_at	*CEPT1*	1,66E-04	2,099846	Down
213060_s_at	*CHI3L2*	0,00131052	2,8916368	Down
230128_at	*CKAP2*	0,015628582	3,0252607	Down
203641_s_at	*COBLL1*	0,007359529	2,1361105	Down
203642_s_at	*COBLL1*	0,005629572	2,5396304	Down
1562836_at	*DDX6*	0,02511044	2,0845397	Down
212503_s_at	*DIP2C*	9,26E-04	2,212788	Down
222850_s_at	*DNAJB14*	0,005629572	2,0553403	Down
219990_at	*E2F8*	0,017218044	2,1936398	Down
1555996_s_at	*EIF4A2*	0,028180348	2,023231	Down
206102_at	*GINS1*	0,001741233	2,1084065	Down
221922_at	*GPSM2*	0,002576029	2,0255637	Down
220085_at	*HELLS*	0,003709542	2,3137715	Down
200679_x_at	*HMGB1*	4,27E-04	2,002743	Down
230621_at	*IAH1*	1,53E-04	2,028879	Down
206332_s_at	*IFI16*	2,07E-10	2,352239	Down
208965_s_at	*IFI16*	8,91E-04	2,1735976	Down
208966_x_at	*IFI16*	3,93E-10	2,4121406	Down
203819_s_at	*IGF2BP3*	0,008682512	4,002813	Down
203820_s_at	*IGF2BP3*	0,010148156	4,122018	Down
223176_at	*KCTD20*	0,0013064	2,0591123	Down
227152_at	*KIAA1551*	0,00707586	2,0117857	Down
210313_at	*LILRA4*	0,022168344	2,321087	Down
235060_at	*LOC100190986*	0,001798373	2,1002595	Down
235167_at	*LOC100190986*	0,009055208	2,0777555	Down
229026_at	*LOC105379173*	0,020182207	2,4816926	Down
230793_at	*LRRC16A*	0,002684468	2,303797	Down
201151_s_at	*MBNL1*	0,020108812	2,033717	Down
222036_s_at	*MCM4*	4,09E-04	2,1149127	Down
226880_at	*NUCKS1*	1,65E-04	2,667727	Down
223381_at	*NUF2*	0,003363432	2,2747343	Down
219148_at	*PBK*	0,014257969	2,133363	Down
201202_at	*PCNA*	2,63E-04	2,0165021	Down
212094_at	*PEG10*	0,021157807	2,0085702	Down
204285_s_at	*PMAIP1*	0,002559309	2,0691586	Down
230352_at	*PRPS2*	6,56E-05	2,2383318	Down
228273_at	*PRR11*	0,011947047	2,3246174	Down
229147_at	*RASSF6*	0,009268034	3,8838599	Down
233463_at	*RASSF6*	0,003219189	2,6172767	Down
235638_at	*RASSF6*	0,007068038	2,7792645	Down
228455_at	*RBM15*	0,002473682	2,485874	Down
212027_at	*RBM25*	0,005629572	2,0230834	Down
228996_at	*RC3H1*	8,88E-04	2,6803448	Down
214257_s_at	*SEC22B*	1,24E-04	2,0202684	Down
221268_s_at	*SGPP1*	0,033115014	2,0768883	Down
223391_at	*SGPP1*	0,01181035	2,085591	Down
206108_s_at	*SRSF6*	0,002559309	2,4004695	Down
209754_s_at	*TMPO*	0,010170757	2,312462	Down
223949_at	*TMPRSS3*	0,02754625	2,1164422	Down
201291_s_at	*TOP2A*	0,028759345	2,3533485	Down
228588_s_at	*UBE2B*	4,92E-04	2,2184792	Down
235003_at	*UHMK1*	0,002675724	2,2043898	Down
206133_at	*XAF1*	0,017114462	2,0860553	Down
222816_s_at	*ZCCHC2*	0,002387533	2,2362235	Down
205739_x_at	*ZNF107*	0,00147799	2,0554366	Down
243495_s_at	*ZNF652*	0,002730198	2,3722727	Down
236033_at	*ASB12*	1,58E-04	2,0688426	Up
1552354_at	*CBARP*	1,66E-04	2,162268	Up
201289_at	*CYR61*	0,027891243	2,8001204	Up
210764_s_at	*CYR61*	0,035330884	2,2227159	Up
1565483_at	*EGFR*	9,60E-05	4,008361	Up
1565484_x_at	*EGFR*	1,58E-04	2,8769505	Up
1564069_at	*HOTTIP*	1,74E-04	2,0109012	Up
1553657_at	*LOC101930115/// VWA3A*	9,50E-05	2,086649	Up
1559502_s_at	*LRRC25*	1,37E-04	2,1343188	Up
230813_at	*P3H2*	3,33E-04	2,3854458	Up
1557680_at	*SAMD15*	3,33E-04	2,2336347	Up
1568249_at	*SNHG17/// SNORA71B*	1,97E-04	2,6449728	Up
1558641_at	*SPATA24*	5,49E-04	2,0069237	Up
1569206_at	*TCP11L2*	8,09E-05	2,2803137	Up
1554400_at	*TCTE3*	1,26E-04	2,482754	Up
1557450_s_at	*WHAMMP2*	3,17E-04	2,0680783	Up
1553718_at	*ZNF548*	2,81E-04	2,12732	Up

