SOXC and MiR17-92 Gene Expression Profiling Defines Two Subgroups with Different Clinical Outcome in Mantle Cell Lymphoma

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Mantle cell lymphoma (MCL) is a heterogeneous B-cell lymphoid malignancy where most patients follow an aggressive clinical course whereas others are associated with an indolent performance. *SOX4*, *SOX11*, and *SOX12* belong to *SOXC* family of transcription factors involved in embryonic neurogenesis and tissue remodeling. Among them, *SOX11* has been found aberrantly expressed in most aggressive MCL patients, being considered a reliable biomarker in the pathology. Several studies have revealed that microRNAs (miRs) from the *miR-17-92* cluster are among the most deregulated miRNAs in human cancers, still little is known about this cluster in MCL. In this study we screened the transcriptional profiles of 70 MCL patients for *SOXC* cluster and *miR17*, *miR18a*, *miR19b* and *miR92a*, from the *miR-17-92* cluster. Gene expression analysis showed higher *SOX11* and *SOX12* levels compared to *SOX4* ($P \leq 0.0026$). Moreover we found a negative correlation between the expression of *SOX11* and *SOX4* ($P < 0.0001$). *miR17-92* cluster analysis showed that *miR19b* and *miR92a* exhibited higher levels than *miR17* and *miR18a* ($P < 0.0001$). Unsupervised hierarchical clustering revealed two subgroups with significant differences in relation to aggressive MCL features, such as blastoid morphological variant ($P = 0.0412$), nodal presentation ($P = 0.0492$), $CD5^+$ ($P = 0.0004$) and shorter overall survival ($P < 0.0001$). Together, our findings show for the first time an association between the differential expression profiles of *SOXC* and *miR17-92* clusters in MCL and also relate them to different clinical subtypes of the disease adding new biological information that may contribute to a better understanding of this pathology.

INTRODUCTION

Mantle cell lymphoma (MCL) is a clinically and biologically heterogeneous disease characterized by genetic instability and numerous oncogenic events. Its genetic hallmark is the translocation t(11;14)(q13;q32), which juxtaposes the *Cyclin D1* gene to the *IGH* (immunoglobulin heavy chain) locus, leading to *Cyclin D1* overexpression (Swerdlow et al., 2008). Consequences of *Cyclin D1* overexpression in MCL include cell cycle re-entry and progression, particularly since other cell cycle components such as CDK4 and CDK6 are also overexpressed in these tumors (Pérez-Galán et al., 2011). Additionally, most MCLs carry a high number of recurrent secondary chromosomal aberrations that contribute to the additional oncogenic events necessary for disease progression (Royo et al., 2011). These secondary abnormalities result in alterations of coding and non-coding genes involved in cell-cycle regulation, DNA damage

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response and survival signaling pathways among other oncogenic relevant mechanisms (Jares et al., 2007; Navarro et al., 2013; Musilova and Mraz, 2015). Although, in the last years, the median overall survival of MCL patients has improved considerably, it is still considered as one of the poorest prognoses malignancies among B-cell lymphomas (Campo and Rule, 2015). Generally, MCL displays an aggressive clinical behavior with poor response to current therapeutic strategies and a median survival ranging between 3 and 5 years. Nonetheless, some patients die of diseases associated features in <6 months, whereas others survive more than 10 years (Caballero et al., 2013). In this matter, in the past years, numerous attempts have been made to identify molecular biomarkers that allow the stratification of MCL patients into different risk groups.

Members of the *SOX* (*SRY box*-containing) gene family encode a group of transcription factors that achieve important tasks during cell fate determination and differentiation (Lefebvre et al., 2007). To date, more than 20 *SOX* gene family members have been identified and they are divided into eight subgroups (A-H) according to the degree of homology within and outside the DNA-binding high-mobility group (HMG) domain. *SOX4*, *SOX11*, and *SOX12* constitute the *SOXC* family of transcription factors that are coexpressed in embryonic neural progenitors and in mesenchymal cells in many developing organs (Penzo-Méndez, 2010). Among them, *SOX11*, is commonly associated with embryonic neurogenesis and tissue remodeling, and is not expressed under normal conditions in any adult normal tissue (Dy et al., 2008). Human *SOX11* is homologous to *SOX4* with 55% amino acid identity within the C-terminal transactivation domain and 86% identity for the HMG domain (Bergsland et al., 2006). *SOX4* is a prominent transcription factor involved in lymphocytes of both B- and T-cell lineage and is crucial for mice B lymphopoiesis (Schilham et al., 1996). In addition, it has been found to bind and regulate the promoter sequence of Dicer, a microRNA biogenesis factor, enhancing its activity (Jafarnejad et al., 2013). Similarly to its closest relatives *SOX4* and *SOX11*, *SOX12*, which is also extensively expressed during several development stages in mammals, is has also been involved in the differentiation and maintenance of numerous cell types during embryogenesis, and observed uniformly expressed in neural and mesenchymal tissue during embryonic development, as well as in the heart, liver, thymus, spleen and adult pancreas (Jay et al., 1997; Dy et al., 2008; Hoser et al., 2008). In contrast, *SOX11* and *SOX12* have no known lymphopoietic function and are not usually expressed in B cells. However, several studies have shown aberrant *SOX11* nuclear protein expression and mRNA levels in MCL patients, establishing that this gene could be a reliable biomarker to distinguish this malignancy from other lymphoid neoplasms (Ek et al., 2008; Dictor et al., 2009; Mozos et al., 2009).

