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Target genes for miR-150 and miR26 and their possible role in B-cell lymphomas; new drug targets for the future?

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Abstract

B-cell lymphoma is a type of cancer that is difficult to completely cure. Therapy consists mainly of radiation and chemotherapy, and aims for bringing the cancer into remission. Several non-Hodgkin lymphomas are known. In this thesis the non-Hodgkin lymphomas diffuse large B-cell lymphoma, Burkitt lymphoma, acute lymphoblastic leukemia and B-cell chronic lymphocytic leukemia and Hodgkin lymphoma are discussed. As for most types of cancer, expression of certain genes is aberrant in B-cell lymphomas. One mechanism in which expression can be altered is by microRNAs (miRs). MiRs can bind to mRNA via the RISC complex and in this manner control gene expression (targeting). A lot of research is carried out to find miRs that target genes involved in cancer. In B-cell lymphomas also several expected targets and miRs are known. Two miRs, miR-150 and miR-26, are being discussed in this thesis. Their genes of interest are selected on the expected role they play in the development, prognosis and treatment of B-cell cancers. The most promising target genes show involvement in lymphocyte cancers, lymphomas and several cell mechanisms involved in cancer, as for example DNA damage response, apoptosis and cell proliferation. Therefore, these genes and their miRs are probably usefull for new therapy targets in the future.

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Introduction

Diffuse large B-cell lymphoma (DLBCL)

This type of non-Hodgkin lymphoma is worldwide the most frequent diagnosed lymphoma. Thirty percent of all lymphoid cancers are DLBCLs. First symptoms are a rapidly enlarging lymph node somewhere in the body, although in about 40% of the patients DLBCL is developing outside of the lymph node. In 15% of the cases the bone marrow is also affected. It is not a typical age related malignancy, and morphology, therapy response and immune phenotype differs between patient cases. DLBCL is frequently treated with anthracycline-based cytostatics, for example doxorubicin, and has a heterogeneous outcome [1]. Low-dose chemotherapy is often sufficient to treat this lymphoma, and vincristine and prednisone are also used [2]. This type of cancer is mildly lethal. 60-70% of the patients survive after treatment with rituximab in combination with chemotherapy [1].

Burkitt lymphoma (BL)

Burkitt lymphoma is a non-Hodgkin B-cell lymphoma that was discovered by Denis Burkitt in an endemic malaria area in Africa in the 1960's. Later, BL was also seen in the rest of the world, mainly in children and adults with a deprived immune system [3]. This type of lymphocytic cancer is highly aggressive. The malignant B-cells proliferate fast, and as in more types of cancer c-myc expression is aberrant [2,4]. The t(8;14)(q24;q32) translocation is a typical hallmark in BL. Due to this translocation the c-myc gene is overexpressed by juxtaposing with a locus for heavy chain immunoglobulin (IGH). Juxtaposing is when genes are placed next together in the genome by a mutation as for example translocation. In diagnosing BL, identifying this translocation is an important factor [5].

BL has some phenotypic and morphologic characteristics in common with DLBCL. For example, the t(8;14)(q24;q32) translocation is also seen in 5-10% of DLBCL patients. DLBCL is a twentyfold more common than BL, so a t(8;14) translocation is not necessarily BL. These two cancer types differ in treatment and management, and should be distinguished from each other when forming a diagnose [2, 4].

The Epstein-Barr herpes virus (EBV) is often found in endemic BL, as seen in for example the earlier mentioned malaria areas in Africa. However, in Western countries, EBV is seldom found in BL. EBV positive BL cells show a latent infection [6]. Research in BL is often carried out in EBV-positive cell lines, like Daudi and Raji, and EBV-negative cell lines, like BJAB and Ramos [7].

Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (ALL) is the most common cancer in children, this type of cancer makes up for 25% of all pediatric malignancies. In contrast with the past, nowadays ALL is well treatable, with survival rates up to 93,5%. The term 'acute' refers to the fast disease progression, with lethality arising after weeks when ALL is not adequately treated. [8]. ALL has its origin in the bone marrow. The immature lymphocytes are being overproduced and replace the healthy lymphocyte progenitor cells.

