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New molecular markers in mantle cell lymphoma: studies of cannabinoid receptors, 5-lipoxygenase and SOX11

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ABSTRACT

Mantle cell lymphoma (MCL) is a malignant B-cell lymphoma that accounts for 5-10% of all lymphomas and tends to occur in older adults with a higher incidence in males. The genetic hallmark of this neoplasm is the t(11;14) translocation which results in overexpression of nuclear cyclin D1. The t(11;14) translocation is not sufficient for the development of MCL. Additional oncogenic events are required.

By global gene expression analysis of MCL, the cannabinoid receptor type 1 and 2 (CB1 and CB2), the key enzyme in leukotriene synthesis 5-lipoxygenase (5-LO) and transcription factor SOX11 were found to be highly express in MCL compared to reactive lymph nodes. In this thesis, the possible roles of these genes in MCL were investigated.

In paper I, the expression of CB receptors in the B-cell lymphomas was analyzed by quantitative real time PCR, Western Blot and immunohistochemistry. We found that the majority of B-cell lymphomas expressed CB1 and/or CB2 and that cannabinoids induced cell death in CB1 and CB2 expressing MCL and B-CLL cell lines. Moreover, a metabolically stable synthetic cannabinoid reduced tumor burden in mice xenografted with human MCL. From this study, we can conclude that CB receptors are broadly expressed in B-cell lymphomas and can be a potential target for therapy.

In paper II, the expression of 5-LO in the different subsets of normal B cells and corresponding B-cell lymphomas was investigated. Using reverse transcriptase PCR, Western Blot, and immunohistochemistry, we found that mantle zone, but not germinal centre B cells expressed high amounts of 5-LO. Similarly, primary MCL expressed high levels of 5-LO, while most of follicular lymphomas lacked 5-LO expression. Furthermore, MCL cell lines were capable of producing leukotrienes under certain conditions. Thus, our results strongly indicate that the expression of 5-LO in lymphomas can mimic the expression in the developmental stage of the B cells from which lymphomas arise.

In paper III, immunohistochemical analysis was used to assess a series of B-cell lymphomas. We found that nuclear SOX11 expression appears to detect most MCL, but not B-CLL or follicular lymphomas. SOX11 can therefore be considered as a new diagnostic marker. Importantly, a few MCL lacked nuclear SOX11 expression. Patients with SOX11 negative MCL had worse overall survival compared to those with nuclear expression.

In paper IV, the role of SOX11 in the pathogenesis of MCL was further analyzed. A siRNA knock down system in MCL cell line Granta 519 was used. After the reduction of more than 80% SOX11 mRNA and protein expression, we performed Affymetrix array to analyze the effect of SOX11 on global gene expression. A total of 26 genes were significantly downregulated in SOX11 siRNA treated cells compared to control cells. These genes were validated in gene expression data from two series of primary MCL. In these cohorts there was a strong correlation between SOX11 expression and the expression of DBN1, SETMAR and HIG2. Moreover, using ChIP, we found that SOX11 can directly target *DBN1*, *SETMAR* and *HIG2* in MCL.

The conclusions from our SOX11 studies are that SOX11 is a new diagnostic marker for MCL and that SOX11 may be of prognostic importance in MCL. *DBN1*, *SETMAR* and *HIG2* are directly targeted by SOX11 in MCL.

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