MicroRNAs (miRNAs) are short (~22 nts) endogenous singled-stranded non-coding RNAs that play central regulatory functions in gene expression and transcriptional control by targeting messenger RNAs (mRNAs) and triggering its degradation and/or translational interference (Morris and Mattick, 2014). It is well known that miRNAs are mechanistically involved in several biological pathways, including development, cell proliferation, differentiation, survival, metabolism, genome stability, invasion and angiogenesis, which impact and affect tumor development and maintenance (Lin and Gregory, 2015). Some tumor-related miRNAs are also causally involved in oncogenesis due to their impact in deregulation of oncogenes and tumor suppressor genes, and may have prognostic significance as has been previously shown in certain lymphoid neoplasms (Fabbri and Croce, 2011). In line with this, a large amount of experimental data demonstrate the oncogenic properties of *miR17-92* cluster in both hematological malignancies and solid tumors, being involved in cell cycle and cell death regulation (Hayashita et al., 2005; Diosdado et al., 2009; Navarro et al., 2009). Moreover, the oncogenicity of this cluster may involve several mechanisms including the cooperation with the *MYC* oncogene to decrease apoptosis (Coller et al., 2007; Matsubara et al., 2007; Tagawa et al., 2007).

Therefore, the aim of the present study was to assess the importance of gene expression patterns of *SOXC* cluster and *miR17*, *miR18a*, *miR19b*, and *miR92a* genes, members of the cluster *miR17-92* in MCL patients. Gene expression profiles exhibited by our set of patients were analyzed and correlated with clinico-pathological characteristics of the disease.

MATERIALS AND METHODS

Patients

Our cohort consisted in FFPE (Formalin-Fixed Paraffin-Embedded) samples from 70 newly diagnosed MCL patients (41 males; median age: 64.5 years; range: 37–87 years), consecutively referred to our Institution. Diagnosis was based on morphology and immunophenotyping

TABLE 1. Clinical Features of Patients with MCL According to Clusters Obtained from SOXC and miR 17-92 Expression Profiling

Clinical features	Total	Cluster A	Cluster B	*P*
Number of cases (%)	70	35 (50)	21 (30)	
Median Age (range)	63.53 (34–87)	64.31 (37–87)	64.84 (34–77)	0.8648
Morphology (%)				
Clasical	53/71 (74.7)	20/35 (57.2)	17/20 (85)	**0.0412**
Blastoid	18/71 (25.3)	15/35 (42.8)	3/20 (15)	
Ki67 (%) (mean ± SE)	38.7 ± 3.2	43.1 ± 3.9	29.5 ± 4.9	**0.0471**
SOX11 positive (%)	30/40 (75)	30/30 (100)	0/10 (0)	**<0.0001**
CD5 positive (%)	41/51(80.4)	30/31(96.7)	11/20 (55)	**0.0004**
Cyclin D1 (%)	69/71(97.2)	34/35 (97.1)	21/21 (100)	1.000
Clinical and pathologic data				
Nodal presentation (lymph node >1 cm) (%)	53/71 (74.6)	27/33(81.8)	12/21 (57.1)	**0.0492**
Splenomegaly (%)	17/53 (32)	9/31 (29)	8/21(38.1)	0.5556
Hepatomegaly (%)	7/43 (16.3)	4/29 (13.8)	3/14 (21.4)	0.4098
Mean WBC count ($x10^9$/L) (range)	9.6 (1.1–35.7)	10.4 (1.1–35.7)	8.3 (3.5–26.8)	0.3804
Mean LDH (IU/L) (range)	370.8 (154–780)	387.6 (167–578)	314.3 (154–780)	**0.0363**
Mean Hb (g/dL) (range)	11.65 (6–16.4)	12.51 (9–16.1)	10,55 (6–16.4)	**0.0491**
Mean Plt count ($x10^9$/L) (range)	175.88 (25–439)	160.8 (25–381)	193.8 (19.2–43.9)	0.2370

WBC: white blood cells; Plt: platelets; Hb: hemoglobin; LDH: lactate dehidrogenase; SE: standard error.
Bold values represent statistically significant *P* values.

