available at www.sciencedirect.com www.elsevier.com/locate/molonc

Knock-down of SOX11 induces autotaxin-dependent increase in proliferation *in vitro* and more aggressive tumors *in vivo*

Paolo Conrotto^{a,b,1}, Ulrika Andréasson^{a,b,1}, Venera Kuci^{a,b}, Carl A.K. Borrebaeck^{a,b}, Sara Ek^{a,b,*}

^aDepartment of Immunotechnology, Lund University, BMC D13, SE-221 84 Lund, Sweden

^bCREATE Health, Lund University, BMC D13, SE-221 84 Lund, Sweden

ARTICLE INFO

Article history:

Received 9 June 2011

Accepted 1 August 2011

Available online 22 August 2011

Keywords:

SOX11

Proliferation

Autotaxin

NOD-SCID mice

MCL

Z138

ABSTRACT

The transcription factor SOX11 is a novel diagnostic marker for mantle cell lymphoma (MCL), distinguishing this aggressive tumor from potential simulators. Recent data also show that the level of SOX11 correlates to *in vitro* growth properties in MCL, as well as the clinical progression. We have previously shown that MCL-associated pathways, such as Rb-E2F, are dysregulated leading to decreased proliferation upon overexpression of SOX11, emphasizing the impact of SOX11 on MCL-specific gene expression and growth control. However, it remains to be determined which growth regulatory pathways that are induced upon SOX11 knock-down, leading to an increased cellular growth. Consequently, we established a model cell line with constitutive down-regulation of SOX11. The highly proliferative features of this cell line were investigated by gene expression analysis, proliferation assay, cell cycle distribution and potential to induce tumors in NOD-SCID mice. Our *in vitro* studies demonstrated a SOX11-dependent regulation of MCL-specific gene expression. In addition, we identified autotaxin (ATX) to be regulated by SOX11. Our results clearly showed a correlation between SOX11 level and cellular growth rate, which was dependent on ATX, as well as a direct relation between the level of SOX11 in tumorigenic cells and the growth rate of these tumors in NOD-SCID mice.

© 2011 Federation of European Biochemical Societies.

Published by Elsevier B.V. All rights reserved.

1. Introduction

The neural transcription factor SOX11 (Penzo-Mendez, 2010) has recently gained great interest as a diagnostic marker for mantle cell lymphoma (MCL) (Chen et al., 2009; Dictor et al., 2009; Ek et al., 2008; Fernandez et al., 2010; Mozos et al.,

2009; Wang et al., 2008). Apart from MCL, SOX11 is also present in a subset of Burkitt's lymphoma, B- and T-cell lymphoblastic leukemia (Dictor et al., 2009; Mozos et al., 2009), and some hairy cell leukemias (Chen et al., 2009), while other major lymphoma subtypes, including FL, completely lack expression of SOX11. We have previously shown that SOX11 is epigenetically

Abbreviations: autotaxin, ATX; counts per minute, cpm; fetal bovine serum, FBS; immunohistochemistry, IHC; interference, iRNA; intravenously, i.v; mantle cell lymphoma, MCL; phosphate buffered saline, PBS; quantitative real-time polymerase chain reaction, Q-RT-PCR; room temperature, RT; scramble, SCR; western blot, WB.

* Corresponding author. Department of Immunotechnology, Lund University, BMC D13, SE-221 84 Lund, Sweden. Tel.: +46 46 222 38 24; fax: +46 46 222 42 00.

E-mail addresses: paolo.conrotto@immun.lth.se (P. Conrotto), ulrika.andreasson@immun.lth.se (U. Andréasson), venera.kuci@immun.lth.se (V. Kuci), carl.borrebaeck@immun.lth.se (C.A.K. Borrebaeck), sara.ek@immun.lth.se (S. Ek).

¹ These authors contributed equally to this study.

1574-7891/\$ – see front matter © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

doi:10.1016/j.molonc.2011.08.001

regulated, (Gustavsson et al., 2010) although the underlying events leading to a differential methylation pattern remain to be determined. However, SOX11 has different partnering transcription factors (Kuhlbrodt et al., 1998; Wiebe et al., 2003) and its function must consequently be discussed in the relevant cellular context. We have recently identified SOX11 as a growth regulatory molecule in MCL, as transient knock-down and overexpression of SOX11 *in vitro* changed the proliferation rate (Gustavsson et al., 2010). In addition, clinical data suggest a correlation between SOX11 expression levels and the aggressiveness of disease. In fact, the expression of SOX11 in MCL is associated with improved survival (Wang et al., 2008), although other data point towards a different role of SOX11 in non-indolent MCL (Fernandez et al., 2010). However, both these observations need to be confirmed in larger clinical cohorts.

In this study, we use the MCL cell line Z138 to create a model cell line (Z138-SOX11^{knocked}) with constitutively reduced levels of SOX11, using a retroviral transduction of specific shRNA. As control we used the same cell line carrying a scrambled shRNA sequence (Z138-SCR). The established cell lines were characterized by determining their, (i) gene expression (ii) functional characteristics and (iii) *in vivo* tumor formation. Our data clearly demonstrates that SOX11 directly regulates a large number of genes, previously shown to be specifically expressed in MCL compared to non-malignant B cell populations or other B cell lymphomas, i.e. MCL-specific genes (Obrador-Hevia et al., 2009). This is consistent with SOX11 being a disease-defining antigen, both phenotypically and functionally. Validation was focused on these genes, known to be consistently deregulated in primary MCL (Obrador-Hevia et al., 2009). Proliferation and cell cycle analysis further confirmed our previously suggested growth-regulatory role of SOX11 (Gustavsson et al., 2010). For the first time, we show that this growth regulatory effect is, at least in part, mediated by the increased levels of the secreted plasma lysophospholipase D enzyme autotaxin (ATX), known to be associated with aggressiveness of disease (Braddock, 2010; Samadi et al., 2011). Of clinical interest, the increased proliferation of Z138-SOX11^{knocked} was translated into a more aggressive tumor behavior *in vivo*, as shown using NOD-SCID mice.

