scientific reports

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The expression of *MALAT1* long non-coding RNA is associated with good prognosis in mantle cell lymphoma

Elena María Fernández-Garnacho[1], Cristina Martínez-Muñoz[1], Ferran Nadeu[1,2], Guillem Clot[1,2,3], Juan García Valero[1,2], Ferran Araujo-Ayala[1,2], Ares Martínez-Farran[1], Anna De Bolòs[1,2], Dolors Colomer[1,2,4], Pablo Mozas[1,4], Andrea Rivero[1,4], Eva Giné[1,2,4], Armando López-Guillermo[1,2,4], Itziar Salaverria[1,2], Cristina López[1,2,4], Sílvia Beà[1,2,4], Virginia Amador[1,2], Santiago Demajo[1], Pedro Jares[1,2,5], José Ignacio Martín-Subero[1,2,6], Patricia Pérez-Galán[1,2], Elías Campo[1,2,3,5] & Lluís Hernández[1,2,7]✉

MALAT1 **is a long non-coding RNA (lncRNA) with altered expression in many cancers but poorly studied in B-cell neoplasms like mantle cell lymphoma (MCL), a very aggressive B-cell lymphoma. To elucidate the role of *MALAT1* in MCL we have investigated its expression in 219 primary tumors using microarray data. We also analyzed the expression of its natural antisense transcript (*TALAM1*), which is key for the processing of *MALAT1* RNA to its active isoform. High expression of *MALAT1/TALAM1* lncRNAs was consistently linked to favorable clinical behavior in MCL, independently of other alterations previously described to influence MCL prognosis. In concordance with the observed clinical association, we found that the increased expression of these lncRNAs was inversely associated with gene signatures related to a proliferative and activation phenotype (MCL35-proliferation/BCR signaling signatures) in MCL samples from lymph nodes as peripheral blood. Finally, functional studies of *MALAT1* in MCL primary samples showed that its levels were downregulated by microenvironmental stimuli and EZH2 activity, a known epigenetic factor induced by pro-proliferative stimuli and previously related with poor MCL prognosis. Altogether, these data support the favorable prognostic value of *MALAT1* expression in MCL, and the *MALAT1* dual role as oncogene or tumor suppressor in different neoplasms.**

Keywords Long non-coding RNAs, *MALAT1*, *TALAM1*, Lymphoma, MCL, Biomarker

Long non-coding RNAs (lncRNAs) are key regulators of the expression of protein-coding genes and play critical roles in both physiological and pathological contexts, including cancer[1,2]. Among them, *MALAT1* is one of the most frequently implicated lncRNAs in oncogenesis primarily through its involvement in the regulation of essential cellular pathways[3]. Consequently, *MALAT1* has been associated with various cancer-related processes such as cell proliferation, migration, invasion, apoptosis and angiogenesis[4].

TALAM1 is a natural antisense transcript at the *MALAT1* locus which has been functionally related to positively regulate *MALAT1* levels by promoting its 3′-end cleavage and maturation[5]. Although the clinical relevance of *TALAM1* expression remains unexplored, preliminary studies in solid tumors have reported its induction specifically in tumor cells, and its cooperation with *MALAT1* to determine cancer aggressiveness[6,7].

Multiple studies have described *MALAT1* expression as a clinical biomarker, predominantly associated with poor prognosis in solid tumors3, and B-cell lymphomas (B-NHL), such as mantle cell lymphoma (MCL)[8]. However other reports have suggested that elevated *MALAT1* levels may correlate with favorable outcomes in lymphoid neoplasms such as diffuse large B-cell lymphoma[9–12]. These seemingly contradictory findings

[1]Lymphoid Neoplasm Program, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona, Spain. [2]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain. [3]Departament de Fonaments Clinics, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain. [4]Hematology Department, Hospital Clínic of Barcelona, Barcelona, Spain. [5]Pathology Department, Hospital Clínic of Barcelona, Barcelona, Spain. [6]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain. [7]Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Centre Esther Koplowitz (CEK), Rosselló 153, Barcelona 08036, Spain. ✉email: hernan@recerca.clinic.cat

underscore the need for further investigation in well-characterized patient cohorts to account for potential confounding factors, such as molecular subtypes or genetic alterations with distinct prognostic implications.

In this study, we examine the biological and clinical significance of *MALAT1* deregulation in MCL, an aggressive and largely incurable lymphoma. While microRNAs have been extensively studied in MCL, the contribution of lncRNAs remains poorly understood[13–15].

Results

MALAT1 and *TALAM1* expression and prognosis in MCL

We investigated the expression of *MALAT1* and *TALAM1* in mantle cell lymphoma (MCL) and assessed their potential clinical and pathological relevance across three independent patient cohorts. These cohorts included data on cytological variants (small-cell, classical, blastoid), tumor sample origin (peripheral blood [PB] and lymph nodes [LN]), sample type (purified tumor cells vs. whole lymph node), and clinical outcomes.

In the cohort#1 from Saba et al.[16] *MALAT1* expression showed no significant differences across MCL cytological variants, sample origin, or tumor cell purification status (Supplementary Fig. S1). In cohort #2, comprising 122 nodal MCL cases[17], patients with high levels of *MALAT1* ($N = 25$) or *TALAM1* ($N = 48$) had a significantly longer OS than cases with low expression ($N = 97$ or $N = 74$, respectively) ($P = 0.038$ and $P = 0.010$, respectively) (Fig. 1A). This association between high *MALAT1/TALAM1* expression an improved prognosis was validated in cohort#3, which included 44 leukemic MCLs ($P = 0.024$ and $P = 0.045$, respectively) (Fig. 1B)[16]. Notably, this cohort also included *SOX11* expression status, a recognized prognostic marker in MCL[19], but no significant differences in *MALAT1* or *TALAM1* expression were observed between *SOX11*-positive and negative cases (Supplementary Fig. S2A). Consistent with findings of cohort#1, no significant differences in *MALAT1* and *TALAM1* expression were detected among cytological variants (Supplementary Fig. S2B) or other prognostic markers, including alterations in *TP53*, *CDKN2A* or copy number changes (Supplementary Table S1).

MALAT1 and *TALAM1* expression and prognostic-related gene signatures in MCL

To investigate the association between elevated *MALAT1* and *TALAM1* expression and favorable clinical outcomes in MCL, we analyzed their relationship with previously described gene expression signatures linked to related biologically and clinically relevant processes in both nodal and leukemic MCL.