standards according to the World Health Organization (WHO) criteria (Swerdlow et al., 2008). Clinical features of our MCL cohort are detailed in Table 1. All samples were obtained at diagnosis or prior to any treatment. Nodal presentation was observed in 53 patients, meanwhile the rest of them were derived from: tonsils (12), colon (2), stomach (1), orbit (1), and parotid (1). Treatment was based on clinical features of the disease. Patients under the age of 65 years and fit were treated with multiagent protocols including R-CHOP (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone), R-DHAP (Dexamethasone, Cytarabine [Ara-C], Cisplatin), ARA-C (Arabinosylcitosine), plus hematopoietic stem-cell transplantation. Patients older than 65 years or unfit were treated with R-CHOP and fit older cases also received bone marrow transplantation. The median of follow up was 13 months (range: 0–70 months). The study was approved by the local institutional Ethics review board. All individuals provided their informed written consent. In addition, mononuclear cells from 12 healthy normal individuals and 8 lymph node benign inflammatory biopsies were included as controls. Mean expression values of controls are detailed in Supporting Information Table S1.

Immunohistochemistry

Standard immunohistochemistry (IHC) staining for MCL differential diagnosis was performed using 4- to 5-μm-thick FFPE sections from each tumor sample with the panel of antibodies: CD5 (clone SP19), CD23 (clone SP23), CCDN1 (clone SP4), Bcl-2 (clone 124) (Cell Marque, Rocklin, CA) and CD20 (clone L26) (Dako, Carpinteria, CA). The proliferating index was measured by Ki-67 (clone SP6) (Cell Marque, Rocklin, CA) and estimated by the percentage of Ki-67^+ cancer cells among all the counted tumor cells. SOX11 (clone CLO142) (Bio SB, Santa Barbara, CA) staining was assessed in 40 FFPE tissue sections. Appropriate positive and negative controls were run alongside.

RNA Extraction

Total RNA was isolated using the RecoverAll® kit (Ambion, Austin, TX), following manufacturer's guidelines with minor modifications. Briefly, samples were placed in a 1.5-mL microcentrifuge tube containing 1 mL of xylene, vortexed and incubated at 50°C for 3 min to melt the paraffin. The material was then centrifuged at 14,000 r.p.m. for 10 min at room temperature to pellet the specimen, after which the xylene was carefully removed and the pellet was washed three times with 1 mL of 100% room temperature ethanol and air-dried at room temperature for 45 min. Following deparaffinization, tissue was protease digested by incubating the pellet in 200-mL digestion buffer and 4 mL protease at 80°C for 15 min. For total RNA isolation, 240 mL of isolation additive was added to the sample, followed by vortexing and addition of 550 mL of 100% ethanol. The

mixture was then loaded onto a prepared filter and collection tube according to the manufacturer's procedure. The sample was centrifuged for 30 sec at 10,000 r.p.m. Flow through was discarded and filter washed twice with wash buffer. Nuclease digestion was obtained by adding 60 mL DNase master mix, containing 6 mL DNase buffer, 4 mL DNase, 50 mL nuclease-free water, to the center of each filter and incubated for 30 min at room temperature. The filter was subsequently washed three times according to the manufacturer's protocol, and RNA was eluted with 60-mL preheated nuclease-free water. RNA quality and quantity were determined by spectrophotometry at 260 nm. All eluted RNAs were immediately aliquoted into 10 mL volumes each and stored at −80°C until all extractions were accomplished.

Reverse Transcription (RT) and Quantitative Real-time PCR (qRT-PCR)

For *SOXC* cluster analysis, RT and cDNA synthesis was carried out from 1 μg of total RNA previously treated with DNAse (Ambion, Carlsbad, CA) for 10 min at 95°C, 60 min at 37°C and 10 min at 95°C to inactivate the enzyme, in a 20 μL final volume. cDNA obtained was stored at −20°C until use. Then, *SOX4*, *SOX11*, and *SOX12* gene expression analysis were performed using quantitative real time PCR (qRT-PCR) in a LightCycler system (Roche Diagnostics, Mannheim, Germany), based on TaqMan technology. The housekeeping gene *GUSB (β-glucoronidase*) (Hs00939627_m1, Applied Biosystems, Foster City, CA, USA) was used to normalize sample-to-sample differences in cDNA input, RNA quality and RT efficiency. Briefly, the PCR reaction was performed by using 4 μl of each RT reaction, 5X TaqMan Master Mix (Roche Diagnostics, Mannheim, Germany), 1X TaqMan Gene Expression Assays for *SOX4* (Hs00268388_s1), *SOX11* (Hs00846583_s1) and *SOX12* (Hs00272869_s1) (Applied Biosystems, Foster City, CA), respectively, in a 20 μL final volume reaction.