2. Material and methods

2.1. Retroviral production

Two different iRNAs, (i) one targeting SOX11 mRNA (5'-CAAG-TATGTTGGTACGTTATGTT-3') (Eurofins MWG Operon, Ebersberg, Germany) and (ii) the other targeting a scrambled sequence (SCR) (5'-AGTACTGCTTACGATACGGTTTT-3'), were cloned into the retroviral vector, pRSMX_PG, a kind gift from Louis M Staudt, (NCI-Bethesda, MD, USA), as previously described (Ngo et al., 2006). In brief, sequences were cloned into the vector between the two restriction sites BglI and HindIII. Retroviral production was performed in Phoenix cells (American Type Culture Collection (ATCC)) (Rockville, MD) cultivated in DMEM (Invitrogen, San Diego, CA, USA) supplemented with 2 mM L-glutamine (Invitrogen) and 10% fetal bovine serum (FBS, Invitrogen). At day of transfection the cells

were 80% confluent and the media was changed to also include 1% Sodium Pyruvate (Invitrogen) and 1% HEPES 1 M (10 mM final concentration) (Invitrogen). The cells were transfected with a cocktail of DNA vectors (retroviral vectors pRSMX_PG-SCR or pRSMX_PG-SOX11^{knocked}, 10 µg, Gag pol (pcDNA3.MLVgp) 6 µg, RD114 coding viral envelop 5 µg). To enable satisfying transfection, Calcium Phosphate Transfection Kit (CAPHOS) (Sigma-Aldrich St. Louis, USA) was used, according to the manufacturer's protocol. The cell supernatant containing retroviruses was harvested after 48 and 72 h, filtered to remove cellular debris, and stored at -80 °C. The viral titre was determined using the GFP gene.

2.2. Infection of target cells

The MCL cell line Z138 (kindly provided by Dr. Martin Dyer, Leicester University) was used to create a model cellular system for constitutive SOX11 knock-down. The cells were cultured under standard conditions (humidified atmosphere, 5% CO₂, 37 °C) and maintained in R10 medium (RPMI-1640, Thermo Scientific HyClone, Logan, UT, USA) supplemented with 10% FBS (Invitrogen) and 1% 2 mM L-glutamine (Invitrogen). The cell cultures were kept in log phase at a density of 0.75–0.8 × 10⁶ cells/ml. Viral transfection was carried out at a multiplicity of infection (MOI) of 2, during which the media was changed to R2 (RPMI-1640 (Invitrogen) supplemented with 2% FBS (Invitrogen)), with the addition of 8 µg/ml polybrene (Sigma-Aldrich). After over-night incubation, the modified cell lines were washed with PBS and cultivated in R10 for an additional 24 h before addition of 5–20 µg/ml puromycin (Sigma-Aldrich) to allow selection of successfully transfected cells. The virally transduced and modified versions of the Z138 wild type (WT) cell lines, carrying SOX11 specific or scrambled shRNA sequences are referred to as Z138-SOX11^{knocked} and Z138-SCR, respectively. The WT Z138 cell line is referred to as Z138-WT.

2.3. Verification of SOX11 levels by quantitative real time PCR (Q-RT-PCR)

Lysis of cells, synthesis and amplification of cDNA were performed, using the Fast SYBR[®] Green Cell-to-CT kit (Applied Biosystem, CA, USA), according to manufacturer's protocol. Briefly, 1 × 10⁵ cells were washed in PBS, lysed and treated with DNase for 5 min, the lysates were reverse-transcribed with random primers and RT Enzyme Mix (Applied Biosystem) in SYBR[®] RT Buffer at 37 °C for 60 min, followed by enzyme inactivation at 95 °C for 5 min.

For the amplification of SOX11 cDNA, the PCR reaction was performed as described previously (Gustavsson et al., 2010). For confirmation of the six genes identified by DNA microarray data TaqMan-based Q-RT-PCR was performed, using TaqMan[®] Fast Universal PCR Master Mix (Applied Biosystem) and the TaqMan assays (all from Applied Biosystem) Hs00165563_m1 (paraoxonase 2, PON2), Hs00189310_m1 (drebrin 1, DBN1), Hs00195010_m1 (FARP1), Hs00905125_m1 (autotaxin, ATX), Hs00191446_m1 (Homer homolog 3, HOMER3), Hs00544069_m1 (L1 cell adhesion molecule, L1CAM). 18S (Hs99999901_s1) was used as reference gene. All data were analyzed with 7500 software v2.0.3 (Applied Biosystem).

2.4. Protein purification, quantification and analysis

Cells were harvested, washed with PBS and incubated on ice in 200 μ l lysis-buffer (1% NP40 (Sigma–Aldrich)/Protease Inhibitor cocktail (Roche, Basel, Switzerland) in PBS) for 30 min and cellular debris were removed by centrifugation (16,000 $\times$ g, 4 °C for 15 min). The protein concentration in each sample was measured using the BCA Kit (Thermo Scientific), according the manufacturer's protocol. Briefly, samples were mixed with BCA reagents, incubated at 37 °C for 20 min, and the absorbance was measured at 562 nm with a series of BSA dilutions (5–100 μ g/ml) used as standard. To perform western blot (WB) cell lysates, containing 25 μ g of protein, were loaded on NuPAGE 10% Bis-Tris gels (Invitrogen) and electrophoresis was performed under reducing conditions for ~60 min at 100 V. The proteins were then blotted onto PVDF membrane (Amersham Hybond-P, GE Healthcare, Uppsala, Sweden) for 40 min at 15 V. The PVDF membrane was blocked and washed with 5% milk/PBS over-night prior to staining (45 min, room temperature (RT)) with the primary antibody rabbit-anti-SOX11 (Atlas Antibodies Stockholm, Sweden) and the endogenous control antibody rabbit-anti-GAPDH (Abcam Cambridge, UK). Detection was performed using HRP-labeled swine anti-rabbit antibody (Dako Glostrup, Denmark) and Super-Signal West Femto Max Sensitivity Substrate (Thermo Scientific) according to manufacturer's protocol. The luminescent signal was detected with the Fluor-S MultiImager CCD camera and QuantityOne software (Bio-Rad Laboratories, California, USA).

2.5. Gene expression analysis

One million cells were washed in PBS and re-suspended in 200 μ l Trizol (Invitrogen). Preparation of tRNA, preparation of HuGene ST 1.0 arrays and raw data extraction was performed as previously described (Gustavsson et al., 2010) and in accordance with the instruction of the manufacturer (Affymetrix Inc., Santa Clara, CA). Quantile normalization using RMA and quality control was performed in the Expression Console 1.0 software (Affymetrix Inc.). Genes that had a 1.2 fold change comparing Z138-SCR vs. Z138-SOX11^{knocked} were selected for further analysis. Ingenuity Pathway Analysis (9.0 version 3206, Ingenuity Systems, Redwood City, CA) was used to extract information on cellular function and localization.