We first examined the correlation of *MALAT1/TALAM1* levels with the proliferation signature (MCL35 assay), a robust prognostic marker in nodal MCL[17]. When treated as continuous variables across cohort #2, *TALAM1* expression showed a significant negative correlation with the proliferation signature (r=-0.204; $P = 0.024$), while *MALAT1* exhibited a similar trend but did not reach statistical significance (r=-0.101; $P = 0.204$) (Supplementary Fig. S3). Consistently, comparison of the proliferation signature scores between clinically relevant *MALAT1/TALAM1* expression groups revealed lower proliferative signatures scores in the high expression groups, with statistical significance observed only for *TALAM1* ($P = 0.323$ and $P = 0.023$, respectively) (Supplementary Fig. S4).

As the proliferation signature comprises two gene subsets with opposite associations to proliferation and prognosis[17], we performed a separated analysis to better characterize the relationship between *MALAT1/TALAM1* expression and cell proliferation in nodal MCL. Unsupervised clustering of normalized expression in 122 nodal MCL samples of (cohort#2) identified two main gene clusters (Cluster#1 of 4 genes and Cluster#2 of 13 genes, previously reported by Scott et al.[17] to be associated with lower and higher tumor proliferation, respectively) and three sample groups: two with opposing expression patterns of the clusters, and one with an intermediate expression (Fig. 2A).

Tumors with high expression of genes of Cluster #1 were associated with better prognosis than those with high expression of Cluster #2 (Fig. 2B), consistent with previous findings[17]. Correlation analysis revealed a significant positive correlation between *MALAT1/TALAM1* and the low-proliferation Cluster #1 ($r = 0.321/P = 0.003$ and $r = 0.215/P = 0.018$, respectively), and a significant inverse correlation with the high proliferation Cluster #2 (r=-0.181/$P = 0.046$ and r=-0.239/$P = 0.008$) (Fig. 2C). These results were further supported by consistent differences in mean expression of the two clusters between groups defined by clinically significant *MALAT1/TALAM1* levels (Supplementary Fig. S5). Together these findings indicate that *MALAT1/TALAM1* expression in nodal MCL is inversely associated with tumor cell proliferation, as measured by gene signatures previously linked to the proliferation and prognosis.

We next assessed the relationship of *MALAT1/TALAM1* levels and a B-cell receptor (BCR) activation signature in leukemic MCL (cohort#1), given that the proliferation signature is less predictive in peripheral blood (PB) samples[16]. We used a of 27-gene BCR signature described by Saba et al.. where overexpression correlates with poor prognosis[18]. Although the BCR signature was generally higher in nodal MCL compared to PB samples, some PB cases retained elevated BCR signaling, which was associated with a more aggressive disease[18]. Notably, *MALAT1* expression was significantly lower in PB MCL samples with high BCR signature scores compared to those with a low score of this signature in the 15 informative cases of the cohort#1 from Saba et al. ($P = 0.008$) (Supplementary Fig. S6).

In summary, these results demonstrate that *MALAT1* and *TALAM1* expression, particularly *MALAT1*, is concordantly associated with gene expression signatures indicative of reduced proliferation and BCR signaling in LN and PB MCL samples, respectively, both of which are linked to improved patient prognosis.

MALAT1 expression and microenvironment stimulation in MCL patient-derived lymphoma spheroids (PDLS)

To explore the functional relevance of *MALAT1* expression in MCL, we initially selected two tumors from cohort#3 with available cryopreserved PB cells to generate patient-derived lymphoma spheroids (PDLS). MCL#1