Reverse transcription for miRNAs was performed using TaqMan® MicroRNA Reverse Transcription Kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, cat. no: 4366597). TaqMan microRNA expression assays of *miR17* (002308), *miR18a* (002422), *miR19b* (000396), and *miR92a* (000431) (Applied Biosystems, Foster City, CA) were used to provide specific primers for reverse transcription and quantitation of mature miRNAs. *RNU6B* (001093, Applied Biosystems, Foster City, CA) was used to normalize sample-to-sample differences in cDNA input, RNA quality and RT efficiency. The relative expression fold change of *SOXC* and *miR17-92* clusters were calculated using the $2^{-\Delta\Delta Ct}$ method. All reactions were performed in triplicate.

Statistical Analysis

All statistical evaluations were performed using GraphPad Prism Version 6.0 (2012). The analysis of mRNA expression data was done using the Mann–Whitney test. Spearman correlation was used to analyze gene expression profiles. Group-wise comparison of the distribution of clinical and laboratory variables was performed with the Student *t* test (for quantitative variables) and the χ^2 or Fisher's exact test (for categorical variables). Receiver operating characteristic (ROC) curves analysis was applied to calculate gene expression cut-off values, with the highest sensitivity and specificity. A hierarchical clustering using Euclidean distance was employed to segregate patients groups based on the qRT PCR expression patterns displaying the MultiExperiment Viewer. Overall survival (OS) was defined from the date of diagnosis to the death of the patient or last follow-up. Survival curves were estimated by the Kaplan–Meier method and compared with the log-rank test. For all tests, $P < 0.05$ was regarded as statistically significant.

RESULTS

SOXC and miR17-92 Gene Expression Profiling in MCL

Previous analyses (Dy et al., 2008; Bhattaram et al., 2010) have suggested that *SOXC* family of transcription factors compete for the similar transcriptional targets *in vivo* and that may have partially overlapping functions *in vitro*. It was therefore of our initial interest to assess the mRNA levels of the *SOXC* transcription factor family members *SOX4*, *SOX11*, and *SOX12*, in primary MCL cases. Gene expression analysis (GEA) of this cluster revealed that *SOX11* and *SOX12* were upregulated in 70 and 74% of the total cohort, respectively, taking into account the cut-off obtained by ROC curves (*SOX11*: 1.76; *SOX12*: 2.61). Conversely, *SOX4*, the other member of this cluster, was found downregulated in 49% of the patients analyzed (cut-off: 1.95). Gene expression levels of *SOX11* (2.23 ± 0.16) and *SOX12* (3.32 ± 0.16) were significantly higher than *SOX4*

