REPORT
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THE IMMUNE TUMOR MICROENVIRONMENT IN PATHOGENESIS AND CONTROL OF MANTLE CELL LYMPHOMA

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1. Summary of the project

Mantle cell lymphoma (MCL) is an aggressive mature B-cell lymphoma, but some patients have an indolent behaviour. The differential expression of SOX11 is one of the major factors that distinguish conventional aggressive and the more indolent leukaemic non-nodal MCL subtypes (nnMCL). We have previously demonstrated the oncogenic role of SOX11 promoting the interaction of tumour cells with endothelial and stromal cells, that support angiogenesis, invasion, and drug resistance in MCL. Despite these findings, little is known about how non-malignant cells and secreted proteins by the tumour microenvironment (TME) affect disease development and prognosis in MCL. We have generated preliminary data of the immunological ecosystem of the MCL TME, using the immune-Gene Expression Profiling NanoString Platform and the secretome in ex-vivo co-culture experiments by mass spectrometry analyses. These results suggest that the immune response in SOX11-expressing aggressive MCL is downregulated compared to nnMCL and normal lymph node (LN) tissues. By immunohistochemistry, we have observed increased levels of FOXP3+ T regulatory (Treg) cells in SOX11-positive compared to -negative LN MCL cases. Moreover, we have identified immune checkpoint proteins and secreted immune factors associated to worse survival in MCL. Our project proposes an integrative transcriptomic, proteomic and cell biology study to understand the composition and the complex interactions between tumour cells and its immune TME, in different MCL tissue samples. After validation in large series of MCL cases, the clinical impact of immunological factors and the influence of SOX11 expression in this MCL TME will be analyzed.

Our preliminary results suggest that the tumour microenvironment (TME)-related immune system (Tumour immune microenvironment (TIM)) controls tumour progression, aggressiveness, and drug resistance in relapse MCL patients. Moreover, SOX11 expression in MCL cells may play an important role in regulating the transcription and secretion of key factors related to tumour-nontumoral adjacent cells protective interactions and immune evasion in aggressive MCL.

Our main objective is to identify immunological mediators of MCL progression and control, as biomarkers with significant challenges for future therapies. Moreover, our project proposes 3D in vitro and in vivo PDX MCL systems where small molecules, blocking antibodies and other immune therapies would be preclinically assayed.

2. Results obtained (papers generated and brief abstract of the results published on it):

De Bolòs A, Sureda-Gómez M, Carreras-Caballé M, Rodríguez ML, Clot G, Beà S, Giné E, Campo E, Balsas P, Amador V. (2024).SOX11/PRDX2 axis modulates redox homeostasis and chemoresistance in aggressive mantle cell lymphoma. *Scientific Reports.* 14(1):7863. doi: 10.1038/s41598-024-58216-2. PMID: 38570586

SRY-related HMG-box gene 11 (SOX11) is a transcription factor that plays an oncogenic role in MCL by promoting a protective crosstalk with the tumour microenvironment, also increasing angiogenesis, immunosuppression and improving tumour cell survival. Recently, we have characterized the nodal MCL microenvironment. However, the precise mechanisms by which MCL cells adapt to survive and grow within the microenvironment remain unknown.
Uncontrolled tumour growth produces high levels of reactive oxygen species (ROS), in response to oxidative stress. Several drugs used to treat cancer are highly dependent on ROS-mediated cytotoxicity. However, tumour cells adapt to survive under this stress conditions through the induction of multiple tumorigenic processes. Oxidative stress caused by the imbalance between ROS and the antioxidant system has been largely associated with drug resistance and cancer progression.

In this paper, we analyzed redox homeostasis in mantle cell lymphoma (MCL.)By gene set enrichment analysis, we observed an upregulation of oxidative stress-related genes and a significant higher production of ROS in SOX11-positive compared to SOX11-negative MCL cases. Within them, we identified peredoxine2 (*PRDX2*) as one of the most significant upregulated antioxidant genes, which significantly correlated with SOX11 overexpression, ROS production in MCL cells and associated with worse overall survival of patients. Moreover, *PRDX2* gene upregulation showed a significant negative prognostic value independently of two other MCL risk factors, high expression of the proliferation signature and copy number alterations (CNA).

Interestingly, PRDX2 expression was reduced upon SOX11 knockout (SOX11KO). Moreover, we observed that PRDX2 expression and ROS production were induced by hypoxia in SOX11-positive, but not in SOX11-negative MCL cell lines.
To decipher the role of PRDX2 in MCL redox homeostasis, we used knockdown PRDX2 (PRDX2KD) in MCL cell lines. In vitro cell death experiments, under hypoxic conditions, showed that SOX11KO and PRDX2KD MCL cells had increased ROS levels and ROS-mediated tumour cell death upon treatment with drugs, compared to control cells. Together, these results suggested that SOX11-positive MCL could adapt to survive to chemotherapy by countering ROS levels through the increase of PRDX2 expression levels in MCL.

