ABSTRACT

Non-Hodgkin lymphoma (NHL) is a highly heterogeneous group of blood malignancies. Fast diagnosis and application of appropriate treatment can ensure the 5-year survival rate up to 90%, but it differs strongly depending on the NHL type and disease stage. The complex process of development and differentiation makes B cells especially prone to genetic aberrations. Immunoglobulin heavy chain locus (IGH) undergoes VDJ recombination, somatic hypermutation and class switch recombination, which are all necessary to establish a wide range of high-affinity antibodies. The expression from *IGH* locus is controlled by its enhancers: Eμ and 3' regulatory regions 3'RR1 and 3'RR2. IGH locus remodeling involves DNA-double strand breaks, which pose a threat of illegitimate rearrangements. Indeed, recurrent translocations placing oncogenes under the regulation of *IGH* enhancers are a hallmark of NHL. Diffuse large B-cell lymphoma (DLBCL) is the most frequently diagnosed subtype of NHL and is often characterized by t(14;18)(q32;q21) IGH/BCL2 juxtaposing an apoptosis regulator - BCL2 - with *IGH* enhancers. Burkitt Lymphoma (BL) belongs to the fast growing NHLs. It is more rare and associated with the t(8;14)(q24.1;q32) MYC/IGH. Similarly to DLBCL, in BL MYC is juxtaposed with *IGH* enhancers which leads to its increased expression. MYC is a transcription factor, involved in the control of several cellular processes, such as growth, proliferation and apoptosis. Oncogene deregulation by *IGH* enhancers is an early pathogenic event setting a B-cell on a path towards malignancy. Survival and proliferation of lymphoma cells often depends on the expression of the translocated oncogene. Despite wide knowledge about roles of *IGH* enhancers in normal B-cells, our current understanding of their functioning in malignant B-cells is limited.

The research undertaken in this doctoral dissertation broaden the knowledge regarding *IGH* enhancers is NHL. In the review article **Enhancing B-Cell Malignancies—On Repurposing Enhancer Activity towards Cancer** (Kasprzyk ME, Sura W, Dzikiewicz-Krawczyk A, 2021) I explored how enhancers can contribute to the formation of malignant B-cells. I especially focused on *IGH* enhancers, providing the summary of the current literature reports of their roles in normal B-cell development and in lymphoma cells. Based on the available knowledge, I described the interactions between Eμ and 3'RRs and listed mouse models used in studies. In the original article **CRISPRi screen identifies core regions in IGH enhancers essential for non-Hodgkin lymphoma cells survival** (Kasprzyk ME et al., in preparation) I validated the results obtained by our CRISPR/dCas9 screen targeting I*GH* enhancers in BL and DLBCL. CRISPRi screen allowed for identification of precise regions within Eμ and 3'RRs that are necessary for survival of NHL cells. I showed that inhibition of those essential regions in IGH enhancers lowers lymphoma cells proliferation and downregulated the expression of translocated oncogenes. In

BL, I was able to rescue the observed phenotype by MYC overexpression. Moreover, I showed that blocking of the Eμ significant region leads to B-cell receptor (BCR) loss on the cell surface, which is necessary for proper functioning and survival of B-lymphocytes. I also performed chromatin-enriched RNA-Seq and confirmed ongoing transcription in *IGH* enhancers and their core regions. I validated enhancer RNAs (eRNAs) expression from *IGH* enhancers essential regions in a wide panel of B-cell lymphomas as well as patient derived samples. In the second original article **7-[[(4-methyl-2-pyridinyl)amino](2-pyridinyl)methyl]-8-quinolinol (compound 30666) inhibits enhancer activity and reduces B-cell lymphoma growth – A question of specificity** (Kasprzyk ME et al., 2021) I tested the compound 30666 proposed recently as a specific *IGH* enhancers inhibitor. My research showed that the compound 30666 indeed alters the *IGH* enhancers activity as indicated by lowered expression of translocated oncogenes, differential expression of eRNAs and global changes of enhancer-specific histone modifications. Although compound 30666 negatively affected cell survival, I demonstrated that the effect was not limited to B-cell lymphomas.