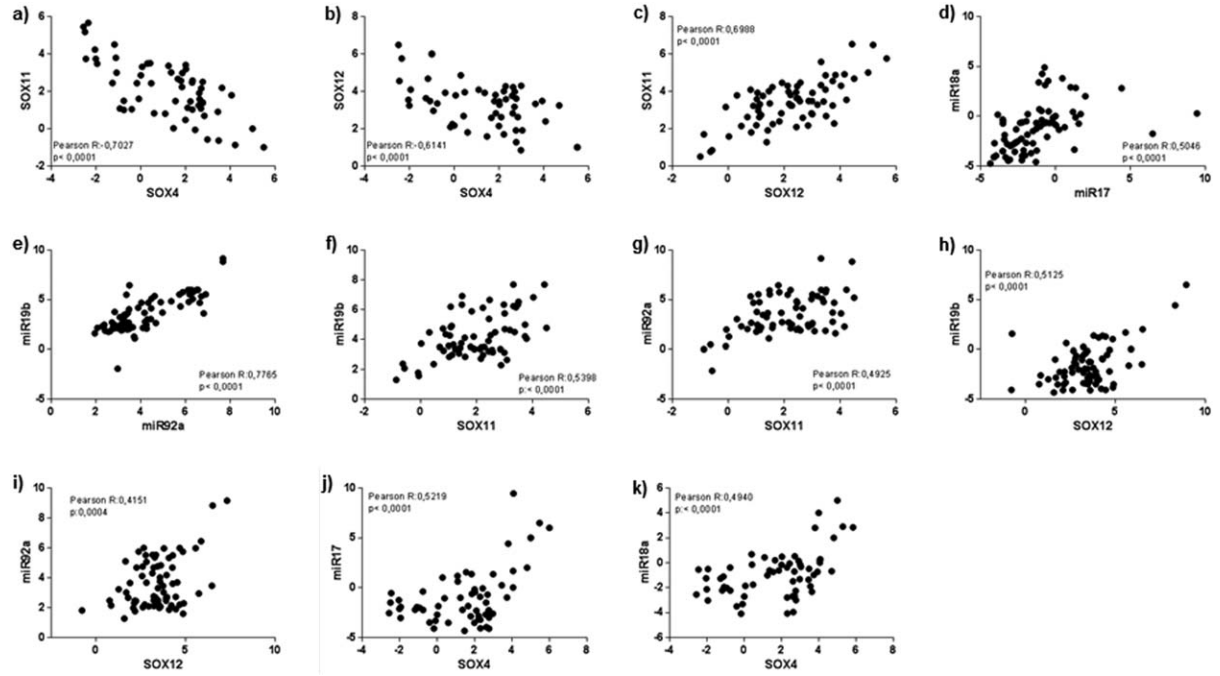

Figure 1. Correlations between gene expression levels among *SOXC* cluster and *miR17, miR18a, miR19b*, and *miR92a* members in MCL patients.

(1.33 ± 0.24) ($P \leq 0.0026$). To better characterize *SOXC* expression profiling in our cohort, we further evaluate if *SOX11* negative MCL patients included in this study may have positive levels of other *SOXC* genes. Intriguingly, we found a negative relationship between gene expression levels of *SOX11* and *SOX4* ($P < 0.0001$). This particular finding in our cohort may suggest that all *SOXC* genes are not engaged in the same regulatory axis in MCL as has been specified before (Wasik et al., 2013). We also explored whether there was any correlation between the expression levels of the three *SOXC* genes. Interestingly, we identified a negative correlation between the expression levels of *SOX4* when compared to *SOX11* ($P < 0.0001$; $r = -0.7027$) (Fig. 1a). Additionally and despite of a more established heterogeneity found in the expression levels than the other two members of the cluster, *SOX12* also revealed an inverse relationship when compared to *SOX4* ($P < 0.0001$; $r = -0.6161$) (Fig. 1b). Moreover, we found a positive correlation between the expression levels of *SOX11* and *SOX12* ($P < 0.0001$; $r = 0.6988$) (Fig. 1c). In addition, *SOX11* protein expression was measured by IHC in 40 samples and 75% of them (30/40) were positive showing an intense and relatively homogeneous nuclear pattern (Supporting Information Fig. S1).

On the other hand, GEA from members of the *miR17-92* cluster analyzed here revealed that *miR19b* and *miR92a* showed upregulation in 73.2% and 71.8% of the samples (cut-off *miR19b*: 2.01; *miR92a*: 1.17) and *miR17* and *miR18a* were downregulated in 60 and 69% of the whole cohort, respectively (cut-off *miR17*: -1.27; *miR18a*: -067) . Furthermore, we also detected that *miR19b* (4.2 ± 0.17) and *miR92a* (3.59 ± 0.21) levels were significantly higher than those exhibited by *miR17* (-1.42 ± 0.28) and *miR18a* (-1.73 ± 0.22) ($P < 0.0001$). Likewise we did with the *SOXC* cluster, we found positive correlations between the mRNA expression pattern exhibited by *miR17* and *miR18a* ($P < 0.0001$; $r = 0.5046$) (Fig. 1d) and by *miR19b* and *miR92a* ($P < 0.0001$; $r = 0.7765$) (Fig. 1e), respectively.

In addition, we explored the relationship between the transcription patterns exhibited among both clusters. Interestingly, we found positive correlations between the expression levels of *SOX11* and *miR19b* ($P < 0.0001$; $r = 0.5398$) (Fig. 1f) and also between *SOX11* and *miR92a* ($P < 0.0001$; $r = 0.4925$) (Fig. 1g). Similarly, *SOX12* also was positively correlated with *miR19b* ($P < 0.0001$; $r = 0.5125$) (Fig. 1h) and with *miR92a* ($P < 0.0004$; $r = 0.4151$) (Fig. 1i). We also found a positive association between gene expression levels of *SOX4* and *miR17* ($P < 0.0001$; $r = 0.5219$)

(Fig. 1j) and with *miR18a* ($P < 0.0001$; $r = 0.4940$) (Fig. 1k).

Unsupervised Hierarchical Clustering with the Concordantly Deregulated Genes

Because of the striking deregulation seen in the expression levels of genes evaluated in the present study; we used unsupervised hierarchical clustering to determine whether the transcriptional differences among *SOXC* cluster and *miR17-92* members analyzed here could segregate our MCL patient set. As shown in Figure 2, patients from our cohort were clustered in two distinct groups: one of them, which we called *Cluster A* (35 patients), exhibiting a high *SOX11/SOX12/miR19b/miR92a* signature and the other, displaying high *SOX4/miR17/miR18a* transcriptional levels, which we named *Cluster B* (21 patients). Equally, *Cluster A* and *Cluster B*, were gathered apart and independently from controls (Supporting Information Table S1). Furthermore, *Cluster A* and *B* encompassed 80% of our set of patients, meanwhile the remaining 20% of our cohort could not be clustered due to a more heterogeneous transcriptional activity. These marked differences in the gene expression signatures in both, *Cluster A* and *Cluster B*, prompted us to hypothesize that patients in each group could be displaying similar pathobiological features and also that these differences could be modulating or at least partially be implicated in moderating different behaviors in each group.