2.6. Assessment of cellular growth in relation to ATX expression

Cells were kept in log phase for 24 h prior to the experiment. Before addition of the ATX inhibitor, cells were extensively washed in PBS and diluted to 1 $\times$ 10⁶ cells/ml in fresh R10 with (10 μ M final concentration) or without the ATX inhibitor S32826 (Echelon Biosciences, Salt Lake City, USA). After additional 24 h, 50,000 cells/well were seeded in a 96 well plate, in triplicates. Proliferation assays were performed, as described previously (Ortega-Paino et al., 2008).

2.7. Cell cycle analysis of Z138 and modified derivatives

The different cell lines were diluted with R10 to 0.2, 0.6, 0.8, 1 and 2 million cells/ml and cultivated in 24 well plates at 37 °C. After 24 h, cells were counted and cell cycle analysis performed. Briefly, approximately 400,000 cells were harvested, washed once, re-suspended in 2 ml cold PBS (Sigma–Aldrich) followed by addition of 5 ml 96% ethanol (Altia Corporation, Rajamäki, Finland) and incubated over-night at –20 °C. Fixed cells were spun down and re-suspended in 500 μ l PBS (Thermo Scientific Hyclone) with 50 μ g/ml propidium iodide (PI, Sigma–Aldrich), 0.1 mg/ml RNase (Fermentas Life Sciences, Burlington, ON, Canada) and 0.05% Triton X-100 (Sigma Chemical Co., St Louis, USA). All samples were incubated 40 min at 37 °C, washed with PBS and analyzed on FACS Canto™ II (BD Biosciences, San Jose, CA, USA). Data were analyzed using FCS Express MultiCycle plug-in model 6 (De Novo Software, LA CA, USA).

2.8. Transplantation of human tumor cells into NOD-SCID mice

At least 3–7 days before transplantation, the culture media for Z138-SOX11^{knocked} and Z138-SCR was supplemented with 2–5 μ g/ml puromycin (Sigma–Aldrich) to select transfected cells. 18–24 h prior to transplantation the viability of cells was confirmed to be above 90% and cells were diluted to 800,000 cells/ml, to ensure exponential growth at time of transplantation. At the day of transplantation, cells were washed, re-suspended in R10 and kept at RT until transplantation (1–3 h). Injections were performed at four different time points (each defined as one experiment) with equal number of animals injected with Z138-SCR and Z138-SOX11^{knocked} in gender matched groups. Animal experimental procedures were performed strictly in accordance with the related University Ethics Committee (approval M229-09). Briefly, six to ten weeks old NOD-SCID mice (The Jackson Laboratory, Sacramento, California, USA, (bred at Lund University for generations)) were intravenously (i.v.) injected in the tail vein with 0.5, 5 or 10 $\times$ 10⁶ cells (Z138-SCR or Z138-SOX11^{knocked}) or R10 as control. After transplantation the mice were monitored daily and their weight controlled twice a week. Symptoms of tumor growth were identified 21–56 days after injection and included slow movements, hunchbacked feature, shaggy coat, weight loose, paralyzed hind legs and death. As soon as symptoms arouse the mice were sacrificed using carbon dioxide or cervical dislocation. Sacrificed mice were kept cold until dissection.

2.9. Analysis of tumor cells post transplantation

Organs from the mice were analyzed using three different methods: (i) flow cytometry, (ii) immunohistochemistry (IHC) and (iii) histological examination. (i) Single cell suspensions were prepared from visible tumors, liver, lungs, heart, kidneys, spleen and brain. Furthermore, cells from bone marrow and blood were collected. Cells were stained with CD19-PE (Dako), CD38-PE (BD Biosciences) and HLA-DR-APC (BD Biosciences) and analyzed using FACS Canto™ II (BD Biosciences). Gating on size and granularity, based on Z138-WT cells, confirmed maintained scatter profile, comparing cells pre- and

post-transplantation. Similarly, analysis of CD19, CD38 and HLA-DR confirmed a maintained phenotype; (ii) Tissues, facial tumors and brains were fixed in formalin for 48 h, paraffin embedded and analyzed using IHC. Briefly, 2–4 μ m-thick paraffin tissue sections were deparaffinized and rehydrated in a water bath. Sections were incubated with the primary antibody rabbit-anti-SOX11 (Atlas Antibodies) or mice-anti-CD20 (Dako) at RT and the signal was detected with REAL EnVision detection system (Dako), containing the secondary goat-anti-rabbit/mouse (CD20) or goat-anti-rabbit (SOX11) antibody conjugated to a peroxidase conjugated polymer. The reaction was visualized by 3,3'-diaminobenzidine (Dako) and counter-stained with Mayers hematoxylin (Sigma–Aldrich). (iii) Histological examination was performed at National Veterinary Institute (SVA) in Uppsala, Sweden. At day 29 post injection, three mice were sent to SVA following the general procedures determined by the animal facility at Lund University. Upon arrival, the mice were sacrificed and tissue specimens collected at necropsy were fixed in neutral buffered formalin. The brain, bone marrow, heart, intestines, kidney, liver, lungs, lymph node, skeletal muscle, skin, spinal cord, spleen, testis and stomach were routinely processed for histology, embedded in paraffin, sectioned at 4 μ m and stained with hematoxylin and eosin.

3. Results

3.1. Generation of a constitutively SOX11 silenced Z138 cell line

In order to study the genes and the corresponding biological functions regulated by SOX11, we modified the MCL cell line

Figure 1 – Verification of SOX11 mRNA and protein down-regulation. Q-RT-PCR and WB confirms down-regulation of SOX11 in Z138-SOX11^{knocked} compared to Z138-SCR and Z138-WT cells.

Z138 (Medeiros et al., 2006) through retroviral infection of the pRSMX vector (Ngo et al., 2006), carrying SOX11-specific shRNA or a scrambled control. Four iRNA sequences targeting different regions of SOX11 mRNA were initially tested. SOX11-specific iRNA sequence-1 showed the most prominent effect on SOX11 knock-down, as assessed by WB analysis (data not shown), and was selected for all the further analysis. The generated stable cell lines were named Z138-SOX11^{knocked} and Z138-SCR, respectively. The down-regulation of SOX11 mRNA and protein in Z138-SOX11^{knocked} compared to Z138-SCR and Z138-WT was confirmed by Q-RT-PCR and WB (Figure 1).

