The role of SOXC transcription factors in B-cell development and lymphoid malignancies

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ABSTRACT

Mantle cell lymphoma (MCL) accounts for 5-10% out of all Non-Hodgkin lymphomas (NHLs) and is one of the most aggressive forms of lymphomas with a median survival of less than 5 years. Currently, MCL is considered to be an incurable disease.

MCL is characterized by the t(11;14)(q13;q32) CCND1/IGH translocation that results in high expression of cyclin D1. This translocation takes place at the pre-B cell stage and is generally recognized as the hallmark and primary oncogenic event in the evolution of MCL. Recently, the neural transcription factor SRY (sex-determining region Y) box 11 (SOX11) gene was found to be expressed in over 90% of all MCLs. The SOX11 protein is not detected in the vast majority of other lymphomas or mature B-cells and its expression is independent of cyclin D1 status. Moreover, SOX11 has been proposed to have a functional role in the pathogenesis of MCL and may not only serve as a diagnostic biomarker.

In this thesis, the functional role of the SOXC genes (SOX4, SOX11 and SOX12) have been studied in several different ways, both in MCL primary samples/cell lines and in non-MCL related cells with focus on the SOX11 gene.

The SOXC transcription factors are known to compete for the same target genes. For the first time in MCL, the SOXC genes were quantified by qPCR in a set of MCL patients and MCL cell lines. As previously reported, SOX11 expression was high in MCL, but also SOX12 mRNA levels were found to be higher compared to non-malignant B-cells, whereas the expression levels of SOX4 varied. Further, expression of the SOXC genes correlated in SOX11 positive MCL (determined by immunohistochemistry). How SOX11 gene expression in MCL is regulated was also addressed by studying its promotor region. The promotor region of SOX11 was found to be hypomethylated in MCL patients and cell lines, but also in non-malignant B-cells indicating regulation by other epigenetic mechanisms than promotor methylation.

Fast and accurate differentiation between similar entities of lymphoma is important since MCL has a more aggressive clinical course. Although having certain distinctive phenotypical markers, MCL and B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL) are both CD19+, CD20+ and usually CD5+, which could complicate diagnosis by flow cytometry. We developed a method to accurately implement SOX11 in the diagnostic flow panel that consistently detected SOX11 protein in ex vivo isolated MCL cells, but not in CLL/SLL. When conjugated SOX11-antibodies are available, this method could be implemented in the clinic for CLL/SLL with aberrant immune phenotypes or rare cyclin D1- MCLs.

The expression levels of SOX11 were further studied in a relatively large group of MCL patients (n=102) by qPCR to determine a cut-off for SOX11-negative MCL and to investigate how quantitative expression related to positivity/negativity by IHC. A cut-off was defined, which resulted in misclassification of only 2/102 by qPCR and IHC. However, for the IHC SOX11+ cases, the qPCR analysis was not able to find a natural cut-off that would identify cases with low expression. When grouping the samples based on expression (10% lowest expression versus the remaining cases), nodal disease was less frequent (p=0.01) and lymphocytosis more frequent (p=0.005) in the qPCR SOX11low-cases. Leukemic non-nodal MCL often expresses low levels of SOX11. The quartile of patients with the lowest SOX11 expression had significantly shorter overall survival in the group of patients who did not receive autologous stem cell transplantation.

Studies were conducted in primary murine B-cells and a murine pro-B cell line to study Sox11 oncogenic potential and role in differentiation in early B-cells. In the studied cell types, Sox11 did not per se act as an oncogene. Instead the rate of proliferation was reduced in the pro-B cell line and these cells changed morphology upon expressing the Sox11 gene. Gene expression analysis revealed upregulation of early cell cycle and cellular adhesion genes upon introduction of the Sox11 gene in the pro-B cells. Despite high similarity to Sox4 (important for B-cell survival and development), no obvious effect on selected B-cell differentiation stage associated genes were detected, which suggest that the effects of Sox11 are context dependent and might differ in pro-B cells compared to MCL and during embryogenesis.