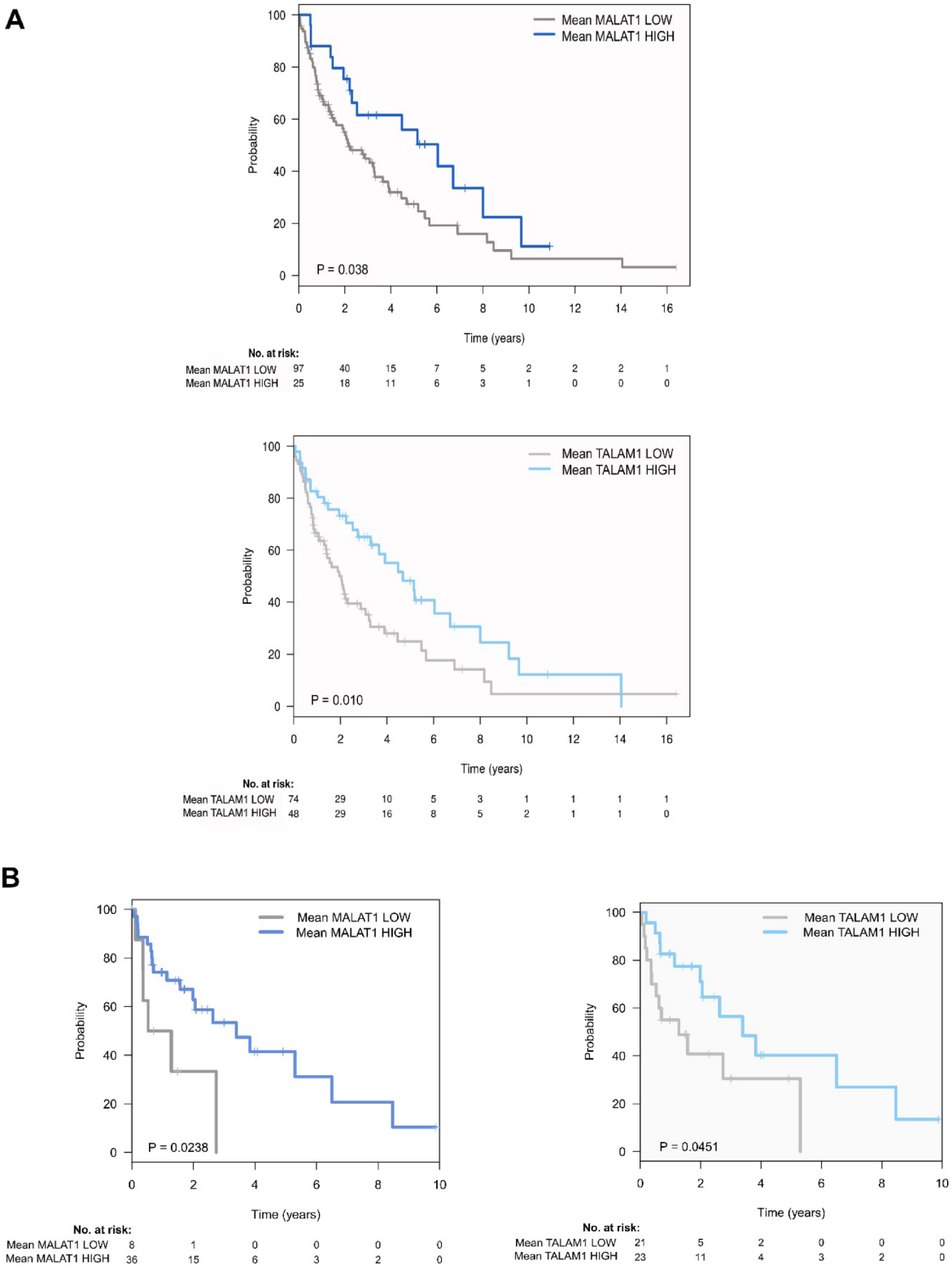
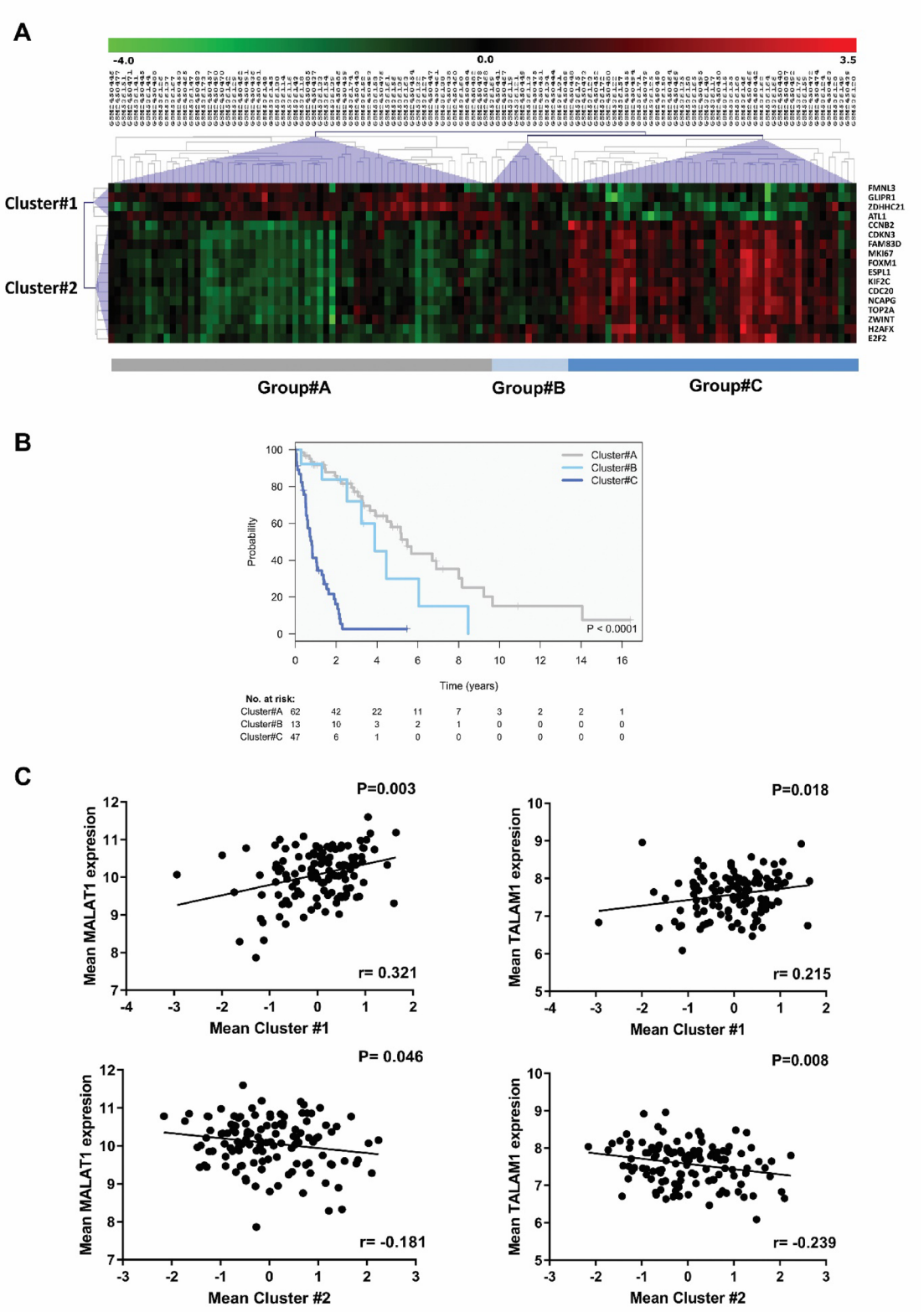


Fig. 1. A high *MALAT1/TALAM1* expression is associated with a more prolonged overall survival in MCL. (**A**) Kaplan-Meier curves for overall survival (OS) of patients according to *MALAT1* or *TALAM1* expression in nodal MCL samples of cohort #2. High *MALAT1/TALAM1* expression levels are significantly associated with a longer OS. (**B**) Kaplan-Meier curves for overall survival (OS) of patients according to *MALAT1* or *TALAM1* expression in MCL samples from peripheral blood of cohort #3. High *MALAT1/TALAM1* expression levels were significantly associated with a longer OS.



was a classical *SOX11*-positive case with low *MALAT1* level, while MCL#2 was *SOX11*-negative showing high *MALAT1* levels, based on the prognostic cutoff defined for this cohort.

We first assessed proliferation/viability in PDLS cultured for four days with a combination of microenvironmentally stimuli, including pro-proliferative factors (IL-4, CD40L) and BAFF[20], versus BAFF alone. As expected BAFF alone resulted in significantly reduced growth in both cases, consistent with its known role in supporting survival but not proliferation in MCL cells (Fig. 3A)[19]. Interestingly, *MALAT1* expression was significantly upregulated in both PDLS-MCL cases cultured without pro-proliferative factors (Fig. 3B). Given the proposed role of *EZH2* as a mediator of *MALAT1* oncogenic effect in MCL cell lines, we quantified

◀**Fig. 2**. *MALAT1/TALAM1* expression is associated with low proliferation according to previously validated MCL prognostic gene signatures. (**A**) Hierarchical clustering of the MCL proliferation gene signature expression (MCL35) in the 122 cases of cohort#2. As previously described[17], three main groups of samples were identified, cluster groups #A and #C with clear opposite expression profiles of the two gene clusters (#1 and #2), and cluster #B with intermediate profiles. (**B**) Kaplan-Meier curve of OS showing that MCL cases with higher expression of cluster#1 genes were associated with a better prognosis, whereas high expression of cluster#2 genes was found in cases with poorer outcomes. (**C**) Scatter plots showing significant linear correlations of *MALAT1/TALAM1* with Cluster#1 (positive correlations) and with Cluster#2 genes (negative correlations).