3.2. SOX11 regulate cellular growth rate

We have previously shown that transient knock-down of SOX11 induced an increased growth rate (Gustavsson et al., 2010) and analysis of the established Z138-SOX11^{knocked} and Z138-SCR cell lines confirmed that also constitutive knock-

Figure 2 – SOX11-related differences in growth rate. (a) Growth rate was assessed through cell counting using trypan blue exclusion and revealed a SOX11-related difference in growth rate. (b) Cell cycle analysis confirmed an increased fraction of cells in S phase in Z138-SOX11^{knocked} compared to Z138-SCR and Z138-WT cells.

Table 1 – Genes deregulated upon SOX11 knock-down.

Symbol	Gene title	FC	Role in cell ^b	Localization ^b
ATX, ENPP2 ^a	Autotaxin, ectonucleotide pyrophosphatase/phosphodiesterase 2	1.77	Chemotaxis, invasion, proliferation ^c	Plasma membrane
GNPDA1	Glucosamine-6-phosphate deaminase 1	1.45	Unknown	Cytoplasm
LTA	Lymphotoxin alpha (TNF superfamily, member 1)	1.40	Apoptosis, activation, proliferation ^c	Extracellular space
TRO	Trophinin	1.33	Adhesion, growth, proliferation	Plasma membrane
LGALS1	Lectin, galactoside-binding, soluble, 1	1.31	Cell death, apoptosis, proliferation ^c	Extracellular space
STAT1	signal transducer and activator of transcription 1, 91 kDa	1.28	Apoptosis, expression, proliferation ^c	Nucleus
HSPB1	Heat shock 27 kDa protein 1	1.26	Apoptosis, cell death, growth ^c	Cytoplasm
SRGN	Serglycin	1.26	Apoptosis, morphology, proliferation ^c	Extracellular space
IGSF6	Immunoglobulin superfamily, member 6	1.26	Unknown	Plasma membrane
IFNAR1	interferon (alpha, beta and omega) receptor 1	1.25	Activation, cell death, apoptosis ^c	Plasma membrane
VAV1	vav 1 guanine nucleotide exchange factor	1.24	Proliferation, development, apoptosis ^c	Nucleus
EGR3	Early growth response 3	1.24	Apoptosis, development, proliferation ^c	Nucleus
SATB2	SATB homeobox 2	1.23	Migration, remodeling, development, invasion	Nucleus
ANXA2	Annexin A2	1.23	Binding, fusion, organization ^c	Plasma membrane
FCER2	Fc fragment of IgE, low affinity II, receptor for (CD23)	1.23	Apoptosis, activation, phagocytosis ^c	Plasma membrane
AOC3	amine oxidase, copper containing 3 (vascular adhesion protein 1)	1.23	Migration, adhesion, cell rolling ^c	Plasma membrane
IL4R	Interleukin 4 receptor	–1.20	Proliferation, differentiation, apoptosis ³	Plasma membrane
IL6	Interleukin 6 (interferon, beta 2)	–1.23	Differentiation, proliferation, apoptosis ^c	Extracellular space
CD22	CD22 molecule	–1.24	Activation, proliferation, apoptosis ^c	Plasma membrane
FCN1	Ficolin (collagen/fibrinogen domain containing) 1	–1.24	Unknown	Extracellular space
HOXB2	Homeobox B2	–1.24	Migration, differentiation	Nucleus
PON2 ^a	Paraoxonase 2	–1.24	Plasma membrane	
HNF1A	HNF1 homeobox A	–1.25	Transcription, apoptosis, proliferation ^c	Nucleus
MMP9	Matrix metalloproteinase 9	–1.26	Migration, invasion, apoptosis, proliferation ³	Extracellular space
MAST4	Microtubule associated serine/threonine kinase family member 4	–1.26	Unknown	Unknown
DLG5	Discs, large homolog 5 x(Drosophila)	–1.27	Plasma membrane	
PTPRG	Protein tyrosine phosphatase, receptor type, G	–1.28	Growth, proliferation, colony formation, survival	Plasma membrane
ICK	Intestinal cell (MAK-like) kinase	–1.28	Unknown	Cytoplasm
L1CAM ^a	L1 cell adhesion molecule	–1.29	Outgrowth, guidance, adhesion, migration ^c	Plasma membrane
DBN1 ^a	Drebrin 1	–1.29	cytoplasm	
KIAA1545	HBV X-transactivated gene 9 protein	–1.30		
OSBPL3	Oxysterol binding protein-like 3	–1.37	Unknown	Cytoplasm
FARP1 ^a	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1	–1.37	Unknown	Unknown
MERTK	c-mer proto-oncogene tyrosine kinase	–1.41	Proliferation, apoptosis, phagocytosis ^c	Plasma membrane

(continued on next page)

Table 1 – (continued)

Symbol	Gene title	FC	Role in cell ^b	Localization ^b
NR4A1	Nuclear receptor subfamily 4, group A, member 1	–1.42	Apoptosis, proliferation, cell death ^c	Nucleus
SOX11 ^a	SRY (sex determining region Y)-box 11	–1.43	Expression in	Nucleus
GAB2	GRB2-associated binding protein 2	–1.45	Proliferation, differentiation, migration	Cytoplasm
AEBP1 ^a	AE binding protein 1	–1.45	Proliferation	Nuclear
HOMER3 ^a	homer homolog 3 (Drosophila)	–1.54	Plasma membrane	
MDK	Midkine (neurite growth-promoting factor 2)	–1.57	Migration, proliferation, differentiation ^c	Extracellular space
ZNF711	Zinc finger protein 711	–1.65	Unknown	Nucleus
CD27	CD27 molecule	–1.67	Apoptosis, proliferation, cytotoxicity ^c	Plasma membrane
RHOBTB3	Rho-related BTB domain containing 3	–1.73	Unknown	Unknown
ASAP2	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	–2.17	Phagocytosis, migration, generation	Nucleus

a These genes are identified as MCL-specific by Obrador-Hevia et al. (2009).

b As identified by Ingenuity Pathway Analysis 9.0 version 3206.

c These descriptions are truncated due to limited space.

down of SOX11 resulted in altered growth characteristics (Figure 2a). Of note, the cellular growth was assessed, using various cell densities and the difference in growth rate between Z138-SOX11^{knocked} and Z138-SCR was most pronounced in log phase growth (Figure 2a). The difference in growth rate indicated that the cell cycle may be affected by the SOX11 knock-down. This was confirmed by comparing frequencies of cells in G₀/G₁, S and G₂/M phase, where the Z138-SOX11^{knocked} cell line had a low frequency of cells in G₀ and an increased fraction (40–45%) of cells in S phase, compared to Z138-SCR (25–30%) (Figure 2b).