Overall, our results suggest that SOX11 through PRDX2 may be playing a role in the adaptation to hypoxia and oxidative stress, protecting tumour cells from drug-mediated cell death through the modulation of ROS levels and its mediated cytotoxic response. Our results suggest a mechanistic pathway explaining oxidative stress-mediated cytotoxic response and propose PRDX2 as promising therapeutic alternative for patients with chemoresistant/refractory MCLs.

Sureda-Gómez M[*], Iaccarino I[*], De Bolòs A, Meyer M, Balsas P, Richter J, Rodríguez ML, López C, Carreras-Caballé M, Glaser S, Ferran N, Jares P, Siciliano MCh, Bellan C, Tornambè S, Boccacci R, Clot G, Leoncini L, Campo Elias, Siebert R, Amador V[†#], Klapper W[†]. (2024). SOX11 and Epstein–Barr virus may substitute each other in the pathogenesis of Burkitt Lymphoma. ***Blood. Accepted. ([#]Corresponding author)***
These manuscripts have recently been accepted for publication in Blood journal, one of the most important reference journals in the field of haematological diseases.
SOX11 is a transcription factor overexpressed in mantle cell lymphoma (MCL), a subset of Burkitt lymphomas (BL) and precursor lymphoid cell neoplasms but is absent in normal B-cells and other B-cell lymphomas. SOX11 has an oncogenic role in MCL but its contribution to BL pathogenesis remains uncertain. Here, we observed that the presence of Epstein-Barr virus (EBV) and SOX11 expression were mutually exclusive in BL. SOX11 expression in EBV- BL was associated with an *IG*::*MYC* translocation generated by aberrant class switch recombination, while in EBV-/SOX11- tumours the *IG*::*MYC* translocation was mediated by mistaken somatic hypermutations.

Interestingly, EBV- SOX11 expressing BL showed higher frequency of *SMARCA4* and *ID3* mutations compared to EBV-/SOX11- cases. By RNA-sequencing, we identified a SOX11-associated gene expression profile, with functional annotations showing partial overlap with the SOX11 transcriptional program of MCL. Contrary to MCL, no differences on cell migration or BCR signalling were found between SOX11- and SOX11+ BL cells. However, SOX11+ BL showed higher adhesion to VCAM-1 than SOX11- BL cell lines. Here we demonstrate that EBV- BL comprises two subsets of cases based on SOX11 expression. The mutual exclusion of SOX11 and EBV, and the association of SOX11 with a specific genetic landscape suggest a role of SOX11 in the early pathogenesis of BL.

Sureda-Gómez M, Balsas P, Rodríguez ML, Nadeu F, De Bolós A, Eguileor A, Kilus M, Castellano G, López C, Giné E, Demajo S, Jares P, Martín-Subero JI, Beà S, Campo E, Amador V. (2023). Tumorigenic role of musashi-2 in aggressive mantle cell lymphoma. *Leukemia.* 37(2):408-421. doi: 10.1038/s41375-022-01776-x. PMID: 36509891

SOX11 (SRY-related high-mobility-group box 11) is expressed during embryogenesis but largely absent in most adult differentiated tissues. SOX11 dysregulation has been implicated in several diseases including neurodevelopmental disorders, and cancers. SOX11 has been proposed as a potential biomarker for diagnosis and prognostic for several solid and aggressive lymphoid tumours, including mantle cell lymphoma (MCL) and Burkitt lymphoma.

In this paper we found that SOX11 could be mediating stemness features in aggressive MCL, regulating the expression of stem cell-related genes, which could be associated with the chemotherapy resistance and relapses, frequently observed in MCL.
MCL frequently responds to initial treatment, although later development of resistance is common, relapsing with more aggressive disease. These results suggest an MCL-initiating cell population with stem cell features, usually associated with resistance to standard therapies that could explain why MCL is still an incurable lymphoma, despite an adequate rate of complete remission to frontline treatments.

In this paper, we have shown that SOX11+ MCLs had significantly higher enrichment in leukaemic and haematopoietic stem cells gene signatures compared to SOX11- MCL primary cases. Moreover, we identified a new oncogene involved in the MCL

pathogenesis, Musashi-2 (MSI2). MSI2 emerged as one of the most significant upregulated stem cell-related genes in SOX11+ MCLs. MSI2 upregulation was significantly associated with, higher clonogenic growth, survival upon drug treatments and poor overall survival independently of other high-risk features of MCL. In vivo, we demonstrated that MSI2-knockdown cells had reduced tumorigenic engraftment into mice bone marrow and spleen compared to control cells in xenotransplanted mouse models. Interestingly, we have proved that MSI2 specific inhibitor (Ro 08-2750) decreased the expression of genes related to apoptosis and stem cell features and significantly reduced clonogenic growth, tumour cell survival and chemoresistance in MCL cells.

