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Acute leukemia is a clonal disease of the bone marrow that is characterized by an accumulation of immature cells due to induction of proliferation and a block in differentiation. The overgrowth of malignant cells in the bone marrow hampers the production of normal blood cells which leads to anemia, thrombocytopenia and leukopenia. Patients with acute leukemia therefore experience fatigue, shortness of breath, easy bruising, bleeding and recurrent infections. Grossly two types of acute leukemia can be discriminated; acute myeloid leukemia (AML) which finds its origin in the immature cells of the myeloid lineage and acute lymphoid leukemia (ALL) which originates from lymphoid progenitors. In adults with acute leukemia, AML is the most prevalent, followed by ALL of the B-cell lineage and less frequently T-cell ALL. Treatment of AML and ALL consists of chemotherapy sometimes followed by an autologous or allogeneic stem cell transplantation although treatment protocols differ for each type of acute leukemia. Despite the fact that most patients achieve a complete remission after initial treatment, relapse often occurs. Relapsed AML is difficult to treat and second complete remissions are seldom achieved. The overall survival of patients with AML and ALL is therefore only 40%. Diagnosing and classifying acute leukemia is based on the combined interpretation of the results obtained by different methods, such as cytomorphology, cytogenetics, immunophenotyping and molecular mutation analysis. As AML and ALL have different morphological characteristics, express diverse immunophenotypical markers and are associated with typical cytogenetic and molecular aberrations, discrimination between the two is often straightforward. However, occasionally a patient with an acute leukemia with an undefined morphology lacking clear marker expression and specific chromosomal or molecular alterations is presented. Although these cases comprise less than 3% of the newly diagnosed acute leukemias, they form a diagnostic hurdle for hematologists as treatment decision greatly depends on the lineage of origin. An example that demonstrates the challenges in diagnosing an acute leukemia with ambiguous lineage is described in Chapter 2 of this thesis. In this chapter a 65-year old woman is presented with an acute leukemia with monocytoid morphology, erythrophagocytosis and B-lymphocytic marker expression combined with the myeloid marker myeloperoxidase. This leukemia cannot be classified as either AML or ALL, and is termed mixed phenotype acute leukemia (MPAL). These leukemias form a separate entity within the World Health Organization (WHO) classification of acute leukemia and are associated with a poor prognosis. The poor outcome of patients with MPAL is in part the result of the high prevalence of drug efflux pump expression and the high proportion with cytogenetic abnormalities. However, failing to respond to induction therapy might also be caused by the wrong choice of therapy (i.e. AML-related therapy for lymphoid leukemia, or vice versa). It is therefore important to unravel the underlying lineage of these ambiguous leukemias and new tools that could aid therapy decision making are needed.
In Chapter 3 we describe the potential of microRNA expression profiling to classify acute leukemias of unknown origin. MicroRNAs are small single stranded RNA molecules that regulate protein expression mainly by binding target mRNAs resulting in translational inhibition or mRNA degradation. They are involved in all biological processes, including proliferation, differentiation, migration and apoptosis, in both physiological as well as pathophysiological conditions. MicroRNA expression is specific for the cell type and developmental cell stage and can be influenced by extracellular stimuli derived from the microenvironment. In cancer, but also during infections and in autoimmune disease, their expression can change and can contribute to the character of the disease. Due to the strict regulation of microRNAs their expression profiles are highly specific for particular cell types and can be can be used to classify tumors. As leukemias of ambiguous lineage are difficult to classify using the traditional diagnostic tools microRNA expression profiling could be a useful way to obtain more information about the lineage of origin.

In Chapter 3 of this thesis we investigate the microRNA expression profiles of 17 ambiguous lineage leukemias and compare these to the expression profiles of genuine AML, B-ALL and T-ALL cases. First, we confirmed that microRNA expression profiles can be used to discriminate between AML, B-ALL and T-ALL and found new as well as known lineage specific microRNAs. Secondly, we showed that miRNA expression profiling can accurately classify of ambiguous leukemia as either of a myeloid or lymphoid genetic background. Moreover, none of the MPAL cases segregated as a separate group and all the cases clustered together with true AML and ALL cases. Defining MPAL as a separate entity within the classification of acute leukemias seems therefore incorrect and leukemias of ambiguous lineage should therefore be analyzed for their microRNA expression profiles to determine the lineage of origin and the associated most optimal treatment strategy. Future microRNA expression analyses could help hematologists in their therapy decision making and hopefully improve clinical outcome of MPAL.

Chapter 5 and 6 of this thesis focuses on microRNAs highly expressed in normal and/or leukemic stem cells (LSC) in AML. Like in normal hematopoiesis, AML is organized as a hierarchy with a the top a small subpopulation of leukemic cells with stem cell properties, the LSC. LSC and normal hematopoietic stem cells (HSCs), responsible for reconstitution of all the blood cells after therapy, share phenotypic marker expression, have both self-renewal capacity and give rise to more differentiated progenitors. LSC are thought to be responsible for relapse and their eradication, while sparing HSC, is needed to improve the prognosis of AML patients. With the aim of finding specific anti-LSC therapeutic targets we performed a microRNA expression profiling of LSC and the more differentiated leukemic progenitor cells as well as of normal HSC residing within the same AML bone marrow (Chapter 6). One of the most discriminating microRNAs highly expressed in residual HSCs is miR-551b. In Chapter 5 we study the expression of miR-551b in normal hematopoiesis and leukemia. We show that expression of miR-551b is highest in HSC and multipotent progenitors and decreases upon differentiation.
suggesting that miR-551b expression is associated with normal stem cells. This led to the hypothesis that AML cases with high miR-551b expression might be a stem cell derived AML with stem cell features and chemotherapy resistance. Upon malignant transformation the intrinsic resistance and regenerating characteristics of HSCs could be inherited by the leukemia conferring poor prognosis. Indeed we find leukemias with high expression of miR-551b to be associated with an undifferentiated phenotype and the expression of stem cell genes. Patients that highly express miR-551b have a lower complete remission-rate, higher chance of relapse and a poorer outcome compared to patients with low expression. Moreover, miR-551b remained an independent prognostic indicator even when analyzed together with well-established prognostic indicators like cytogenetics, WBC and important molecular aberrancies. Patients in complete remission with undetectable or low levels of minimal residual disease but with high expression of miR-551b also had a higher chance of relapse and poorer prognosis compared to patients with low levels of miR-551b. Possibly, miR-551b expression could add value to current prognostication models and direct therapy decision.

Chapter 6 of this thesis focuses on miR-126. Profiling of HSC, LSC and leukemic progenitors revealed higher expression of miR-126 in HSC compared to LSC but also showed a higher expression in LSC compared to leukemic progenitors. These results suggested that miR-126 is a stem cell associated microRNA that might be involved in stemness. Indeed, targeting of miR-126 in AML cell lines induced apoptosis and resulted in growth inhibition in vitro and reduced tumor growth in a xenograft mouse model. In patient derived AML cells knockdown of miR-126 also resulted in apoptosis reducing the number of CD34+CD38- LSC and decreasing their clonogenic capacity and long term survival in vitro. In contrast, normal HSC are not harmed by miR-126 targeting. In fact, a slight increase of normal CD34+CD38- cells after miR-126 knockdown is observed. This makes miR-126 a promising new target for future AML specific therapy sparing normal hematopoiesis and possibly even reducing the time to hematological recovery due to stimulation of residual HSC.

This thesis ends with a general discussion and future perspectives on microRNA research in AML. The findings of this theses are discussed with a focus on the relevance and implications for the clinic and highlight the potential and hurdles of future microRNA directed therapy.