3.3. Gene expression analysis and TaqMan verification of target genes

To identify genes associated with altered SOX11 expression level, transcriptional analysis was performed. Bioinformatic analysis using fold change criteria (>1.2) identified 7000 genes with a differential expression in Z138-SOX11^{knocked}, compared to Z138-SCR. Since SOX11 has been shown to identify MCL phenotypically, we focused on those differentially regulated transcripts that previously have been identified by us, as specifically over expressed in MCL (Ek et al., 2006, 2002). This procedure resulted in identification of 44 transcripts (Table 1), present in primary MCL tumors as supposed to normal tissue, as well as regulated upon SOX11 knock-down in Z138. Furthermore, eight of these differentially regulated genes overlapped with a previously identified list of 30 genes, consistently deregulated in MCL compared to normal tissue or other lymphomas, as reviewed by Obrador-Hevia et al. (2009) (marked in Table 1). Of note, similar to SOX11, several of the identified genes (Table 1) have important functions in development of the CNS or neuron function. These genes include, DBN1 (Kojima and Shirao, 2007; Wang et al., 2010), FARP1 (Zhuang et al., 2009), HOMER3 (Ehrensgruber

et al., 2004) and L1CAM (Schafer and Altevogt, 2010). This suggests that SOX11 regulate these genes, not only in MCL, but also in other tissues. Moreover, many of the identified genes are functionally involved in proliferation, apoptosis and migration, which may explain the functional effect seen upon SOX11 knock-down (see Cellular function Table 1).

Based on their presence in primary MCL tumors and their relation to proliferation or neural development, six genes were selected for independent validation, using target-specific TaqMan probes. These analyses confirmed a co variation with SOX11 expression levels for most genes, ATX being an exception showing a strong up-regulation in response to the SOX11 knock-down (Figure 3).

3.4. Functional role of ATX

The knock-down of SOX11 resulted in an increase in proliferation and a shift in cell cycle distribution (Figure 2a and b). Consequently, we wanted to assess if ATX and its downstream effector molecules were involved in this growth regulatory effect. In brief, ATX is a phospholipase D (PLD) that generates lysophosphatidic acid (LPA) but also shingosine-1-phosphate (S1P) from lysophosphatidylcholine (LPC) and sphingo-sylphosphorylcholine (SPC), respectively (Clair et al., 2003; Umezū-Goto et al., 2002) and is thus a major regulator of cell proliferation.

To assess the role of ATX-induced proliferation in Z138-SOX11^{knocked} and Z138-SCR cells, the ATX-specific inhibitor S32826 was used to specifically block the ATX-related enzymatic activity. Cells were kept in log phase with or without the inhibitor for 48 h and growth rates were assessed by ³H-thymidine incorporation. Repetitive experiments confirmed an increased growth rate of Z138-SOX11^{knocked} compared to Z138-SCR cells, thus demonstrating a dependence on ATX

Figure 3 – Technical verification of SOX11-regulated genes. Verification of mRNA levels of six SOX11-regulated genes were performed using TaqMan assays and revealed a co-regulation of (a) DBN1, PON2, HOMER3, FARP and L1CAM with SOX11 while (b) ATX showed a major up-regulation upon SOX11 knock-down.

for the increase in proliferation in Z138-SOX11^{knocked} cells (Figure 4).

3.5. In vivo analysis of the tumorigenic potential of SOX11^{knocked} MCL cells

To evaluate if the increased growth rate observed for the SOX11 knock-down cells *in vitro* had any effect on the tumorigenic potential of cells *in vivo* Z138-SOX11^{knocked} and Z138-SCR controls cells were i.v. transplanted into NOD-SCID mice. Both Z138-SOX11^{knocked} and Z138-SCR showed tumorigenic properties and generated tumors after three to eight weeks. A dissemination of tumor cells to CNS and brain was identified through histological examination, showing also extensive accumulation of lymphocytic cells in the meninges, around vessels in the brain, and in the spinal cord (Figure 5a). This finding was confirmed by flow cytometry of cells isolated from brain and tumors and by IHC, using staining for the human B cell marker CD20 (Figure 5b). The localization of the tumor cells into the

Figure 4 – Proliferation measurement and ATX inhibition. Proliferation was assessed for Z138-SOX11^{knocked} compared to Z138-SCR cell lines, confirming an increased growth rate for Z138-SOX11^{knocked} cells. The observed increase in proliferation comparing the Z138-SOX11^{knocked} and control cells could be completely inhibited using the ATX-specific inhibitor S32826. The incorporation of ³H-Thymidine is measured as counts per minute (cpm).

CNS is consistent with the neurological symptoms that the mice show, including paralyzed hind legs. However, tumor cells were also identified in other organs, including liver and spleen (Table 2).

Of note, a clear difference in tumorigenicity was detected between Z138-SOX11^{knocked} and Z138-SCR in the majority of experiments, as determined by the frequency of animals showing symptoms of disease (Figure 5c). To further assess the different tumorigenic potentials, animals injected with Z138-SOX11^{knocked} and Z138-SCR were monitored and time until symptoms of disease or occurrence of spontaneous death was compared. Three of four experiments showed a shorter time to symptoms of disease for animals injected with Z138-SOX11^{knocked} as compared to Z138-SCR (Figure 5d).

Table 2 – Identification of tumor cells in specific organs post transplantation.

Organ	Identified/ investigated ^a
Brain	24/38
Lung	13/21
Kidney	4/21
Spleen	14/22
Liver	9/23
Bone marrow	9/18
Blood	2/3

^a Number of organs with identified tumor cells (Z138-SCR or Z138-SOX11^{knocked}) using flow cytometry compared to the total number of specific organs analyzed.

Figure 5 – Establishment of tumors *in vivo*. The tumorigenicity of Z138-SOX11^{knocked} and Z138-SCR cells was assessed in NOD-SCID mice. (a) Histological examination of brain tissue confirmed involvement of the CNS (arrow-heads) and (b) IHC analysis using antibodies targeting CD20 confirmed a human B cell origin of the established tumors. (c) A trend towards an increased tumorigenicity with reduced SOX11 expression was seen upon transplantation of tumor cells (0.5, 5 or 10 million cells). (d) Survival analysis showed a more rapid development of symptoms related to tumor growth for the Z138-SOX11^{knocked} compared to Z138-SCR cells.

