ABSTRACT

Classical Hodgkin lymphoma (cHL) is one of the most common lymphomas. It is characterized by a bimodal distribution regarding age, which makes it one of the most frequently diagnosed cancers among young adults. The currently used therapeutic methods, which mainly include chemotherapy and radiotherapy, allow for a durable remission reflected by the 5-year survival rate in over 80% of newly diagnosed patients. Recently approved immunotherapy is highly effective in relapsed refractory patients for whom standard therapy is insufficient. Despite the high survival rate, standard therapy is characterized by many adverse effects that drastically reduce the quality of life of patients. The adverse effects of chemo and radiotherapy, as well as the difficulty in treating relapsing patients, are the reasons for the search for new therapeutic methods that take into account the molecular mechanisms of cHL pathogenesis. Therefore, this dissertation aims to characterize changes in cHL biology at the genetic and epigenetic levels, which may allow a better understanding of this disease.

The first aim of this dissertation was the identification of B-cell specific transcription factors that are lost in cHL. Although Hodgkin and Reed-Sternberg (HRS) cells in most cases arise from pre-apoptotic germinal center B-cells (GCB cells), they do not express typical B cell markers. On the contrary, the phenomenon of loss of B cell identity has been well characterized and is an essential mechanism for HRS cells to survive and avoid elimination by the immune system. The presented results showed the loss of the ELF1 transcription factor, so far not described in the context of cHL. The absence of ELF1 protein was demonstrated in HRS cells in 89% of cases as well as in all analyzed cHL-derived cell lines. Moreover, it was proved that the loss of *ELF1* is the result of epigenetic silencing by DNA methylation of its promoter region and to a lesser extend a consequence of heterozygous deletions targeting the gene.

Considering the importance of epigenetic alterations in cHL, the investigation of the role of microRNAs in this lymphoma was chosen as the second aim of this dissertation. For this purpose, a systematic review of the available literature on microRNA expression profiles in cHL was prepared which allowed the identification of recurrently upregulated (let-7-f, mir-9, mir-21, mir-23a, mir-27a, mir-155 and mir-196) and downregulated (mir-138 and mir -155) microRNAs in cHL. Processes in which the deregulated microRNAs are potentially involved have also been

proposed. These are: impaired B cell development (mir-9, mir-150 and mir-155), NFκB hyperactivation (mir-155 and mir-196) and immune evasion (mir-9 and mir-138).

The third aim of this dissertation was to test the hypothesis whether microRNA deregulation could be a result of abnormal DNA methylation in cHL. For this purpose, based on our previous NGS sequencing results, a group of downregulated microRNAs with a CpG island in their promoter regions was selected. The inverse correlation between expression and DNA methylation was found for four microRNAs (mir-148a-3p, mir-148a-5p, mir-193a-5p and mir-339-3p). In further studies, it was proved that DNA hypermethylation and silencing of mir-148a is not only limited to cHL cell lines but occurs also in microdissected HRS cells.

Functional studies revealed that the restoration of mir-148a expression in cHL cell lines leads to a decrease in the viability of some of the tested cell lines. Moreover, new target genes for mir-148a (*IL-15, HOMER1, SUB1* and *SERPINH1*) have been proposed. The subsequent changes in their expression might be responsible for the affected viability and proliferation of HRS cells.

In summary, the results presented in this dissertation showed the loss of ELF1 protein, not previously described in Hodgkin lymphoma and indicated the mechanisms responsible for this phenomenon. Moreover, on the basis of the available literature, the current state of knowledge on microRNA deregulation in this lymphoma was systematized. Experimental studies allowed to prove that DNA methylation is responsible for the regulation of microRNA expression in a similar way as it was described for protein coding genes.