A trend toward a reduced expression was also observed in DLBCL; however, the difference was not significant ($p = 0.07$). Of note, DLBCL were compared to GC only, GEP from plasmablasts, the postulated counterpart of ABC-type DLBCL not being available. Nonetheless, this choice did not seem to affect the analysis since ABC and GCB-type DLBCL did not show differential *IFI16* gene expression [41].

Thereafter, we studied *IFI16* expression in a validation cohort of cases. When *IFI16* gene expression levels were studied in 64 MCL vs. naïve ($N = 5$) and memory ($N = 5$) B-cell samples, an even more evident difference was observed (-0.375 vs. 0.498 vs. 0.544, ANOVA, p-value = 0.002; Fig. 2A). In fact, again, *IFI16* expression levels were higher than in memory ($p = 0.01$) and naïve ($p = 0.009$) B-cell subtypes.

To improve the strength of these data, we also studied IFI16 protein expression by immunohistochemistry in a series consisting of 11 BL, 26 DLBCL, 24 FL, 13 MZL, and 32 MCL. We found a variable degree of expression with positive cases ranging from 31% in MZL to 73% in BL. Concerning MCL, 19/32 cases were positive (59%). Noteworthy, the intensity of the staining was strong in all positive cases with the exception of all MCL and a few FL, in which the staining was weak (Fig. 2B; Table 2). A similar pattern has also been observed in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), the first lymphoid neoplasm in which a weak IFI16 expression was documented [30]. Overall, these data indicate that MCL significantly down-regulates IFI16, even in cases that do not completely abolish its expression.

3.2. *IFI16* gene down-regulation is associated with increased proliferation and altered metabolism in MCL

We sought to assess whether the expression of IFI16 was associated with the expression of other genes and specific cellular functions. To do so, we studied the transcriptome of B-NHL and normal B-cell subsets, looking for genes with expression levels correlated to those of *IFI16*. We found 93 genes whose expression was significantly related to that of *IFI16* (Pearson correlation >0.5; Fig. 3A; Table 3). Interestingly, at GSEA, they turned out to be significantly involved in interferon response and allograft rejection (as expected based on IFI16 known functions), as well as in other biological processes (Table 4). Of interest, these findings were, at least in part, validated by the evidence that several of the identified molecules are indeed expressed in MCL, showing sometimes a reduced expression mirroring that of IFI16, including *CD22, BCL11A, FGD2, GPR18, PTPRC, HHEX, PPP1CB*, and *CTSS* [42, 43, 44, 45, 46, 47].

We then compared the cases with the highest (25^{th} percentile) or lowest (25^{th} percentile) *IFI16* gene expression with supervised analysis. We found 79 genes differentially expressed in the two groups (Table 5); by GSEA they turned out to be significantly involved in cell cycle, cell death and proliferation as well as in other programs (Table 6). Furthermore, GSEA indicated that the two groups significantly differed as far as certain biological processes related to metabolism were concerned. Particularly, both lipid and glucose metabolism appeared affected (Fig. 3B–E; Table 6). Overall, these data indicated that IFI16 deregulation was associated with relevant changes in cell biology.