4. Discussion

SOX11 has been recognized as an important diagnostic antigen (Chen et al., 2009; Dictor et al., 2009; Ek et al., 2008; Fernandez et al., 2010; Mozos et al., 2009; Wang et al., 2008) with a suppressor-like function (Gustavsson et al., 2010) in MCL. Recent results have shown consistent changes in gene expression in primary MCL samples and MCL cell lines upon transient SOX11 knock-down (Wang et al., 2010). In this study, we used the MCL-derived cell line Z138 to create a model cell line with constitutively reduced levels of the transcription factor SOX11 (Z138-SOX11^{knocked}) and a corresponding control cell line (Z138-SCR). MCL cell lines, including Z138, have previously been described as relevant models for investigations of MCL-associated features (Amin et al., 2003). Z138 has been shown to be derived from a patient with blastoid transformation (Medeiros et al., 2006) and have features related to other MCL cell lines (Tucker et al., 2006), but is also characterized by a more rapid proliferation. Using this cell line and modifications thereof, we demonstrated that constitutive down-regulation of

SOX11 through viral integration of SOX11-specific shRNA lead to an increased growth rate, measured by both cell counting and ³H-Thymidine incorporation. Furthermore, cell cycle analysis revealed an increased frequency of the Z138-SOX11^{knocked} cells in S phase, as compared to Z138-SCR. Of note, other MCL-associated genes with a known growth promoting function, such as cyclin D1, have failed to induce changes in growth rate upon knock-down, as shown by us (Ortega-Paino et al., 2008) and others (Klier et al., 2008). This emphasizes the complexity of growth regulation but also the significance of SOX11 as a central player in growth control.

We have previously shown that overexpression of SOX11 led to dysregulation of signaling pathways normally used by MCL tumor cells, such as the Rb-E2F pathway (Gustavsson et al., 2010). Here we show that the gene expression changes induced by SOX11 knock-down are also tightly linked to MCL-specific features. In a recent review by Obrador-Hevia et al. a set of 30 MCL-specific genes was identified, based on public data from gene expression analysis of primary MCL in at least two independent studies, regardless of the technical platforms used (Obrador-Hevia et al., 2009). Among these 30 genes, SOX11

and seven additional genes were differentially regulated upon SOX11 down-regulation. Of interest, many of the identified genes are important for neuron or normal brain function, emphasizing that the identified target genes not only are regulated by SOX11 in MCL, but also in other contexts, as for example during the fetal development of the central nervous system (CNS) (Ehrengreber et al., 2004; Kojima and Shirao, 2007; Penzo-Mendez, 2010; Schafer and Altevogt, 2010; Zhuang et al., 2009).

In addition, to further analyze the growth regulatory role of SOX11, we focused on the novel finding that ATX is up-regulated upon SOX11 knock-down. The primary product of ATX is the lysophosphatidic acid 1-acyl 2-hydroxyl glycerol 3-phosphate (LPA), which is the primary product of ATX-catalyzed hydrolysis of circulating lysophospholipids, most often lysophosphatidylcholine (LPC) (Federico et al., 2008). LPA promotes growth, survival, differentiation and motility of a range of neuronal cells (Inoue et al., 2008) and is involved in tumor progression (Liu et al., 2009). It has been suggested that ATX is auto-inhibited by its two products LPA and lysophospholipid S1P, regulating its own expression (van Meeteren and Moolenaar, 2007). Of note, ATX can also promote anti-tumorigenic properties as it induces cyclic phosphatidic acid (cPA), which is associated with inhibition of cell growth (Kobayashi and Murakami-Murofushi, 2001) through transphosphorylation of LPA (Tania et al., 2010). However, in lymphomas, ATX seems to be correlated with an increased tumor burden and has been proposed as serum biomarker to follow the clinical course of patients with follicular lymphoma (Masuda et al., 2008). Of note, using the ATX-specific inhibitor S32826 (Ferry et al., 2008) we showed that the increase in proliferation upon SOX11 knock-down could be reversed upon blockage of ATX activity. Thus, the increased SOX11-mediated proliferation is ATX-dependent.

As our model cell line showed increased growth rate upon SOX11 knock-down and the literature suggests that increased levels of LPA promotes angiogenesis and tumor progression, we were also interested to assess the capability of tumor formation *in vivo*. Previous studies suggests that *i.v.* injections, using MCL cell lines such as GRANTA519 in immunodeficient mice, may promote systemic disease, involving liver tumors (Munger et al., 2006; Wang et al., 2007). Z138 has, however, previously been shown not to cause systemic disease in Rag-2 deficient mice (Tucker et al., 2006). In our study, NOD-SCID mice were used and characteristic symptoms of sickness, including neurological symptoms, were noted after three to eight weeks. Multiple analyses, including IHC staining, using the human B cell marker CD20, confirmed tumor growth in the CNS. This is consistent with data for human primary MCL, where involvement of the CNS often is associated with systemic disease and decreased survival (Gill et al., 2009; Valdez et al., 2002). In agreement with our *in vitro* data and previous clinical data (Wang et al., 2008), Z138-SOX11^{knocked} cells showed an increased tumorigenicity and shorter time to survival compared to Z138-SCR control cells.

5. Conclusions

We have for the first time showed that constitutive SOX11 knock-down in a MCL cell line promotes a highly proliferative

feature, which can be reversed by the specific inhibition of ATX. This cell line, with a silenced SOX11 gene, was also shown to have an increased engraftment potential and a more aggressive behavior *in vivo*.

Authorship contributions

PC was involved in the design of the study, responsible for viral work and functional evaluation of modified cell lines *in vitro* and took active part in writing the manuscript. UA contributed to the design of the study, was responsible for the *in vivo* studies and evaluation of the data and took active part in writing the manuscript. VK took active part in the functional evaluation of modified cell lines. C.A.K.B contributed to the design of the study and revision of the manuscript. SE was responsible for the design of the study, interpretation of data and writing of the manuscript.

Conflicts of interest

SE and CAKB have previously filed a patent for the diagnostic, prognostic and therapeutic use of SOX11.