Correlation Between *SOXC* and *mir17-92* Expression Profiles and MCL Prognostic Factors

To assess the differences in the transcriptional signatures seen before, we explored potential associations between *SOXC* and *miR17-92* gene expression profiles and several clinical features of MCL tumors. Table 1 list the clinical features of both *Cluster A* and *Cluster B*, which not differ significantly with respect to age, Cyclin D1 protein expression, splenomegaly, hepatomegaly, white-blood cell and platelet counts. However, they did differ significantly with respect to important features of the pathology, such as tumor morphology, tumor presentation, CD5 expression, proliferation index and overall survival. In this manner, clinical data analysis showed increased lactate dehydrogenase serum levels ($P = 0.0363$) in patients from *Cluster A* compared to those that exhibit *Cluster B* gene expression signature. In this regard, almost

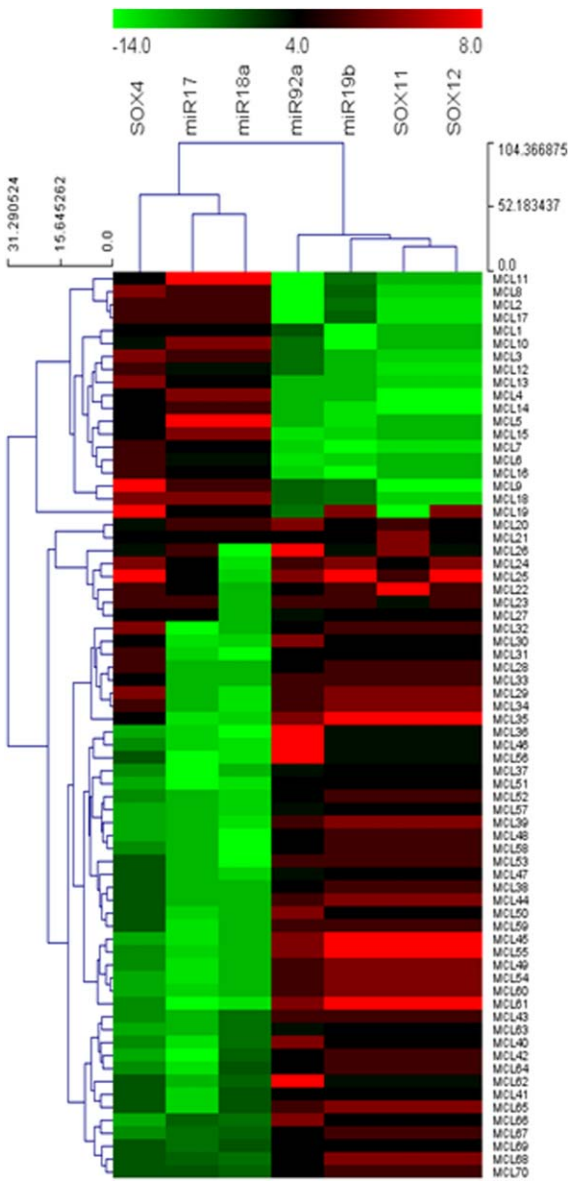

Figure 2. Heat map showing *SOXC* cluster and *miR17, miR18a, miR19b*, and *miR92a* expression levels in MCL samples determined by qPCR (log relative units). The unsupervised hierarchical clustering separates the patients in two main subgroups corresponding to *Cluster A* (*SOX11*$^+$/*SOX12*$^+$/*miR19b*$^+$/*miR92a*$^+$) and *Cluster B* (*SOX4*$^+$/*miR17*$^+$/*miR18a*$^+$) expression. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

half of patients (42.8%) from *Cluster A* presented blastoid morphological variant in comparison with patients from *Cluster B* (15%) ($P = 0.0412$). Moreover, patients from *Cluster A* had nodal presentation in virtually 82% of cases in comparison with *Cluster B* (57.1%) ($P = 0.0492$), as well as, high Ki67 proliferation index (43.1%) in contrast to the proliferating levels that *Cluster B* patients presented (29.5%) ($P = 0.0471$). Additionally, only slightly more than half of *Cluster B* cases expressed