Table 6
Gene set enrichment analysis of genes differentially expressed in cases (referred to Gene Ontology Biological Process gene sets).

Gene Set Name	p-value	FDR q-value
GO LIPID DIGESTION	0	1
GO RESPIRATORY SYSTEM PROCESS	0	0,981
GO REGULATION OF SYNCYTIUM FORMATION BY PLASMA MEMBRANE FUSION	0	1
GO REGULATION OF MYOBLAST FUSION	0	0,737
GO RESPONSE TO MAGNESIUM ION	0	0,691
GO HYPEROSMOTIC RESPONSE	0	0,893
GO MONOCARBOXYLIC ACID TRANSMEMBRANE TRANSPORTER ACTIVITY	0	1
GO SYNAPTIC VESICLE LOCALIZATION	0	1
GO MITOTIC CELL CYCLE	4,26E-10	1,89E-06
GO CELL PROLIFERATION	1,56E-09	2,08E-06
GO CELL CYCLE PROCESS	1,80E-09	2,08E-06
GO CELL CYCLE	1,88E-09	2,08E-06
GO CELL DEATH	8,49E-09	7,53E-06
GO RESPONSE TO UV	2,04E-08	1,51E-05
GO POSITIVE REGULATION OF DNA METABOLIC PROCESS	2,00E-07	1,27E-04
GO NEGATIVE REGULATION OF NITROGEN COMPOUND METABOLIC PROCESS	7,52E-07	4,17E-04
GO CHROMOSOME ORGANIZATION	9,53E-07	4,70E-04
GO RESPONSE TO RADIATION	1,45E-06	6,41E-04
GO MRNA METABOLIC PROCESS	1,68E-06	6,64E-04
GO POSITIVE REGULATION OF RESPONSE TO DNA DAMAGE STIMULUS	1,80E-06	6,64E-04
GO RESPONSE TO LIGHT STIMULUS	2,24E-06	7,64E-04
GO IN UTERO EMBRYONIC DEVELOPMENT	4,09E-06	1,28E-03
GO NEGATIVE REGULATION OF GENE EXPRESSION	4,55E-06	1,28E-03
GO DNA GEOMETRIC CHANGE	4,62E-06	1,28E-03
GO REGULATION OF CELL CYCLE	5,01E-06	1,31E-03
GO REGULATION OF DNA METABOLIC PROCESS	6,81E-06	1,68E-03
GO POSITIVE REGULATION OF TRANSCRIPTION FROM RNA POLYMERASE II PROMOTER	7,87E-06	1,84E-03
GO REGULATION OF CELLULAR AMIDE METABOLIC PROCESS	8,56E-06	1,90E-03
GO REGULATION OF RNA SPLICING	9,47E-06	2,00E-03
GO DNA DEPENDENT DNA REPLICATION	1,03E-05	2,07E-03
GO REGULATION OF ALTERNATIVE MRNA SPLICING VIA SPLICEOSOME	1,48E-05	2,86E-03
GO POSITIVE REGULATION OF DNA REPAIR	1,91E-05	3,52E-03
GO REGULATION OF MRNA METABOLIC PROCESS	2,05E-05	3,64E-03
GO NEGATIVE REGULATION OF CELL CYCLE	2,66E-05	4,39E-03