Acknowledgment

The study was supported by the Lund Institute of Technology (LTH), Bioinvent International AB, the Leukemia and Lymphoma Society (Grant No. R6189-09), Cancerfonden (08/0285 and 09/0390), Smärtafonden (SSF/09-05), CREATE Health, a strategic Center for Translational Cancer Research (www.createhealth.se), Stiftelsen Olle Engkvist Byggmästare and BioCARE – a strategic program for Cancer Research at Lund and Gothenburg Universities. We would like to acknowledge Eva Nilsson for introducing us to the NOD-SCID work, Georg Hansson for his support with *i.v.* injections, Kristina Lövgren for immunohistochemistry staining and tissue preparation, Beata Lindqvist for lentiviral production, Ann-Charlott Olsson for gene expression sample and array preparation and Ann-Sofie Albrekt for bioinformatic support.

REFERENCES

- Amin, H.M., McDonnell, T.J., Medeiros, L.J., Rassidakis, G.Z., Leventaki, V., O'Connor, S.L., Keating, M.J., Lai, R., 2003. Characterization of 4 mantle cell lymphoma cell lines. *Arch. Pathol. Lab. Med.* 127, 424–431.
- Braddock, D.T., 2010. Autotaxin and lipid signaling pathways as anticancer targets. *Curr. Opin. Investig. Drugs* 11, 629–637.
- Chen, Y.H., Gao, J., Fan, G., Peterson, L.C., 2009. Nuclear expression of sox11 is highly associated with mantle cell lymphoma but is independent of t(11;14)(q13;q32) in non-mantle cell B-cell neoplasms. *Mod. Pathol.* 23, 105–112.
- Clair, T., Aoki, J., Koh, E., Bandle, R.W., Nam, S.W., Ptaszynska, M.M., Mills, G.B., Schiffmann, E., Liotta, L.A., Stracke, M.L., 2003. Autotaxin hydrolyzes sphingosylphosphorylcholine to produce the regulator of

