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Functional Characterization of Candidate Genes in the Pathogenesis of B-Cell Chronic Lymphocytic Leukemia (B-CLL)

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B-cell chronic lymphocytic leukemia (B-CLL) is the most frequent leukemia of adults in the Western world. A critical region in chromosomal band 13q14.3 is deleted in more than 50% of patients, making it the most common genomic aberration in B-CLL. This points to a tumor suppressor mechanism localized in the critical region, which is involved in a variety of tumors, especially in B-CLL. Towards elucidation of this tumor suppressor mechanism, the function of candidate genes localized in the critical region as well as their regulation was characterized in this thesis. The regulation of candidate genes down regulated in patients with loss of one copy of 13q14 was analyzed by comparing the expression status of genes with the deletion status of B-CLL patients. No gene dosis effect of a co-regulated gene cluster outside the critical region in 13q was detected. However, due to strong hints for an involvement of an epigenetic regulatory mechanism, candidate genes were analyzed for monoallelic expression. In fact, the monoallelic expression of candidate genes was found in healthy B- and T-cells, which is especially interesting, since loss of the single copy in patients would suffice for complete loss of function of the corresponding gene. Monoallelic silencing in healthy probands thus provides a plausible explanation for the pathogenicity of monoallelic loss of the critical region. It was shown that monoallelic silencing was due to methylation of DNA and histone modifications, pointing to an epigenetic regulatory mechanism in the critical region of B-CLL. Furthermore, the mechanism of imprinting could be excluded because monoallelic expression resulted either from the paternal or the maternal chromosome copy. Since the involvement of an epigenetic regulatory mechanism in the pathogenesis of B-CLL is very likely, replication timing for the two copies on 13q14 was analyzed. It was shown that the two chromosome copies replicate asynchronously, suggesting different chromatin packaging of the two copies. To get an insight into the function of candidate genes localized on 13q14.3, two methods were established to modulate their expression. First, candidate genes were overexpressed in a hematological cell line that shows a deletion of one copy of 13q14. Second, candidate genes were knocked down via *in vitro* transcribed siRNAs in two reference cell lines in order to get strong down regulation of gene expression. The resulting phenotypical effects of both approaches were analyzed by different strategies. One strategy were knowledge-driven experiments performed for those genes with homology to genes of known function. The second strategy was a genomewide approach to identify genes de-regulated upon modulation of the expression of 13q14 candidate genes. The candidate gene *SETDB2* has a homolog *SETDB1* that is a histone methyltransferase. However, no similar enzymatic activity could be detected for *SETDB2*. Similarily, no apoptotic potential for *RFP2* was shown after overexpression of this gene, subsequent staining of apoptotic cells and analysis by FACS, even though several homologous genes have been shown to induce apoptosis. In contrast, it could be shown for *KPNA4* and *KPNA3* that they are directly involved in shuttling of NFκB into the nucleus in U2OS cells. In the second strategy, a genomewide approach, effects resulting from modulation of gene expression of candidate

genes were analyzed on transcription level. The analysis of the microarray data resulting from knock down experiments of candidate genes revealed unexpected regulatory effects of the siRNA used as negative control and targeting the exogenous gene *EGFP*. Since this siRNA should have no effect on endogenous genes and is therefore commonly used as control for RNAi experiments, this finding is of general interest. Target genes identified in the microarray experiments give important insights into the function of 13q14 candidate genes regarding the pathomechanism of B-CLL. For *C13ORF1* and *RFP2*, an involvement in hematopoiesis via NOTCH signalling, in AKT signalling via regulation of *GNAZ* and the ability to repress proliferation via regulation of *WIG1*, which is involved in the *TP53*-dependent cell growth regulatory pathway, was shown. Additionally, *C13ORF1* is likely involved in *TP53*-dependent signal transduction, repression of transcription and ubiquitin signalling, pointing to an important role of *C13ORF1* in the pathogenesis of B-CLL. It was shown that the majority of candidate genes (*RFP2, DLEU2, C13ORF1* and *KPNA3*) are involved in signalling of transcription factor NFκB. The classical as well as the non-classical NFκB pathway has the potential to promote survival of B-cells and plays a major role in B-cell development. Since it has been suggested that malignant cells in B-CLL accumulate rather than proliferate and thus are dependent on receiving the required input signal, the involvement of candidate genes in this pathway has major importance.

In conclusion, we were able i) to shed light on the complex epigenetic regulatory mechanism residing in 13q14 by uncovering monoallelic expression, ii) to show involvement of 13q14 candidate genes in NFκB signaling and finally iii) to identify functional linkage of these candidate genes with several additional signalling pathways, which likely play central roles in the pathogenesis of B-CLL. These findings will in the future refine diagnostic tools, open new avenues for elucidation of the pathomechanism of B-CLL and contribute to identify therapeutical target molecules.