GO POSITIVE REGULATION OF BIOSYNTHETIC PROCESS	2,67E-05	4,39E-03
GO MACROMOLECULE CATABOLIC PROCESS	3,41E-05	5,24E-03
GO DNA CONFORMATION CHANGE	3,50E-05	5,24E-03
GO CELL DIVISION	3,73E-05	5,24E-03
GO PLACENTA DEVELOPMENT	3,79E-05	5,24E-03
GO NEGATIVE REGULATION OF CELLULAR COMPONENT ORGANIZATION	3,80E-05	5,24E-03
GO MITOTIC CELL CYCLE CHECKPOINT	3,90E-05	5,24E-03
GO REGULATION OF RESPONSE TO DNA DAMAGE STIMULUS	4,60E-05	6,00E-03
GO CELLULAR RESPONSE TO DNA DAMAGE STIMULUS	5,24E-05	6,64E-03
GO ORGANELLE FISSION	5,65E-05	6,79E-03
GO REGULATION OF CATABOLIC PROCESS	5,77E-05	6,79E-03
GO POSITIVE REGULATION OF PEPTIDASE ACTIVITY	5,81E-05	6,79E-03
GO NUCLEAR TRANSCRIBED MRNA CATABOLIC PROCESS DEADENYLATION DEPENDENT DECAY	6,50E-05	7,39E-03
GO DNA METABOLIC PROCESS	7,23E-05	8,02E-03
GO LEUKOCYTE HOMEOSTASIS	7,57E-05	8,19E-03
GO REGULATION OF MRNA SPLICING VIA SPLICEOSOME	8,35E-05	8,82E-03
GO POSITIVE REGULATION OF CELL CYCLE	8,82E-05	9,10E-03
GO POSITIVE REGULATION OF GENE EXPRESSION	1,02E-04	1,02E-02
GO EMBRYO DEVELOPMENT ENDING IN BIRTH OR EGG HATCHING	1,04E-04	1,02E-02
GO REGULATION OF CELL CYCLE PROCESS	1,08E-04	1,04E-02
GO CELL CYCLE DNA REPLICATION	1,17E-04	1,08E-02
GO NEGATIVE REGULATION OF CD4 POSITIVE ALPHA BETA T CELL DIFFERENTIATION	1,17E-04	1,08E-02
GO REGULATION OF TRANSCRIPTION FROM RNA POLYMERASE II PROMOTER	1,29E-04	1,17E-02
GO IMMUNE SYSTEM DEVELOPMENT	1,35E-04	1,20E-02
GO REGULATION OF DENDRITE EXTENSION	0,002	0,948
GO POSITIVE REGULATION OF DENDRITE EXTENSION	0,002	0,812
GO RETINOL METABOLIC PROCESS	0,002	0,727
GO METANEPHROS MORPHOGENESIS	0,002	0,731
GO REGULATION OF CILIUM MOVEMENT	0,004	1
GO REGULATION OF OXIDATIVE PHOSPHORYLATION	0,004	0,886
GO TAU PROTEIN BINDING	0,004	0,731
GO E BOX BINDING	0,004	0,758
GO CELLULAR PIGMENT ACCUMULATION	0,004	0,744
GO PIGMENT ACCUMULATION	0,004	0,682
GO MODIFIED AMINO ACID BINDING	0,004	1
GO ISOPRENOID BINDING	0,004	1