- migration, sphingosine-1-phosphate. *Cancer Res.* 63, 5446–5453.
- Dictor, M., Ek, S., Sundberg, M., Warenholt, J., Gyorgy, C., Sernbo, S., Gustavsson, E., Abu-Alsoud, W., Wadstrom, T., Borrebaeck, C., 2009. Strong lymphoid nuclear expression of SOX11 transcription factor defines lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma. *Haematologica* 94, 1563–1568.
- Ehrengruber, M.U., Kato, A., Inokuchi, K., Hennou, S., 2004. Homer/Vesl proteins and their roles in CNS neurons. *Mol. Neurobiol.* 29, 213–227.
- Ek, S., Hogerkrup, C.M., Dictor, M., Ehinger, M., Borrebaeck, C.A., 2002. Mantle cell lymphomas express a distinct genetic signature affecting lymphocyte trafficking and growth regulation as compared with subpopulations of normal human B cells. *Cancer Res.* 62, 4398–4405.
- Ek, S., Andreasson, U., Hober, S., Kampf, C., Ponten, F., Uhlen, M., Merz, H., Borrebaeck, C.A., 2006. From gene expression analysis to tissue microarrays: a rational approach to identify therapeutic and diagnostic targets in lymphoid malignancies. *Mol. Cell Proteomics* 5, 1072–1081.
- Ek, S., Dictor, M., Jerkeman, M., Jirstrom, K., Borrebaeck, C.A., 2008. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. *Blood* 111, 800–805.
- Federico, L., Pamuklar, Z., Smyth, S.S., Morris, A.J., 2008. Therapeutic potential of autotaxin/lysophospholipase D inhibitors. *Curr. Drug Targets* 9, 698–708.
- Fernandez, V., Salamero, O., Espinet, B., Sole, F., Royo, C., Navarro, A., Camacho, F., Bea, S., Hartmann, E., Amador, V., Hernandez, L., Agostinelli, C., Sargent, R.L., Rozman, M., Aymerich, M., Colomer, D., Villamor, N., Swerdlow, S.H., Pileri, S.A., Bosch, F., Piris, M.A., Montserrat, E., Ott, G., Rosenwald, A., Lopez-Guillermo, A., Jares, P., Serrano, S., Campo, E., 2010. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. *Cancer Res.* 70, 1408–1418.
- Ferry, G., Moulharat, N., Pradere, J.P., Desos, P., Try, A., Genton, A., Giganti, A., Beucher-Gaudin, M., Lonchampt, M., Bertrand, M., Saulnier-Blache, J.S., Tucker, G.C., Cordi, A., Boutin, J.A., 2008. S32826, a nanomolar inhibitor of autotaxin: discovery, synthesis and applications as a pharmacological tool. *J. Pharmacol. Exp. Ther.* 327, 809–819.
- Gill, S., Herbert, K.E., Prince, H.M., Wolf, M.M., Wirth, A., Ryan, G., Carney, D.A., Ritchie, D.S., Davies, J.M., Seymour, J.F., 2009. Mantle cell lymphoma with central nervous system involvement: frequency and clinical features. *Br. J. Haematol.* 147, 83–88.
- Gustavsson, E., Sernbo, S., Andersson, E., Brennan, D.J., Dictor, M., Jerkeman, M., Borrebaeck, C.A., Ek, S., 2010. SOX11 expression correlates to promoter methylation and regulates tumor growth in hematopoietic malignancies. *Mol. Cancer* 9, 187.
- Inoue, M., Xie, W., Matsushita, Y., Chun, J., Aoki, J., Ueda, H., 2008. Lysophosphatidylcholine induces neuropathic pain through an action of autotaxin to generate lysophosphatidic acid. *Neuroscience* 152, 296–298.
- Klier, M., Anastasov, N., Hermann, A., Meindl, T., Angermeier, D., Raffeld, M., Fend, F., Quintanilla-Martinez, L., 2008. Specific lentiviral shRNA-mediated knockdown of cyclin D1 in mantle cell lymphoma has minimal effects on cell survival and reveals a regulatory circuit with cyclin D2. *Leukemia* 22, 2097–2105.
- Kobayashi, T., Murakami-Murofushi, K., 2001. Bioactions and synthesis of a novel lipid mediator, cyclic phosphatidic acid. *World Rev. Nutr. Diet.* 88, 228–232.
- Kojima, N., Shirao, T., 2007. Synaptic dysfunction and disruption of postsynaptic drebrin-actin complex: a study of neurological disorders accompanied by cognitive deficits. *Neurosci. Res.* 58, 1–5.
- Kuhlbrodt, K., Herbarth, B., Sock, E., Enderich, J., Hermans-Borgmeyer, I., Wegner, M., 1998. Cooperative function of POU proteins and SOX proteins in glial cells. *J. Biol. Chem.* 273, 16050–16057.
- Liu, S., Umez-Goto, M., Murph, M., Lu, Y., Liu, W., Zhang, F., Yu, S., Stephens, L.C., Cui, X., Murrow, G., Coombes, K., Muller, W., Hung, M.C., Perou, C.M., Lee, A.V., Fang, X., Mills, G.B., 2009. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. *Cancer Cell* 15, 539–550.
- Masuda, A., Nakamura, K., Izutsu, K., Igarashi, K., Ohkawa, R., Jona, M., Higashi, K., Yokota, H., Okudaira, S., Kishimoto, T., Watanabe, T., Koike, Y., Ikeda, H., Kozai, Y., Kurokawa, M., Aoki, J., Yatomi, Y., 2008. Serum autotaxin measurement in haematological malignancies: a promising marker for follicular lymphoma. *Br. J. Haematol.* 143, 60–70.
- Medeiros, L.J., Estrov, Z., Rassidakis, G.Z., 2006. Z-138 cell line was derived from a patient with blastoid variant mantle cell lymphoma. *Leuk. Res.* 30, 497–501.
- Mozos, A., Royo, C., Hartmann, E., De Jong, D., Baro, C., Valera, A., Fu, K., Weisenburger, D.D., Delabie, J., Chuang, S.S., Jaffe, E.S., Ruiz-Marcellan, C., Dave, S., Rimsza, L., Braziel, R., Gascoyne, R.D., Sole, F., Lopez-Guillermo, A., Colomer, D., Staudt, L.M., Rosenwald, A., Ott, G., Jares, P., Campo, E., 2009. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. *Haematologica* 94, 1555–1562.
- Munger, C.M., Vose, J.M., Joshi, S.S., 2006. Dendritic cell-based therapy for mantle cell lymphoma. *Int. J. Oncol.* 28, 1337–1343.
- Ngo, V.N., Davis, R.E., Lamy, L., Yu, X., Zhao, H., Lenz, G., Lam, L.T., Dave, S., Yang, L., Powell, J., Staudt, L.M., 2006. A loss-of-function RNA interference screen for molecular targets in cancer. *Nature* 441, 106–110.
- Obrador-Hevia, A., Fernandez de Mattos, S., Villalonga, P., Rodriguez, J., 2009. Molecular biology of mantle cell lymphoma: from profiling studies to new therapeutic strategies. *Blood Rev.* 23, 205–216.
- Ortega-Paino, E., Fransson, J., Ek, S., Borrebaeck, C.A., 2008. Functionally associated targets in mantle cell lymphoma as defined by DNA microarrays and RNA interference. *Blood* 111, 1617–1624.
- Penzo-Mendez, A.I., 2010. Critical roles for SoxC transcription factors in development and cancer. *Int. J. Biochem. Cell Biol.* 42, 425–428.
- Samadi, N., Bekele, R., Capatos, D., Venkatraman, G., Sariahmetoglu, M., Brindley, D.N., 2011. Regulation of lysophosphatidate signaling by autotaxin and lipid phosphate phosphatases with respect to tumor progression, angiogenesis, metastasis and chemo-resistance. *Biochimie* 93, 61–70.
- Schafer, M.K., Altevogt, P., 2010. L1CAM malfunction in the nervous system and human carcinomas. *Cell Mol. Life Sci.* 67, 2425–2437.
- Tania, M., Khan, A., Zhang, H., Li, J., Song, Y., 2010. Autotaxin: a protein with two faces. *Biochem. Biophys. Res. Commun.* 401, 493–497.
- Tucker, C.A., Bebb, G., Klasa, R.J., Chhanabhai, M., Lestou, V., Horsman, D.E., Gascoyne, R.D., Wiestner, A., Masin, D., Bally, M., Williams, M.E., 2006. Four human t(11;14)(q13;q32)-containing cell lines having classic and variant features of mantle cell lymphoma. *Leuk. Res.* 30, 449–457.
- Umez-Goto, M., Kishi, Y., Taira, A., Hama, K., Dohmae, N., Takio, K., Yamori, T., Mills, G.B., Inoue, K., Aoki, J., Arai, H., 2002. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. *J. Cell Biol.* 158, 227–233.
- Valdez, R., Kroft, S.H., Ross, C.W., Schnitzer, B., Singleton, T.P., Peterson, L.C., Finn, W.G., 2002. Cerebrospinal fluid

- involvement in mantle cell lymphoma. *Mod. Pathol.* 15, 1073–1079.
- van Meeteren, L.A., Moolenaar, W.H., 2007. Regulation and biological activities of the autotaxin-LPA axis. *Prog. Lipid. Res.* 46, 145–160.
- Wang, M., Zhang, L., Han, X., Yang, J., Qian, J., Hong, S., Samaniego, F., Romaguera, J., Yi, Q., 2007. Atiprimod inhibits the growth of mantle cell lymphoma in vitro and in vivo and induces apoptosis via activating the mitochondrial pathways. *Blood* 109, 5455–5462.
- Wang, X., Asplund, A.C., Porwit, A., Flygare, J., Smith, C.I., Christensson, B., Sander, B., 2008. The subcellular Sox11 distribution pattern identifies subsets of mantle cell lymphoma: correlation to overall survival. *Br. J. Haematol.* 143, 248–252.
- Wang, X., Bjorklund, S., Wasik, A.M., Grandien, A., Andersson, P., Kimby, E., Dahlman-Wright, K., Zhao, C., Christensson, B., Sander, B., 2010. Gene expression profiling and chromatin immunoprecipitation identify DBN1, SETMAR and HIG2 as direct targets of SOX11 in mantle cell lymphoma. *PLoS One* 5, e14085.
- Wiebe, M.S., Nowling, T.K., Rizzino, A., 2003. Identification of novel domains within Sox-2 and Sox-11 involved in autoinhibition of DNA binding and partnership specificity. *J. Biol. Chem.* 278, 17901–17911.
- Zhuang, B., Su, Y.S., Sockanathan, S., 2009. FARP1 promotes the dendritic growth of spinal motor neuron subtypes through transmembrane Semaphorin6A and PlexinA4 signaling. *Neuron* 61, 359–372.