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Decreased Expression Levels of Tumor Suppressor MicroRNAs in Hairy Cell Leukemia

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Abstract

Hairy cell leukemia is a chronic, clonal disease of the hemopoetic stem cell. Although our knowledge on its pathogenesis has increased a lot during the previous years, further details are needed to provide a more personalized, and therefore more successful treatment for patients. New advancements in the growing field of epigenetics may contribute to the finding of novel therapeutic target molecules. Similarly to acute leukemia, hairy cell leukemia also possesses a unique microRNA expression pattern. Decreased levels of tumor suppressor microRNAs lead to the elevation of the expression levels of their oncogenic targets. During our investigations on the level of let-7b and miR-124 in the bone marrow sample of a patient suffering from hairy cell leukemia, decreased expression levels have been found in the case of both microRNAs. These alterations also influence the activity of the MAPK signaling pathway, resulting from the regulation of its members by microRNAs including those mentioned above. Our results support new details about the connection between altered microRNA expression levels and signal transduction pathways in hairy cell leukemia.

Keywords: Hairy cell leukemia; Epigenetics; MicroRNA; Signal transduction; Prognosis; Personalized treatment

Abbreviations

HCL: Hairy Cell Leukemia; miRNA: microRNA; MAPK: Mitogen-Associated Protein Kinase; UTR: UnTranslated Region

Introduction

The term epigenetics includes mechanisms that alter gene expression levels without causing any changes in the sequence of the DNA. Besides DNA methylation and histone modification, microRNAs (miRNAs) also fulfill this definition [1]. MiRNAs are small non-coding RNA molecules that regulate gene expression at posttranscriptional level [2].

Besides many other physiological processes, including metabolic and signaling pathways, inflammation and stress response, intact hemopoesis also requires intact miRNA function. During the differentiation of blood cells, the expression levels of miRNAs are strictly determined [3]. Among pathological conditions, solid tumours and leukemia are hot spots in miRNA research: many of them have oncogenic (oncomiRs) or tumor suppressor (anti-oncomiRs) potential, therefore it is not surprising that altered miRNA expression levels are confirmed to have a significant role in both the initiation and progression of a wide variety of malignant diseases including leukemia [4,5]. Moreover, specific changes of the expression levels of several miRNAs have also been found in the different subtypes of leukemia [6,7]. These alterations can be applied in both the differential diagnosis and the prognostication of the disease. Arising from the promising possibilities that may turn back the pathological changes of epigenetic regulation of gene expression, the elements of the epigenetic regulatory machinery including miRNAs have got into the center of interest.

Recent findings refer to the role of miRNAs in the pathogenesis of hairy cell leukemia (HCL) by regulating the expression levels of proteins involved in the mitogen-associated protein kinase (MAPK)-pathway [8]. However, it is not elucidated yet, which miRNAs are responsible for this phenomenon. Our preliminary experimental results may give new details to these observations.

Materials and Methods

During our investigations, the first step was the isolation of the mononuclear cell fraction from the bone marrow sample, taken at the time of diagnosis. An 83 years old male patient, with atrial fibrillation and gastroesophageal reflux as concurrent conditions, was diagnosed with HCL. At the time of the diagnosis high white blood cell count (WBC: 20.5 G/L), anaemia (RBC: 3.47 T/L), low platelet count (PLT: 58 G/L), enlarged spleen and axillary lymphadenomegaly were the leading clinical features. Flow cytometric examination of the bone marrow described approximately 8-10% hairy cells with CD79b+/CD81+/CD24+/FMC7+/CD25+/CD11c+ immunphenotype in a rather hypocellular environment. These hairy cells were detected in the periphery at 50%.

Following the Ficoll-Hypaque density gradient centrifugation and the subsequent separation of the total RNA fraction with Trizol reagent, reverse transcription and qPCR reaction were performed. Expression levels of two tumor suppressor miRNAs, let-7b and

miR-124 were evaluated with the help of stem-loop structured primers [9]. According to the online available miRNA target prediction databases, these miRNAs are involved in the regulation of the expression levels of the proteins of the MAPK-pathway. As a reference, the level of miR-26b was considered. Expression levels of the two anti-oncomiRs in the bone marrow sample of the patient were compared to that of immortalized lymphoblasts derived from healthy members of a genetically characterized family (1000 Genomes Project, LCL CEU 1459, Coriell Institute).

Results and discussion

In the case of both miRNAs we observed much lower expression levels in the sample of the patient, than in the lymphoblastoid cells used as control (data not shown). These results correlate with the previously mentioned findings that disturbed function of miRNAs regulating the members of the MAPK-pathway may have pathogenetic role in HCL [8].

Let-7b is the member of the tumor suppressor let-7 family, the first discovered microRNAs in Caenorhabditis elegans, 1993 [10]. Although the altered expression level of let-7b has been detected both in solid tumours [11] and some kinds of hematological malignancies including multiple myeloma, little is known about its possible pathogenetic role in HCL [12]. Mir-124 is also a well-known anti-oncomiR, whiches expression level is generally decreased in leukemia, in a large part resulting from epigenetic silencing [13]. Similarly to let-7b, the function of miR-124 has not been characterized yet in HCL. Let-7b regulates ras, and MAPK itself is a target of miR-124 (Figure 1).

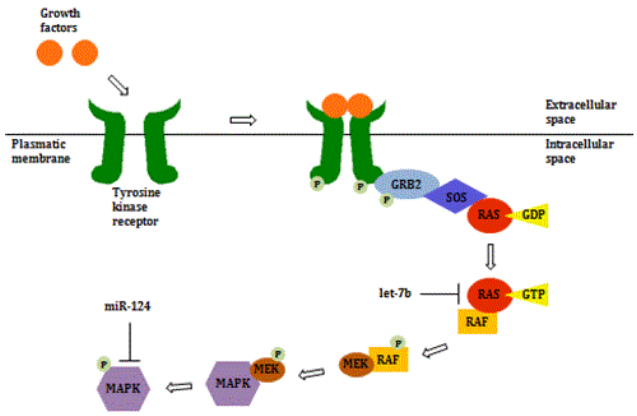

Figure 1: Regulation of the MAPK-pathway by anti-oncomiRs let-7b and miR-124

Therefore, decreased levels of these anti-oncomiRs are supposed to lead to the elevation of the expression levels of their oncogenic targets, ras and MAPK, resulting in a pathologically increased activity of the MAPK signaling pathway. This increased activity can contribute to the increased and uncontrolled proliferation rate that features leukemic cells.

Besides the suspected pathogenetic role of altered let-7b and miR-124 levels, the investigation of their expression levels may also be interesting in the context of prognostication; however, to confirm any prognostic connections, further experiments will be necessary to perform, involving a large number of patients suffering from HCL. It is important to emphasize, that one microRNA is only a single element of the enormous regulatory circuit that determines the gene expression pattern of leukemic cells, and each microRNA regulates several target genes, but in some cases, strong evidence supports their prognostic significance. For example, upregulation of miR-155 independently identifies high-risk patients in cytogenetically normal acute leukemia [14].

Similarly to other pathologically changed epigenetic regulatory mechanisms in malignant diseases, the alterations of microRNA expression levels are also reversible, due to the emerging possibilities that can either increase or decrease the level of them. Therefore, anti-oncomiRs including let-7b and miR-124 can be regarded as promising novel therapeutic targets in HCL: the elevation of their expression level may be combined with both epigenetic therapies and conventional chemotherapeutic drugs, which may contribute to a more personalized treatment approach in the future.

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