Treatment often consists of chemotherapy, as in more leukemia's, and the main goal is to bring the cancer into remission. After that has succeeded, other treatments are required, for example radiation therapy, more chemotherapy or a stem cell transplant [9].

There are several types of ALL, depending on the stage of development in which the immature lymphocyte (blast) becomes malignant. As ALL cells have a restricted proliferation, one can hypothesize that to maintain this type of leukemia a small group of progenitor cells is responsible. However, data for long-term culturing of ALL cells is scarce, so no reliable information on growth potential and cloning ability of ALL progenitor cells is available yet [10].

B-cell chronic lymphocytic leukemia (CLL)

CLL, chronic lymphocytic leukemia, is a type of non-Hodgkin lymphoma mainly seen in elderly people. It is the most common leukemia in the Western world, and is highly variable in disease progression and survival. CLL often goes in a remissive state when patients are treated, but a relapse happens in almost every patient. When this occurs, CLL becomes a chronic and non-curable disease [11]. In this stage, survival ranges from months to decades. Some patients experience almost no symptoms and become of age similar to a control group, other patients quickly go into a therapy-required state.

Due to this chronic state, current therapy strategies are aimed for palliation in the advanced stage with symptoms. Treatment in an early stage did not significantly positively affect the survival of CLL patients. However, strategies that involve stem cell transplantation and antibody-chemotherapy are being developed, but not all of them are effective enough considering cost and toxicity [12].

Hodgkin lymphoma (HL)

Hodgkin lymphoma (HL), or the original term Hodgkin's disease, is a cancer of the lymph nodes, where only 1-2% of the tumor consists of typical cancer cells called Reed-Sternberg (RS) cells. The remaining 98-99% consists of surrounding normal T-cells, B-cells, plasma cells, neutrophils and eosinophils, fibroblasts and stromal cells. The affected lymph node loses its original structure. The tumor expands and survives because the RS-cells and the surrounding cells communicate via cytokines and probably induce anti-apoptotic signals and signals that promote proliferation [13]. The disease is often seen in young adolescents or adults of age 15 to 34 years, and people aged over 50 years. The overall 5-year relative survival for HL is 85,1% [14].

Due to the small proportion of RS-cells in HL, research is proven to be difficult. However, the current opinion on RS-cells is that they are derived from B-cells, because RS-cells showed increased somatic Ig mutations, which indicates a B-cell origin. A part of the lymphomas show presence of Epstein-Barr virus (EBV), where the genome of this virus is monoclonal. This suggests that EBV is an early step in the development of HL. EBV can directly influence HL onset by activating certain pathogenic mechanisms, or indirect via indicating a underlying immunodeficiency. Further research discovered that factors like sex, age and ethnicity are associated with the effect EBV has on HL. In the Western world, HL is less common (20-50%) compared to developing countries (>60%). A reason for this higher prevalence can be the same as for BL. Inhabitants of endemic malaria areas often have a deprived immune system, which makes them more vulnerable to eventual EBV infection [13].

Micro RNAs

A striking quote from Chen et al defines in an elegant way what function micro-RNAs have: *"MicroRNAs play a role in 'fine tuning' the physiological and pathological differentiation process, by which cells can rapidly regulate dynamic events such as cell-lineage decisions and morphogenesis."* [7]

MicroRNAs (miRs) are small RNAs that are about 22 nucleotides in length. They origin from a stem-loop RNA precursor, the primary transcript, that is coded in the genome. The classic definition of a miRNA depends on three criteria. These state that microRNA has to be made visible by laboratory experiments as for example Northern blotting. Also, the miRNA should have a precursor from which it originates, and that precursor must have a hairpin structure, where the miRNA is located in the hairpin-stem. At last, Dicer should process the mature RNA, and this can be proven by knocking out Dicer in a test animal and demonstrate the stacking of precursor RNA [15]. MicroRNAs regulate gene expression by binding to the target mRNA via the RISC complex (*figure 1*). Since 2001, when the first microRNAs were discovered, an immense load of information can be found on the large number of different miRNAs and their possible, expected and proven target genes.

