ABSTRACT

Cutaneous T- cell lymphomas (CTCLs) are a large, heterogenous group of T-cell skin malignancies. Among many CTCL subtypes, Sézary Syndrome (SS) and mycosis fungoides (MF) are the two most common clinical variants. Although characterized by great differences in gene expression, mutations, and chromosomal aberrations, the causes of CTCLs remain unknown. Many genetic alterations and dysregulation of signaling pathways have been reported in the CTCLs, however, the exact molecular mechanism of the pathogenesis is still to be unraveled. Due to the lack of diagnostic markers and the presence of multiple clinical presentations, the diagnosis of CTCL is difficult, resulting in an average of 6 years of confirmation from the disease onset. What's more, to date, there is no cure for CTCL, and the therapy mainly focuses on relieving symptoms and inhibiting disease progression. Because of that, there is a need for further intensive studies on CTCL, not only to understand tumor biology, but also to find a potential diagnostic marker and finally introduce new therapeutic strategies. We found that the *transmembrane protein coding 244 gene* (*TMEM244*) is ectopically expressed in all SS patients, SS-derived cell lines, and, to a lower extent, in MF and a fraction of T-cell lymphomas, but not in B-cell malignancies and peripheral blood mononuclear cells (PBMC) of healthy individuals. Therefore, the main aim of this dissertation was to identify the mechanisms of *TMEM244* expression and its function in CTCL biology that will allow to better understand this disease.

The first specifc aim of this dissertation was the identification of the mechanism responsible for *TMEM244* activation. Epigenetic dysregulations are known to play an important role in the development and progression of SS as it was shown that SS cells are characterized by widespread changes in DNA methylation. The presented results showed a negative correlation between *TMEM244* expression and promoter methylation in patient samples and T-cell lines. Moreover, by using the CRISPR-dCas9 epigenome editing system it was proved that demethylation of selected CpGs in the *TMEM244* promoter region activated *TMEM244* expression in examined cell lines, suggesting methylation to be a mechanism responsible for the regulation of its expression.

Considering elevated levels of *TMEM244* in CTCL patients and the fact that the *TMEM244* gene role has not been investigated yet, establishing the function and the coding potential of *TMEM244* was the second aim of this dissertation. By applying the GFP competition test, it was demonstrated that *TMEM244* is necessary for cellular growth in CTCL cells, therefore it might be considered a new potential therapeutic target for the treatment of CTCL. Furthermore, by using

RNA fractionation followed by qRT-PCR as well as RNA-FISH assay, cytoplasmic localization of *TMEM244* transcript was identified. Although *TMEM244* transcript is localized in the cytoplasm, by using the Western Blot method it was shown that it does not encode a protein but is rather a long non-coding RNA (lncRNA), whose specific function is still to be discovered.

The third aim of this dissertation was to examine *TMEM244* expression in a subpopulation of blood cells of healthy individuals and to address whether its expression may serve as an easy diagnostic tool for SS. By applying flow cytometry and qRT-PCR it was established that in physiological conditions generally higher expression of *TMEM244* was observed either in CD4+ or in CD8+ subsets of memory cells (CD4RO+) of peripheral blood mononuclear cells (PBMC), which is in line with the immunophenotype of Sézary cells. Additionally, as it was shown by applying qRT-PCR, *TMEM244* expression in either CD4+ T-cells or the whole population of PBMC of SS patients, can be used to distinguish this lymphoma from diseases with similar clinical presentations such as MF and non-malignant erythroderma and as a result significantly improve the diagnosis.

In summary, the results presented in this dissertation show that specific DNA demethylation of promoter is responsible for *TMEM244* expression. Moreover, *TMEM244* is necessary for the growth of CTCL cells and despite its annotation, does not code a protein but is rather a lncRNA, not previously described. Experimental studies allowed us to prove that analysis of *TMEM244* expression could be used as an easy and cheap blood diagnostic marker to distinguish SS from diseases with similar clinical presentation. Additionally, based on the available literature, the current state of knowledge on the heterogeneity of malignant cells in this lymphoma was summarized.