Our results indicates that MSI2 might play a key role in sustaining stemness and tumour cell survival, representing a possible novel target for therapeutic interventions in MCL to inhibit drug resistance and relapse in aggressive MCLs.

Balsas P, Veloza L, Clot G, Sureda-Gómez M, Rodríguez ML, Masaoutis C, Frigola G, Navarro A, Beà S, Nadeu F, Giné E, López-Guillermo A, Martínez A, Ribera-Cortada I, Engel P, Quintanilla-Martínez L, Klapper W, Campo E, Amador V.(2021). SOX11, CD70 and Treg cells configure the tumor immune microenvironment of aggressive mantle cell lymphoma. *Blood.* 138(22):2202-2215. doi: 10.1182/blood.2020010527. Blood. 2021. PMID: 34189576

While improvements in next generation sequencing (NGS) technology have greatly increased our understanding of the intrinsic abnormalities of MCL, the role of extrinsic signals is poorly known. Several studies had highlighted the central role of the tumour microenvironment (TME) in the pathogenesis of MCL. However, the characterization of the diverse tumour niches and comprehension of the crosstalk between tumour cells and surrounding cell within the MCL microenvironment remain largely unknown.
In this paper, my research group has characterized the nodal tumour microenvironment of SOX11+ and SOX11- MCL primary samples. We discovered that SOX11+ nodal MCLs have a significant immunodepressed TME, with lower T-cell intratumoral infiltration and a reduced expression of MHCI/II-like and T-cell costimulation and signalling activation related transcripts compared to negative cases. Lower T-cell infiltration significantly associated with poor clinical outcome.
My group also identified an immune checkpoint, CD70, upregulated in SOX11+ compared to negative cases, associated with an immune unbalance of the tumour

microenvironment characterized by increased number of effector regulatory T (Treg) cell infiltration, higher proliferation, and aggressive clinical course.

Here, my lab was the first to describe the nodal TME in MCL, discovering that SOX11 expression in MCL is associated with an immunosuppressive microenvironment characterized by CD70 overexpression in tumour cells, increased regulatory T cells (Treg cell) infiltration and downmodulation of antigen processing, and presentation and T-cell activation that could promote MCL progression. Characteristic CD70 upregulation in aggressive SOX11+ cMCLs has opened the possibility to treat MCL with anti-CD70 CART-cells immunotherapies.

Moreover, in collaboration with principal investigators of the lymphoid neoplasm program and other research groups, we have identified altered genes that could distinguish between patients with different types of non-Hodgkin lymphoma or Leukemia to identify new candidates and networks responsible for the aggressive behaviour of these neoplasms that may be potential candidates for therapeutic interventions (***Morsy MHA, et al., Blood 2024; Li Y, et al., J Mol Biol. 2022; Domostegui A, et al., J. Blood. 2021***).

3. Relevance with possible future implications

1. Mantle-cell lymphoma (MCL) is an aggressive and still incurable lymphoma. Targeted therapies have improved outcome but to a degree. Chimeric antigen receptor T-cells (CART-cells) targeting CD19 have also shown a remarkable efficacy in patients with relapsed/refractory (R/R) MCL. However, long-term results with these products have revealed a progression-free survival around 2 years, including early and late relapses. Recently, we have identified CD70 overexpressed in aggressive compared to indolent nodal MCLs and non-tumour reactive lymph nodes. CD70 upregulation significantly correlated with SOX11 overexpression, blastoid/pleomorphic cytological morphology, higher proliferation and higher intratumoral infiltration of effector regulatory T-cells and associated with poorest outcome of patients (***Balsas P, et al., Blood. 2021***). CD70 is playing an essential role in tumour progression by promoting immune suppression, as well as tumour cell proliferation and survival in aggressive MCL. Our preliminary data has shown that anti-CD70 blocking antibodies increased IFN-γ secretion and tumour

cell death in MCL/T-cells co-culture systems. In teamwork with haematologists and immunologists at the Hospital Clínic de Barcelona, we are developing a new dual anti-CD19/CD70 CART-cell based immunotherapy, trying to overcome anti-CD19 CART-cell therapy failures. If our dual CART-cells product is superior to anti-CD19 CART-cells, in pre-clinical assays, we plan to manufacture clinical-grade dual anti-CD19/CD70 CART-cells from patients, using the CliniMACS Prodigy system, to clinical translate this product for a phase I clinical trial, aiming to improve outcome and life quality of patients with MCL.