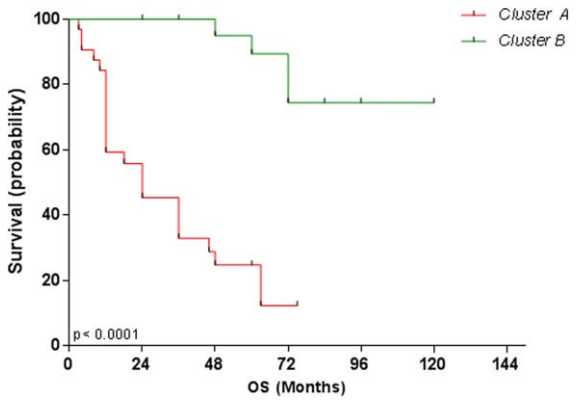

Figure 3. Overall survival of MCL patients according to the gene expression signature displayed in *Cluster A* and *Cluster B*. MCL patients with concomitant high *SOX11/SOX12/miR19b/miR92a* (*Cluster A*) ($n = 35$) exhibited a significant shorter overall survival than patients with high *SOX4/miR17/miR18a* (*Cluster B*) ($n = 21$) ($P < 0.0001$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CD5 (55%), whereas almost all tumors from *Cluster A* (96.7%) were CD5 positive ($P = 0.0004$). Among samples analyzed by IHC, $SOX11^+$ patients were gathered in *Cluster A* meanwhile $SOX11^-$ cases were grouped in *Cluster B* (Table 1). Interestingly, patients from *Cluster B* had a significantly better prognosis hence longer OS (media: 63.13 months) than patients belonging to *Cluster A* (media: 25.9 months) ($P < 0.0001$) (Fig. 3), indicating a more aggressive behavior in the last group of patients.

DISCUSSION

Several studies have shown that *SOXC* group of transcription factors, principally *SOX11*, and the polycistronic miR17-92 cluster are deregulated in MCL having major implications and performing active roles as central regulators of tumor development (Mozos et al., 2009; Navarro et al., 2009; Gustavsson et al., 2010; Iqbal et al., 2012; Vegliante et al., 2013). In the present study, we have explored gene expression patterns of *SOXC* cluster and *miR17*, *miR18a*, *miR19b*, and *miR92a* members of the *miR17-92* cluster in a series of 70 MCL patients and their correlation with biological and clinical characteristics of the disease. Transcriptional profiles of both clusters revealed that tumors exhibited different expression signatures from healthy donors and display a singularly heterogeneous gene expression mark. Interestingly, unsupervised hierarchical clustering analysis revealed two distinctive subsets of tumors showing significant differences in important clinical variables taken into account in the disease. In this way, patients with high expression of *SOX4*, *miR17*, and *miR18a*, grouped in *Cluster B*, exhibited a reduced proliferating signature and CD5 expression. Moreover, patients from this cluster were more frequently associated with a classical morphology, non-nodal presentation of the disease and extended OS. In contrast, cases from *Cluster A*, associated with the high *SOX11*, *SOX12*, *miR19a*, and *miR92a* signature, were linked to more aggressive diseases features performing nodal localization, high proliferation index, presenting blastoid morphological variants in a high number of cases and being prone to have significantly shorter OS in comparison to patients from the other cluster identified here.

The clinical relevance of *SOXC* genes has risen to a high level in recent years as numerous studies have suggested that *SOXC* transcription factors may contribute to tumor prognosis. In this manner, *SOX11* have been shown to be highly expressed in most medullobastomas (Lee et al., 2002) whereas increased *SOX4* expression was also associated with bladder (Aaboe et al., 2006), prostate (Liu et al., 2006), colon (Andersen et al., 2009), and non-small cell lung tumors (Medina et al., 2009). In contrast, it was shown that higher levels of *SOX4* was related to better prognosis in medulloblastomas and ependymoma patients (deBont et al., 2008), but still, slightly is known about the role/s of this gene in MCL. Furthermore, it has been well documented that *SOX11* is deregulated in more aggressive MCL patients and cases with more indolent disease features lack *SOX11* expression (Fernández et al., 2010; Ondrejka et al., 2011; Navarro et al., 2012). Additionally, it also has been established by Vegliante et al. (2013) that *SOX11* upregulation promotes angiogenesis in MCL cells contributing to tumor development by altering the MCL B-cell differentiation program and that *SOX11* silencing promotes the shift from a mature B cell into the initial plasmacytic differentiation phenotype, in both primary tumor cells and an *in vitro* model. Moreover and in line with Vegliante's findings, Palomero et al. (2014) identified that the *platelet-derived growth factor-A* acts as a direct *SOX11* target gene upregulated in MCL cells whose inhibition impaired *SOX11*-enhanced *in vitro* angiogenic effects on endothelial cells. Lastly, *SOX12* was found correlated with microvascular invasion and higher tumor nodule metastasis and indicated poor prognosis in hepatocellular carcinoma patients (Huang et al., 2015). Like Wasik et al. (2013), we noticed a similar transcription signature between the expression levels of *SOX11* and *SOX12* in our MCL cohort, but in contrast to

Wasik's results, we did not observe a fine tuning among the three members of *SOXC* clusters. In fact, our *SOX11*$^{+}$/*SOX12*$^{+}$ MCL patients from *Cluster A* were highly *SOX4*$^{-}$ and vice versa regarding *Cluster B*. Thus, our data point towards that *SOXC* genes may not be corregulated in MCL tumors as it was previously speculated. Therefore, it is logical to hypothesize that *SOXC* genes may be triggered by different activation pathways hence have different effects in tumor cells depending on the context and primary transformation mechanisms.

