1 Characterization of RNA networks regulated by transcription factors in malignant B cells

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1.1 SUMMARY

Transcription factors (TFs) have been identified as key regulators of RNA regulatory networks in aggressive subtypes of chronic lymphocytic leukemia (CLL) and follicular lymphoma (FL). It has been shown that both diseases harbor complex deregulation of interconnected TFs that leads to aberrant mRNA expression profile together with dysregulated expression of post-transcriptional regulators such as RNA-binding proteins and microRNAs. Our preliminary data suggest that one such aberrant transcription factor hub consists of two FOXO-family members (FOXO1, FOXM1), HMGA1, and CEBPB. We plan to explore the RNA interaction networks as well as target DNA-regulatory regions associated with these TFs during the biology of B-cell malignancies

1.2 SCIENTIFIC BACKGROUND

Genom-wide approaches revealed striking changes in epigenome features in mature B cell leukemias and lymphomas as compared to healthy mature B cells. Increased chromatin accessibility in the sites of *de novo* activated super-enhancers and promoter regions has signified a potential role of transcription factors (TF) as regulators of disordered gene expression programs, for example, in chronic lymphocytic leukemia (CLL) (Beekman et al., 2018; Ott et al., 2018). Moreover, epigenome remodeling seems to be associated with the activation and binding of specific transcription factors (such as NFATs, FOXO1, TCF7, or EBF1) (Beekman et al., 2018; Rendeiro et al., 2016). So far studies have focused dominantly on the TFs role in the context of healthy B-cells vs. malignant B cells; however, it remains largely unknown how TFs influence the microenvironmental interactions and how this contributes to the aggressiveness of B cell malignancies.

We performed an in-house developed transcription factor binding motif analysis (TFEA) of patient- gene expression profiles from 20 CLL and 22 follicular lymphoma samples and conducted a systematic gene set enrichment analysis (GSEA). Results of both analyses revealed candidate transcription regulators, which are highly responsible for deregulated transcriptional programs observed in aggressiveness-associated transcription programs of both malignancies. These selected TFs, namely FOXO1, FOXM1, HMGA1, and CEBPB, are essential cellular programs' key regulators in several other malignancies.

FOXO1 is a canonically known tumor suppressor that induces genes involved in cell cycle arrest (Szydlowski et al., 2016). However, our results describe FOXO1's oncogenic character in CLL by contributing to the migration of CLL cells to protective immune niches via upregulation of GAB1 and other genes (Seda et al., 2021). FOXO1 is also known to be aberrantly transcriptionally active in B-cell lymphomas and to be activated by mutations in some cases (Kabrani et al., 2018; Trinh et al., 2013). FOXM1 (Forkhead box M1) is a

proliferation-associated TF that has been classified as a human proto-oncogene and its elevated levels are frequently associated with cancer progression (Reeves, 2001). We found that FOXM1, together with HMGA1 (high mobility group A1), another TF closely associated with aggressiveness and poor prognosis of cancer (De Martino et al., 2019), have higher expression levels in DLBCL patients compared to the normal population (GEPIA2). Interestingly, networking between HMGA1 and FOXM1 was confirmed in other malignancies, such as breast cancer, showing that they modulate the expression of each other (Fiscon et al., 2021). CEBPB (CCAAT/enhancer-binding protein beta) orchestrates complex steps in the fate of hematopoietic cell commitment with a high degree of complexity in protein-protein interactions as demonstrated before (Dittmar et al., 2019). Our preliminary data showed that CEBPB is activated in CLL microenvironment through the BCR signaling and T-cell interactions, which are indispensable for CLL cells survival and proliferation. Importantly, CEBPB is known to interact with FOXO1 and HMGA1 (and FOXO1 also interacts with FOXM1) and each of the three proteins has been described as a transcriptional target of CEBPB (Encode).

Besides a direct impact on RNA transcription, these TFs regulate a number of post-transcriptional regulators of mRNA stability/translation, such as RNA-binding proteins and miRNAs, which further add to the complexity of the regulatory hubs. We believe that the deep ChIP-seq analysis would shed light on the novel regulatory roles of these TFs in the aggressiveness of mature B cell malignancies.

1.3 AIMS OF THE RESEARCH PROJECT

We aim to describe the regulatory landscape of four target transcription factors – FOXO1, FOXM1, HMGA1, and CEBPB, in malignant B cells. Our preliminary data indicate that each of these TFs is involved in the regulation of essential cellular programs such as survival, proliferation or migration, and we plan to identify i) binding sites for selected TFs in the genome of malignant B cells and ii) RNAs regulated by TFs and involved in the deregulation of transcriptional programs orchestrated by these TFs. Based on the preliminary and published data, we expect to reveal a cooperative pattern between these TFs in the activation of transcriptional programs in B cells.

1.4 WORK PROGRAMME

1.4.1 The experimental approach including key techniques or technologies

To study the functions of the TFs in malignant B cells, we will modulate their expression levels by CRISPR-KO technology or lentivirus-based shRNA interference. Subsequently, the cells will be exposed to microenvironment-relevant stimuli for specific time period (Mraz lab) and then subjected to ChIP-seq to identify the activity of TFs at specific regions (Benes lab). This will be supplemented by RNAseq experiments (Mraz lab) to define the effects of TFs on gene expression. We will also be monitoring the direct effect of TFs knockdown on cell proliferation, survival and migration of malignant B cells.

1.4.2 What will be done by which partner and the expected synergies

The project is conducted at CEITEC, and the majority of the experiments will be performed in our laboratories, including optimizing of the pull-down experiments and proliferation/migration assays. ChIP-seq experiments themselves will be conducted at the Genomics Core Facility headed by Dr. Vladimir Benes (EMBL, Heidelberg), and RNAseq experiments will be conducted mainly at CEITEC MU.

1.4.3 Describe the benefit of this research project to RNA research at CEITEC

With this project, we want to reveal the RNA regulatory hubs dysregulated via TFs governing the aggressiveness of malignant B lymphocytes. The TFs were selected based on a Transcription factor enrichment analysis developed by the CEITEC MU Bioinformatics facility. The RNA-regulatory network connected to the TF machinery is coming into the focus since they turn out to be a crucial mechanism participating in the deregulated transcriptome. This is well known for diseases that harbor translocations of transcription factors such as MYC, but until recently remained unrecognized in B cell malignancies without recurrent genetic defects in TFs. Outcomes of this project will help elucidate the role of TFs as master regulators of B cell fate and indicate a direction for targeted therapy.

First, through this collaboration, we will establish state-of-the-art methods to study TFs role in the biology of a model cancer type and integrate CHIPseq with RNAseq data. Second, we believe that this project can yield high-impact discoveries and eventually good quality publications. Thus, this will increase the scientific excellence and reputation of CEITEC. Third, this project brings together research groups with a biological understanding of cancer and high technical excellence.

1.4.4 Future collaborations or joint funding expected to result from work proposed

We perceive this as a pilot project to establish collaboration between two labs that on CHIPseq technics. Thus, at the moment, we do not plan, but do not exclude, for the future, to apply for external funding beyond this INTEG-RNA project.

1.5 REFERENCES

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1.6 BUDGET

7000 EUR
The seeding funds will be used for purchasing the chemistry for the optimization and performing of the CHIPseq experiments and optimization. This also includes the antibodies, constructs, and reagents to perform NGS experiments.

In addition, we will apply for a secondment that will cover travel expenses and approximately 1-week stay at EMBL (alternatively, this could be covered from internal resources at Mraz lab).