EZH2 levels in the PDLS model. *EZH2* was significantly upregulated in the presence of all stimulatory factors but downregulated in the absence of pro-proliferative factors (Fig. 3C). To further investigate the functional impact of *MALAT1*, we silenced its expression in PDLS cultured with BAFF alone. Silencing was effective in both cases (Fig. 4A) and led to increased cell viability/proliferation (Fig. 4B), along with a significant rise in *EZH2* expression (Fig. 4C).

We expanded the study to include two additional PDLS samples from SOX11-positive classical MCL tumors (MCL#3 and MCL#4), also from cohort#3. These, along with MCL#1, were used to examine in more detail the relationship between *MALAT1* and *EZH2*. Treatment with the *EZH2* inhibitor (GSK126) at two different concentrations revealed a dose-dependent increase in *MALAT1* expression, particularly at the higher dose, both in the absence ($P = 0.0039$ to $P = 0.0006$) and presence ($P = 0.021$ to $P = 0.013$) of pro-proliferative factors (Fig. 5). This increase in *MALAT1* was consistently associated with reduced cell growth ($P = 0.0079$ to $P < 0.0001$) (Supplementary Fig. S7). Notably, MCL#4 exhibited a unique pattern in which *MALAT1* levels decreased at four days of treatment with the lower inhibitor dose ($P = 0.005$) but significantly increased with the higher dose ($P = 0.005$) (Fig. 5). This finding in such case which also showed high *MALAT1* levels in PB (cohort#3), suggests a complex regulatory relationship between *EZH2* and *MALAT1* expression in MCL. In spite of the heterogeneity found among the cases, in all of them, *MALAT1* and *EZH2* levels showed a consistent inverse association growing under both in presence and in absence of pro-proliferative factors (Supplementary Fig. S7).

To further characterize the transcriptional impact of *MALAT1*, we performed RNAseq on the same three SOX11-positive PDLS-MCL samples previously studied, comparing *MALAT1*-silenced PDLS to paired controls cultured with pro-proliferative factors. *MALAT1* silencing was confirmed in all cases ($P = 0.003$ to $P < 0.0001$)(Supplementary Fig. S8). Fourteen genes were found differentially expressed under stringent criteria (Supplementary Table S2 and Supplementary Fig. S9). Among the upregulated genes, only *CSNK2B*, a regulatory subunit of casein kinase 2 complex involved in microenvironment-mediated survival pathways in MCL, was of particular interest[20,21].

To validate these findings we analyzed previously published data from four additional MCL PDLS samples[22]. PB tumor cells were cultured with the growth factors IL-4, CD40L and BAFF, with or without monocytes. *MALAT1* levels were significantly higher on day 0 (PB samples) compared to PDLS after six days of culture, particularly in presence of supplementary stimuli from co-culture with monocytes ($P = 0.036$) (Supplementary Fig. S10A). In contrast, *EZH2* expression showed an inverse trend ($P = 0.09$ and $P = 0.07$, with or without monocytes, respectively) (Supplementary Fig. S10A). Pathway enrichment analysis of genes correlated with *MALAT1* confirmed significant negative associations with pathways linked to MCL aggressiveness, proliferation and resistance to cell death (Supplementary Fig. S10B), as well as microenvironment stimuli. These findings support the observed downregulation of *MALAT1* in response to growth factors such as CD40L and IL-4 (Supplementary Table S3A and B) (Supplementary Fig. S11).

Discussion

This study provides novel insights into the clinical and biological role of *MALAT1* and also complementary findings about its natural antisense transcript *TALAM1* in MCL. Our findings indicate that upregulation of both *MALAT1* and *TALAM1* is associated with favorable prognosis in MCL. While a previous study suggested a trend toward poor prognosis of *MALAT1* expression in a small cohort of 40 MCL patients, the statistical significance was marginal and lacked detailed clinical or molecular characterization[8]. In contrast, our results demonstrate a consistent and reproducible association between *MALAT1* overexpression and improved prognosis across two independent and larger cohorts, encompassing both nodal and leukemic samples.

Importantly, *MALAT1* and *TALAM1* expression levels in leukemic MCL samples were not associated with histological subtypes, *SOX11* status or other common genetic alterations with known prognostic relevance in MCL[23]. However, due to the relative small number of cases analyzed, further validation is warranted. We also examined the relationship between the expression of the studied lncRNAs with proliferation and BCR activation signatures, two parameters previously shown to be related to a more aggressive clinical behavior of MCL[17,18]. Notably, both lncRNAs, particularly *MALAT1* in PB samples, showed inverse associations with these signatures.

Functional studies using patient-derived lymphoma spheroids (PDLS) from PB MCL primary samples further supported the clinical relevance of *MALAT1* in MCL. The PDLS-MCL model, previously established using BAFF (pro-survival) and IL-4/CD40L (pro-proliferative) stimuli[20], enabled us to investigate *MALAT1* modulation under different microenvironmental conditions. We observed that *MALAT1* levels increased when PDLS were cultured with BAFF alone, but this effect was suppressed in the presence of pro-proliferative factors, suggesting that such stimuli repress *MALAT1* expression.

To explore the underlying mechanisms, we examined the role of *EZH2*, a known epigenetic mediator of transcriptional programs induced by microenvironmental stimuli in lymphomas and that has been related