2. The pattern of minimal residual disease and consequent relapses observed in MCL suggests the presence of a cell population with higher self-renewal and treatment resistance capabilities. Different research groups have described the presence of cancer stem cell (CSC) populations in MCL, characterized by an increase in drug resistance, clonogenic growth, ALDH activity and tumorigenicity. However, MCL-CSC factors and their contribution to tumour behaviour have not been explored in MCL. Recently, my group identified MSI2, an RNA-binding protein that regulates self-renewal and differentiation in embryonic and haematopoietic stem cells, directly regulated by SOX11. MSI2 upregulation in MCL was significantly associated with a poorest overall survival of patients. MSI2 knockdown (KD) significantly decreased apoptosis-related genes expression and stem cell features, reducing clonogenic growth, tumour cell survival and chemoresistance in vitro, as well as tumorigenic engraftment in xenotransplanted MCL mice models (***Sureda-Gómez M, et al., Leukemia 2023***). MSI2 plays a key role in sustaining stemness and tumour cell survival, highlighting it as potential therapeutic target to inhibit stemness features and relapses in aggressive MCL. Our data have shown that Ro 08-2750, a small molecule that binds selectively to the MSI2 RNA-binding site, leaded to MSI2-loss of function in vitro. However, it proved too toxic to be used for therapy in vivo. In cooperation with Dr RD. Artero at the "Instituto Universitario de Biotecnología y Biomedicina" (BIOTECMED), Universidad de Valencia, we are analyzing the antitumorigenic effect of in vivo non-toxic compounds, based on modified oligonucleotides (gapmers) that can specifically inhibit the oncogenic effects due to MSI2 upregulation in lymphoid cells and patients derived xenotransplanted (PDX) MCL mice models expressing MSI2, to demonstrate that the use of gapmers targeting MSI2 could be a promising therapeutic approach, as target therapy for cancer stem cells, for R/R MCL.

3. SOX11 is a pioneer factor, member of the SOX family of transcription factors (TF) characterized by binding the DNA at the minor groove, through its high mobility group (HMG) domain. The HMG DNA-binding domain is also able to interact with one or more variety of proteins simultaneously, forming protein complexes that could determine SOX-specific functional roles. SOX11 is regulating different genes and oncogenic mechanisms in the pathogenesis of MCL, suggesting that it might be interacting with different protein complexes for its distinct actions. Recently, my group has identified SOX11-specific interactomes by proximity-labelling (BioID2) coupled to mass spectrometry proteomic strategies in MCL cells. STRING analyses have revealed a highly connected interactome, which included the SWI/SNF, histone acetyltransferase, mediator, and DNA repair complexes, as well as interactions with a diversity of TFs involved in tumour development. In parallel, we have identified specific SOX11-genome wide DNA binding sides and direct transcriptional regulated genes, using CUT&RUN- and RNA-sequencing analyses (***De Bolòs A, et al., in preparation***). We have validated direct physical interactions and characterized DNA-binding sides and direct target genes commonly regulated by SOX11 in complex with SMARCA4/BRG1, the catalytic ATPase subunit of the SWI/SNF complex; and with other partners, to identify new oncogenic SOX11-protein-DNA complexes, as well as small molecules that would block these interactions as target therapies for aggressive MCL.

4. SOX11 is absent in normal B-cells and B-cell lymphomas except for precursor cell neoplasms, MCL and Burkitt lymphoma (BL). SOX11 has an oncogenic role in MCL but its contribution to BL pathogenesis remains uncertain. Recently, we observed EBV and SOX11 to be mutually exclusive in BL. Within the group of EBV- BL, SOX11 expression correlated with case switch recombination-mediated *IG*::*MYC* translocation, while in EBV-/SOX11- cases, somatic hypermutation is the cause for *IG*::*MYC*. Interestingly, within EBV- BL, SOX11 expression was significantly associated with mutations in *SMARCA4* and *ID3* genes. By RNA-sequencing, we identified a SOX11-associated gene expression profile that showed partial overlap with the MCL SOX11 transcriptional program (***Sureda-Gómez M, et al., Blood. 2024***). Within EBV- BL, SOX11 expression delineates a molecular subtype of this disease, it having a major role in early BL pathogenesis. We have now analysed the mechanistic-functional role of SOX11 in BL cells, its relationship with direct target gene and transcriptional program changes and *SMARCA4* mutations, to identify its early oncogenic implication in BL pathogenesis and new promising target therapies for BL.

4. Scientific bibliography generated

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