On the other hand, one of the most frequently deregulated miRNA-encoding genes in human cancer is the polycistronic *miR17-92* cluster, which encodes six miRNAs (*miR-17*, *miR-20a*, *miR-18a*, *miR-19a*, *miR-19b*, and *miR-92a*) (Volinia et al., 2006). *miR17-92* cluster was originally described as an *oncomir* because of its oncogenic function in many hematological, thyroid and lung tissues (Mendell, 2008). In addition, *miR17-92* overexpression was found to inhibit proliferation of luminal breast cancer cells by targeting a steroid receptor *coactivator (NCOA3)*, *estrogen receptor* α *(ESR1)* and *Cyclin D1* (Hossain et al., 2006; Yu et al., 2008; Castellano et al., 2009; Yu et al., 2010), the latter one overexpression known to be the major genetic hallmark in more than 90% of MCL patients. However, emerging evidence suggests that loss of function of *miR17-92* cluster might contribute to the development and progression of other types of cancers, implicating that these cluster may also exert tumor suppressor function in some tissues. For example, loss of heterozygosity at chromosome 13q31, where the human *miR17-92* cluster resides, was detected in ~25% of human breast tumors (Eiriksdottir et al., 1998). Moreover, some key studies demonstrated that the six miRNAs encoded by the *miR17-92* cluster are not functionally equivalent when it comes to promoting cell survival and tumorigenesis (Mu et al., 2009; Olive et al., 2009).

In this regards, a recent report showed that overexpression of *miRNA-18a* in MDA-MB-231 cells reduced cell invasiveness, primary tumor growth and lung metastasis *in vivo* and decreasing growth in colorectal cancer cells, acting in consequence, in opposition to other members of the cluster (Humphreys et al., 2013). Simultaneously, *miRNA-18a* inhibition leads to a pro-metastatic effect by targeting of the *Hypoxia Inducible Factor 1*, *Alpha Subunit (HIF1A)* gene (Krutilina et al., 2014).

In addition, it has been noticed that miR-19a and miR-19b were primarily responsible for promoting proliferation (Humphreys et al., 2013). In fact, the two members of the *miR-19* seed family were found to be necessary and sufficient to recapitulate the oncogenic activity of the full cluster (Mu et al., 2009; Olive et al., 2009). These studies also demonstrated that the tumor suppressor *PTEN* is a prominent *miR-19* target, and its suppression can at least partially explain the prosurvival effect of *miR-19* (Mu et al., 2009; Olive et al., 2009). Interestingly, these findings were later found to also apply to a model of T-cell leukemias (Mavrakis et al., 2010). Additionally, many studies determined that aberrant expression of *miR-92a* can be observed in different types of malignant tumors (Li et al., 2014). In line with this, it has been demonstrated that *miR-92a* contributes to tumorigenesis and development by promoting cell proliferation, invasion, metastasis and inhibiting cell apoptosis (Ghosh et al., 2009; Chen et al., 2011; Tsuchida et al., 2011; Niu et al., 2012; Rao et al., 2012).

In summary and to the best of our knowledge, our results show for the first time a combined analysis around the deregulated transcriptional patterns exerted by *SOXC* and *miR17-92* clusters in MCL in comparison with normal lymphoid tissue and distinguishing two subgroups of tumors that differ in prognosis and survival associated clinical variables of the disease. Although, the number of patients in each group is limited, these findings suggest that both clusters exhibit gene expression profiles that may link them to two different subtypes of MCL tumors with marked clinical differences. In agreement with our results, some previous studies have also recognized a MCL subtype mainly associated with leukemic non-nodal presentation, lack of *SOX11* and CD5 expression, lower proliferation index and better outcome (Orchard et al., 2003; Rubio-Moscardo et al., 2005; Fernández et al., 2010; Navarro et al., 2012). Hence, the two clusters of MCL patients identified by the present study with different *SOXC* and *miR17-92* gene expression profiles may contribute to distinguish this better outcome subtype of the disease meanwhile the other subgroup of patients may correspond to the conventional counterpart of the pathology, associated with more aggressive clinical behavior. Understanding the whole comprehensive biological functions of *SOXC* and *miR17-92* clusters is perhaps a significant challenge facing the comprehension of MCL pathobiology.

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