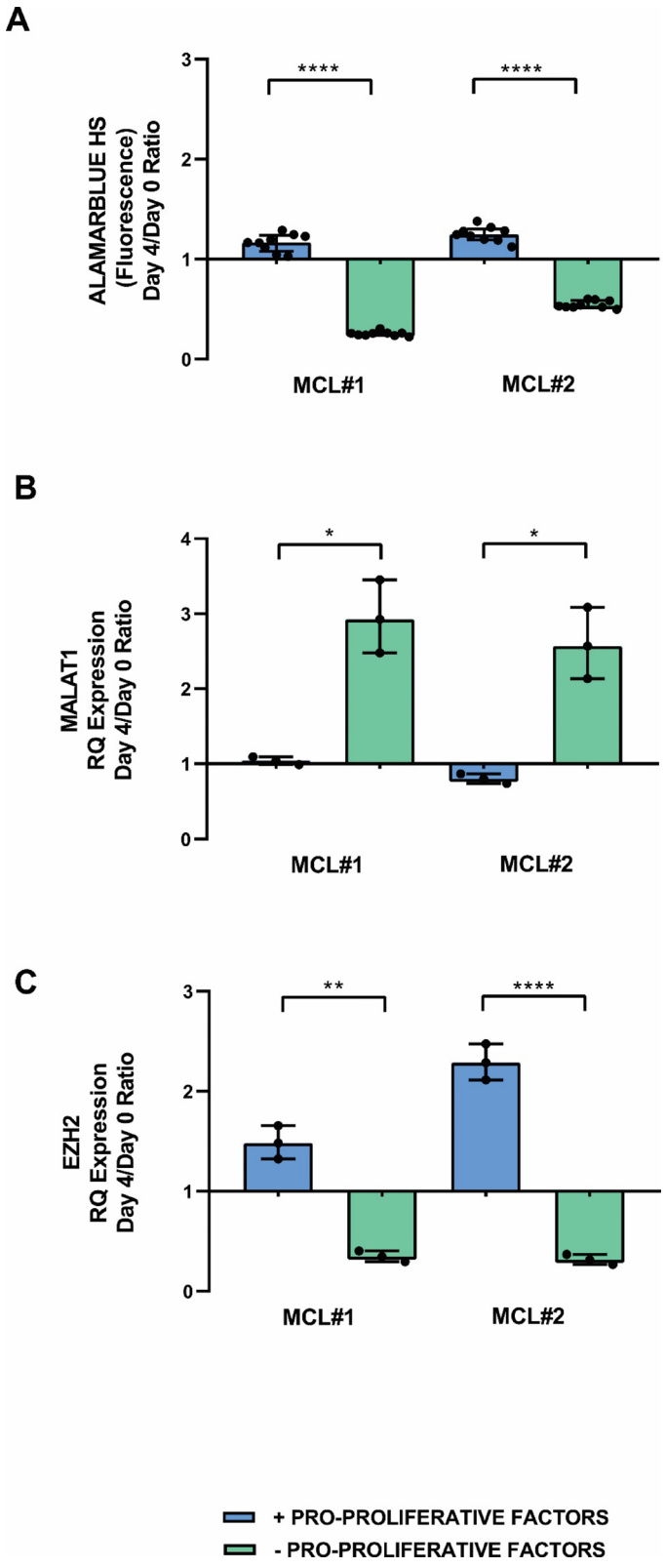


Fig. 3. The pro-proliferative factors in PDLS-MCL boost cell growth and are associated with increased levels of *EZH2* but decreased levels of *MALAT1*. (**A**) Cell growth is significantly higher in PDLS of both cases when pro-proliferative cases are present in the media ($P < 0.0001$). (**B**) *MALAT1* expression significantly increased in both MCL cases cultured when cultured without pro-proliferative factors ($P = 0.020$ in MCL#1 and $P = 0.021$ in MCL#2. (**C**) *EZH2* levels increased in both MCL cases after growing with pro-proliferative factors (MCL#1 $P = 0.0047$ and MCL#2 $P < 0.0001$). RQ: Relative Quantification. Median values and interquartile range are depicted.