3.3. *IFI16 expression is not related to clinical aggressiveness and survival in MCL*

Since IFI16 downregulation appeared to be associated with relevant cellular function modifications [19, 20, 21, 22, 23, 24, 25, 26, 28], we investigated whether its expression was associated with clinical features. *IFI16* gene expression did not significantly differ in indolent (N = 7) vs. classical (N = 15) MCL cases (p = 0.67) (Fig. 4A). Similarly, *IFI16* gene expression was not associated with overall survival when it was evaluated as either continuous variable (Cox-regression, p = 0.785), or by 50th percentiles (p = 0.5, Fig. 4B), or by quartile sub-grouping (p = 0.647, Fig. 4C). This was somehow surprising as MCL prognosis is strictly associated with proliferation [48], which in turn appeared to be associated with IFI16. However, in CLL the prognostic value of IFI16 reduction was limited to the subgroups of patients presenting with CD38 and/or ZAP70 expression [30]. Therefore, we cannot exclude that further investigation in MCL will unveil a prognostic value for this molecule.

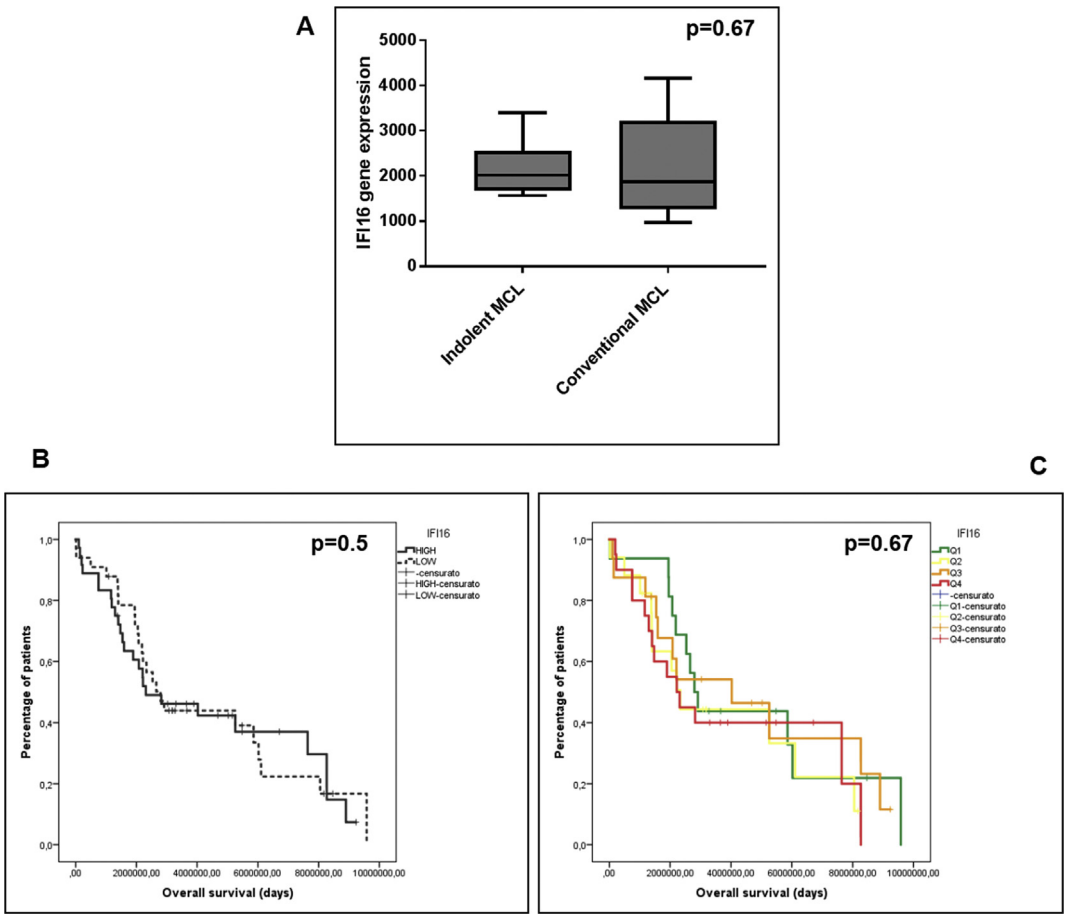

Fig. 4. IFI16 gene expression is not related to clinical aggressiveness and survival in MCL patients. In A, IFI16 gene expression in indolent (N = 7) vs classic (N = 15) MCL cases; in B, overall survival based on IFI16 gene expression (50° percentiles, high vs low); in C, overall survival based on IFI16 gene expression (quartiles, Q1 the lowest, Q4 the highest values).

4. Conclusion

In conclusion, we found that IFI16 is significantly downregulated in MCL, being associated with relevant cellular modifications such as proliferation increase and metabolism activation. Nonetheless the possible functional value of IFI16 down-regulation remains to be assessed and further studies need to be performed to determine its real role in MCL. Similarly, a more extensive evaluation of its prognostic value, in perspective homogeneous series should be assessed. In fact, our study was not specifically designed for clinical correlates, the series being not homogenous and the treatment variable. Finally, the specific potential impact of IFI16 as a diagnostic marker might be assessed in diagnostic accuracy study. In fact, though probably not useful for differentiating MCL from CLL (similarly down regulating it), it might be more relevant for other differential diagnoses such as, for example, CCND1- MCL vs. CD5+ DLBCL, in addition to SOX11.

Declarations

Author contribution statement

P. P. Piccaluga, D. Gibellini: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M. Navari, A. Visani, C. Agostinelli, S. Righi, E. Diani, M.Ligozzi, M. Carelli, C. Ponti, I. Bon, D. Zipeto, S. Landolfo: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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