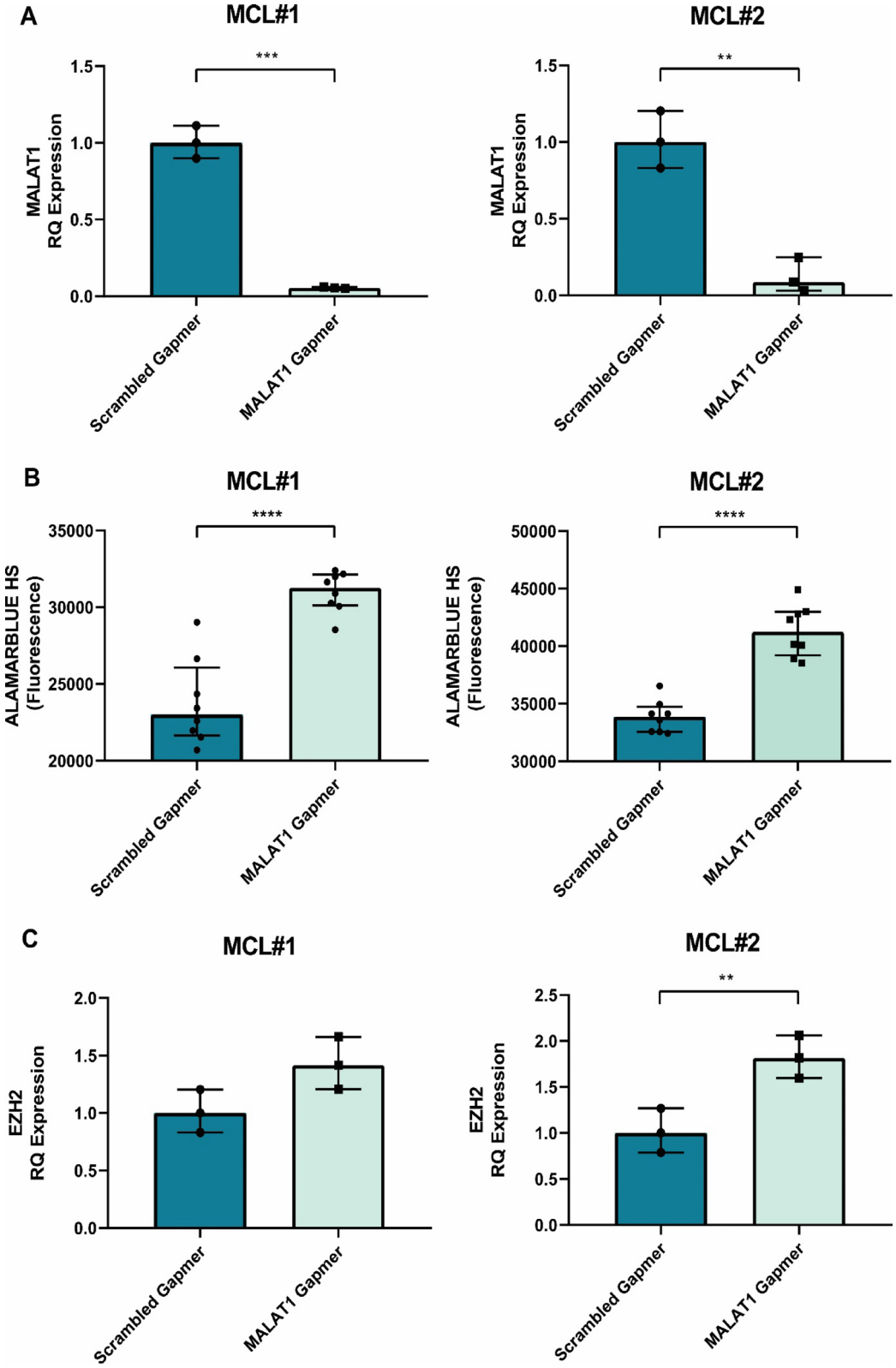


Fig. 4. *MALAT1* silencing induced an increase in both proliferation/survival and *EZH2* expression in MCL PDLS growing without pro-proliferative factors. (**A**) A noticeable significant downregulation of *MALAT1* levels was achieved after 4 days of silencing with a specific Gapmer compared to a scrambled one, in cells from two MCL patients growing only with BAFF (MCL#1 $P = 0.0001$ and MCL#2 $P = 0.0021$). (**B**) *MALAT1* silencing induced a significant increase in viability in the two MCL cases ($P < 0.0001$). (**C**) *EZH2* levels also increased upon *MALAT1* silencing, reaching statistical significance in MCL#2 ($P < 0.0018$). Median values and interquartile range are depicted.

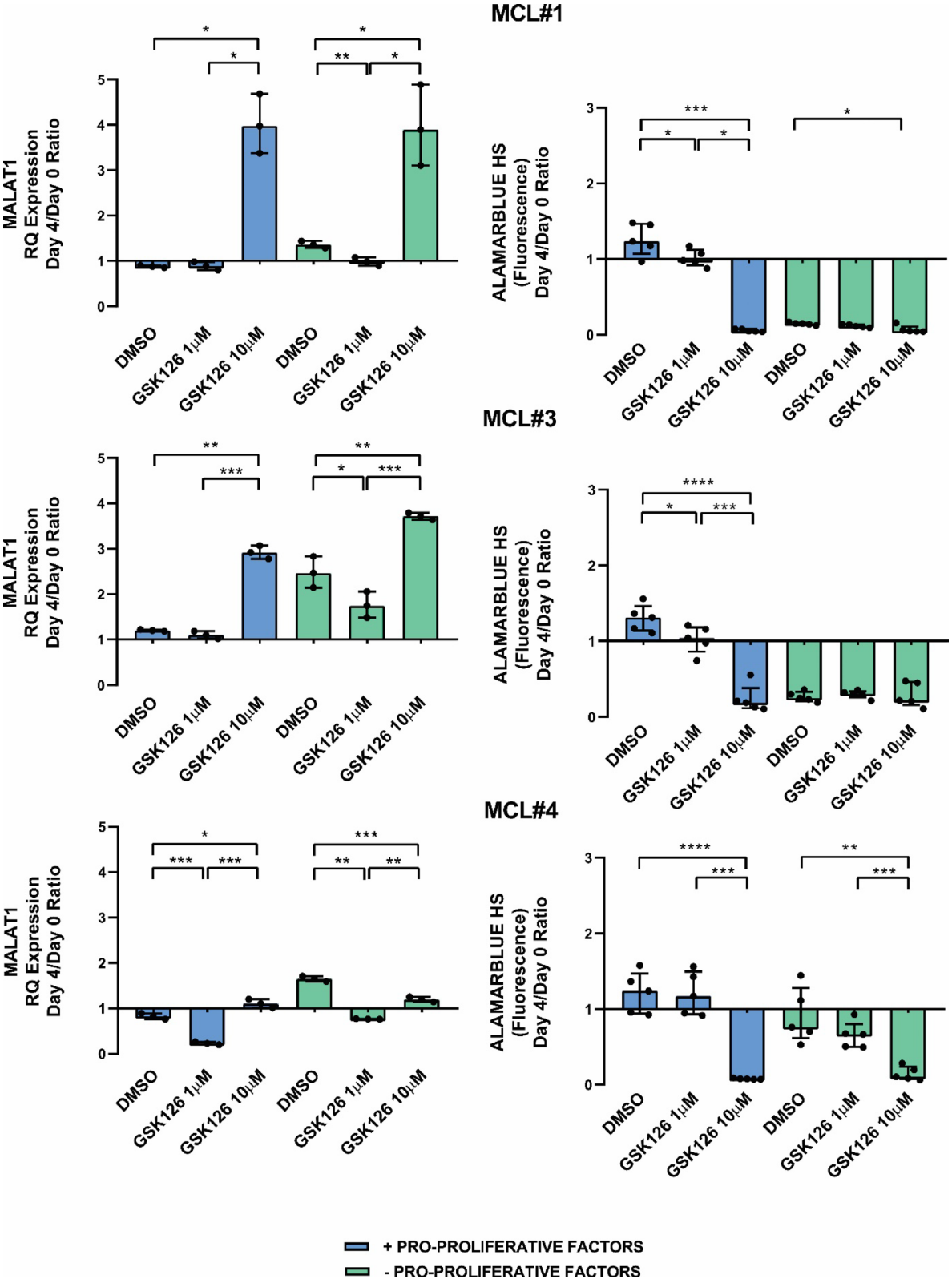


Fig. 5. EZH2 inhibition using GSK126 significantly upregulated *MALAT1* expression in three SOX11 positive MCL PDLS growing with or without pro-proliferative factors. The inhibitor was used at two 10-fold different concentrations, and we could observe a highly significant increase in *MALAT1* expression upon the treatment with the highest dose of the inhibitor in all cases with pro-proliferative factors and nearly all in their absence.

with poor prognosis in MCL[24,25]. EZH2 is also known to interact with *MALAT1* within the PRC2 complex but the related study was done in MCL cell lines and without considering potential mutual regulatory effects between them[8]. In our primary MCL samples, *MALAT1* expression was inversely correlated with *EZH2* levels and directly associated with reduced cell growth. Silencing *MALAT1* led to increased *EZH2* expression in two MCL cases cultured without pro-proliferative factors. This relationship was not observed in RNA-seq data from three SOX11-positive PDLS samples cultured with such stimuli, suggesting that *MALAT1* regulatory influence on *EZH2* is modest compared to the dominant effect of microenvironmental signals. Therefore, our results strongly support the main reason for the inverse association between *MALAT1* and *EZH2* expression to be the effect that the presence of pro-proliferative factors in the media has on them. Further supporting this, treatment with the EZH2 inhibitor GSK126 resulted in a dose-dependent increase in *MALAT1* expression, reinforcing the role of EZH2 as a mediator of microenvironment-driven repression of *MALAT1*.

To explore the broader functional impact of *MALAT1* in MCL pathogenesis, we analyzed RNA-seq data following *MALAT1* silencing in three MCL cases. Only a limited number of differentially expressed (DE) genes were identified. Among these, to our knowledge, only one upregulated gene (*CSNK2B*) has previously been implicated in pathways that promote lymphocyte activation through microenvironmental signals[22], including BCR, Toll-like receptor, CD40, and IL-4 signaling[21]. Although the magnitude of transcriptional changes observed does not indicate a global reprogramming dependent on *MALAT1*, additional experiments comparing *MALAT1* levels in PB samples and their derived PDLS cultured with full growth factor supplementation and monocyte co-culture revealed a consistent decrease in *MALAT1* expression under microenvironmental stimulation. These findings highlight the role of normal cell-derived factors in modulating *MALAT1* expression and their potential impact on MCL cell proliferation and viability. Similarly, *MALAT1*-correlated coding genes showed negative enrichment in pathways associated with aggressive clinical behavior and microenvironmental stimuli, including pro-proliferative factors such as IL-4 and CD40L.

Despite the limited number of patient samples, our results—combined with the absence of correlation between *MALAT1* levels and established genetic prognostic markers—suggest that *MALAT1* contributes to microenvironment-dependent regulation of proliferation in MCL, with possible implications for patient outcomes. Further studies are warranted to increase sample size and dissect the influence of specific microenvironmental factors and cell types on *MALAT1* expression. Moreover, although *TALAM1* expression demonstrated similar prognostic value to *MALAT1* in predicting favorable outcomes, functional experiments were not feasible due to its low expression levels, which limited detection even at maximum RNA input. Additionally, as *TALAM1* is an antisense transcript of *MALAT1*, we cannot exclude the possibility that the observed correlations reflect co-expression rather than independent functional roles. Future work is needed to clarify the biological significance of *TALAM1* in MCL.

In summary, we demonstrate that *MALAT1* and *TALAM1* expression is associated with favorable prognosis in MCL. Functional data on *MALAT1* supports its clinical relevance and suggests that these lncRNAs warrant further investigation to elucidate their mechanisms and target genes. Their potential as therapeutic targets, such as strategies to overexpress *MALAT1* to counteract pro-proliferative signaling, may offer new avenues to enhance existing treatments compromised by microenvironmental influences.

Materials and methods

Samples

This study used previously published genome wide MCL transcriptional data. In particular, MCL microarray data previously generated by us and other studies were obtained from GEO datasets GSE70910 (cohort#1), GSE93291 (cohort#2) and GSE79196 (cohort#3)[16–18], which overall include 59 PB samples and 160 LN MCL samples. Clinical data was available for a total of 166 cases. Overall survival (OS) was determined from the moment of sampling. Cryopreserved MCL patient samples used for the functional studies were obtained from the Hematopathology collection of the Biobank of the Hospital Clínic de Barcelona-IDIBAPS (Spain), in accordance with the Declaration of Helsinki and national regulations, including the approval of the Institutional Review Board of Hospital Clínic de Barcelona. All patients provided written informed consent. For the functional studies a total of 4 patients from cohort#3 were included: one SOX11 negative non-nodal MCL (MCL#2) and three SOX11 positive classical MCL (MCL#1 and MCL#3 from low *MALAT1* expression group and MCL#4 from high *MALAT1* expression group).

Bioinformatic analyses and statistics

Microarray data were normalized with RMA and batch effects were adjusted with ComBat[26]. Although the expression microarrays (Human Genome U133 Plus 2.0 from Affymetrix) were enriched in probe sets for coding genes, they also included several probe sets that hybridize exclusively to the *MALAT1 or TALAM1* transcripts. The expression analysis using probe sets that hybridize to the corresponding strands from where these lncRNAs are transcribed is an approach that has been previously described and validated with independent techniques to be a general reliable measure of the expression of transcripts in antisense orientation[27]. The specificity of the probe sets was confirmed in the Affymetrix database (NettAffx™). Only one probe set was excluded (223579_s_at) at this stage due to lack of specificity. The different probe sets were evaluated by strand, and one additional probe set was excluded as an outlier regarding the lack of significant association with prognosis when considered individually in cohort#2 (224559_at). Finally, the mean values of the remaining probe sets were retained as reliable for measuring the expression levels of *MALAT1* (probe sets 1558678_s_at, 223940_x_at, 224558_s_at, 224567_x_at, 224568_x_at and 226675_s_at) and *TALAM1* (including 223577_x_at, 223578_x_at, 227510_x_at, 228582_x_at, 231735_s_at) transcripts.

Clinical association studies used overall survival (OS) as the primary endpoint, with survival curves estimated with the Kaplan-Meier method. Optimal cut-off points for *MALAT1/TALAM1* expression groups

were obtained using *maxstat*[28]. Student's t-test was used to evaluate the differences in the mean expression of *MALAT1* and *TALAM1* among subtypes and molecular factors with previously described prognostic value in MCL. This test was also applied to analyze differences in the mean proliferation signature between *MALAT1* and *TALAM1* expression groups in leukemic MCL cases with data previously generated by Nanostring[29]. Linear regression on scatter plots was used for representing the correlation between *MALAT1/TALAM1* expression with the proliferation signature clusters. Scatterplots, and box plot graphs were generated using GraphPad Prism v7. Hierarchical clustering analysis for MCL35 signature of proliferation-related genes was performed over their Z-scores using Pearson uncentered metrics implemented in the TIGR package TMeV v4.0 (https://github.com/web-mev/). The analysis of the BCR signature and its relation to *MALAT1/TALAM1* expression was performed using the published data from of cohort#1[16].

For the analysis of RNAseq data, ribosomic RNA reads were filtered out using SortMeRNA (version 4.3.4). Non-ribosomic reads were trimmed using trimmomatic (version 0.39) and transcript-level counts (GRCh38.p13; Ensembl release 105) were calculated using kallisto (version 0.46.1). The tximport package (version 1.30.0) was used to load gene-level counts into R (version 4.3.3). DESeq2 package (version 1.42.1) was used to perform differential expression analysis following authors' recommendations. Cutoff parameters were a log fold change of 0.25 and an adjusted p-value < 0.05. Pathway enrichment analysis was performed on independent gene lists of upregulated and downregulated genes upon *MALAT1* silencing obtained by considering only the log fold change cutoff of 0.25. Metascape webtool v3.5 (http://www.metascape.org/) was used as the software of choice for these analyses[30].

In vitro studies with MCL primary samples

Patient derived lymphoma spheroids (PDLS) from four MCL patients MCL#1 to MCL#4 (see sample section) were generated using a previously described method where the cells were cultivated in presence of several factors stimulating proliferation (IL-4, CD40L, also adding HA-tag to allow proper stimulatory activity of CD40L) and survival (BAFF)[20], or with BAFF alone. *MALAT1* silencing was performed by adding 1μM specific Gapmer (Exiqon, Bionova S.L.) 1-hour post-seeding in comparison to paired PDLS treated with 1μM of a scrambled control Gapmer (Exiqon, Bionova S.L.). The incorporation of Gapmers was achieved by Gymnotic delivery, meaning the involvement of passive entry of the oligos that bind to the cell membranes[31]. The *MALAT1* Gapmer sequence (5'-CGTTAACTAGGCTTTA-3') target al.l three described transcript variants (NR_002819.4, NR_144567.1 and NR_144568.1). The scrambled control Gapmer sequence (5'-AACACGTCTATACGC-3') has no target in the human transcriptome.

Viability/proliferation was assessed on days 0/4 using the Alamarblue HS assay (ThermoScientific), with fluorescence measured after four hours of incubation and using an Infinite M Nano spectrophotometer (TECAN), following the supplier recommendations. The amount of *MALAT1* silencing was also quantified at day 4 by qRT-PCR, normalized using U6 as a reference gene, as described elsewhere[32]. *EZH2* expression normalized with TBP/HTRP1 as endogenous reference genes was also measured by qRT-PCR in three classical/SOX11 + MCL cases using Taqman Fast Advance mastermix and Taqman Assays (*EZH2*: Hs00544830_m1; *TBP*: Hs00427620_m1; *HTRP1*: Hs02800695_m1) (Applied Biosystems, ThermoScientific). Furthermore, the levels *MALAT1* and *EZH2* were also studied in previously published in four MCL PDLS (two classical/SOX11 + and two non-nodal MCL/SOX11-) under the same experimental conditions as used here previously published[22]. In these four PDLS we also studied the genes which expression was related to *MALAT1* levels. Pathway enrichment analyses were performed on correlated genes with *MALAT1* as previously described[32].

RNAseq

Total RNA was extracted using the RNeasy Plus Micro Kit (Qiagen), quantified with Qubit, and quality-checked using Tapestation 4200 (Agilent). RNA was obtained from three SOX11-positive cases used in functional studies comparing *MALAT1* silencing with scrambled controls. Only samples cultured with pro-proliferative factors yielded sufficient RNA for sequencing. Libraries were prepared from 10 ng of total RNA using the TAKARA_SMART-Seq® Total RNA Pico Input (ZapR® Mammalian) kit (following the manufacturer's instructions). Libraries were sequenced using a NextSeq2000 (Illumina, Inc.) in paired-end mode with a read length of 2 × 50 bp, generating more than 60 million paired-end reads per sample.

Data availability

Data provided within the manuscript or supplementary information files that support the findings of this study are available from the corresponding author upon reasonable request. Previous data from elsewhere that were used for some analyses done in this work are available from GEO repository accession numbers GSE70910, GSE93291 and GSE79196. RNA-seq data was deposited at ArrayExpress database at EMBL-EBI under accession E-MTAB-16259.

Received: 12 June 2025; Accepted: 2 February 2026
Published online: 07 February 2026

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Acknowledgements

We are indebted to the Genomics core facility of the Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS).

Author contributions

Conceived and designed the Study: L.H., J.I.M-S. and E.C. Collection and curation of cryopreserved material, clinical and molecular databases of MCL from ICGC/Hospital Clinic de Barcelona: A.M.-F., D.C., P.M., A.R., E.G., A.L.-G., S.B. and I.S. Final sample selection: C.L. and L.H. Microarrays preparation and bioinformatic analysis for lncRNA: E.F-G., G.C., F.N., S.D., P.J., A.D., V.A., and L.H. PDLS experiments; C.M.-M., F.A.-A., J.G., P.P.-G. and L.H. qRT-PCR: C.M.-M. and L.H. Wrote the manuscript: L.H. and E.C. All authors read and approved the final manuscript.

Funding

This research was funded by Spanish Ministry of Science, Innovation and Universities (MCIN) & European Regional Development Fund (ERDF, "A way of making Europe") grant numbers PID2021-123054OB-I00 and PID2024-155962OB-I00 to E.C and PID2021-124894OB-I00 to P.P.G. Fundació La Marato de TV3 ("projecte

finançat per Fundació La Marató de TV3") 201920-30 to L.H. and TAIFOL project, 201933-30 to P.P.G. Interreg POCTEFA program (IMLINFO EFA281/16 and THERAVLINFO EFA123/1 to P.P.G. Suport Grups de Recerca AGAUR (2021-SGR-01172, 2021-SGR-01343 and 2021-SGR-01294 of the Generalitat de Catalunya to E.C., J.I.M-S. and P.P.G., respectively. CIBERONC (ONCG09/2025) to E.C. AGAUR 2018 FIB00696, Generalitat de Catalunya and FSE (European Union) to E.M.F.GJ.I.M-S is and Academia Researcher of the "Institució Catalana de Recerca i Estudis Avançats" (ICREA) of the Generalitat de Catalunya.

Declarations

Competing interests

E.C. has been a consultant for Takeda, NanoString, AbbVie and Illumina; has received honoraria from Janssen, EUSPharma and Roche for speaking at educational activities and research funding from AstraZeneca and is an inventor on 2 patents filed by the National Institutes of Health, National Cancer Institute: "Methods for selecting and treating lymphoma types," licensed to NanoString Technologies, and "Evaluation of mantle cell lymphoma and methods related thereof", not related to this project. F.N. has received honoraria from AbbVie, AstraZeneca, Janssen, and Sophia Genetics for speaking at educational activities. F.N. and E.C. have licensed to Diagnostica Longwood and Sophia Genetics the IgCaller algorithm. D.C. has received honoraria from AbbVie, Sophia Genetics, Thermofisher and AstraZeneca for speaking at educational activities. The remaining authors declare no competing financial interests.

Ethics approval and consent to participate

The cryopreserved samples used for the functional studies were obtained from MCL patients were obtained from the Hematopathology collection of the Biobank of the Hospital Clínic de Barcelona-IDIBAPS (Spain), in accordance with Declaration of Helsinki and national regulations, including the approval of the Institutional Review Board of Hospital Clínic de Barcelona. All patients provided written informed consent.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-38971-0.

Correspondence and requests for materials should be addressed to L.H.

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