In this thesis, the microRNAs miR-150 and miR-26 and their target genes are discussed for their possible role in the development, treatment and prognosis of B-cell lymphomas. The list of target genes for these two types of microRNAs can be found in the not yet published PhD thesis written by Jan Lukas Robertus in 2013 [16].

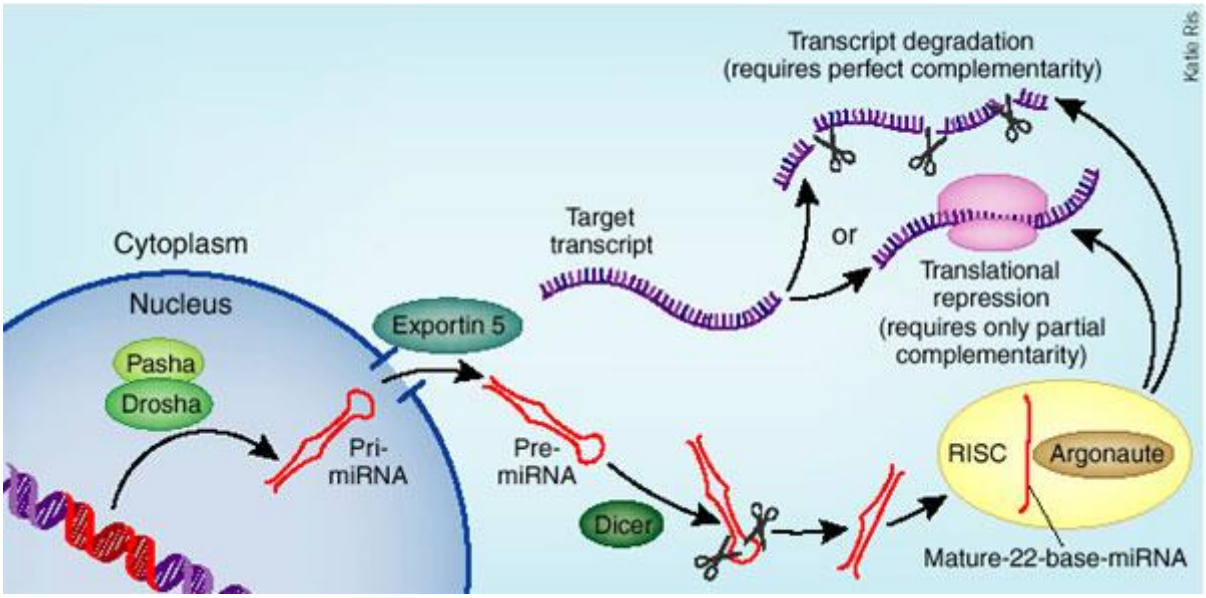

Figure 1: MicroRNA in the cell. The key proteins Drosha and Pasha process the RNA primary transcript to pre-microRNA. Then, Exportin5 exports the pre-microRNA in to the cytosol, where the endoribonuclease Dicer cleaves the pre-microRNA by taking off the loop transcript. After this, one strand of the two microRNA strands is taken up in the RISC complex, where it can function to inhibit RNA by complementary binding and activation of RNase [17].

miR-150 targets

AKT2

AKT2 is a putative oncogene for a RAC-beta serine/threonine protein kinase, which regulates cell proliferation, survival and growth in several types of cells [18]. AKT2 is a target for miR-150, as shown in the leukemic cell line MOTN-1 in a study carried out by Watanabe et al. Expression of AKT2 was diminished in presence of miR-150 expression [37].

The effect on B-cells is not fully clear, but a study carried out by Calamito et al investigated B-cell maturation in chimeric mice that were deficient for Akt1 and/or Akt2. They found that Akt1 and Akt2 co-expression had a key role in the development of marginal zone B-cells and B1-cells, and deficiency of Akt1/Akt2 had no effect on mature or transitional follicular B-cells in the peripheral lymph nodes or on B-cell precursors in the bone marrow. Furthermore, Akt1/Akt2-deficient follicular B-cells that were mixed with wild-type B-cells in vivo showed a decreased survival. Altogether, Akt kinases play an important role in the survival and maturation of peripheral B-cells [18].

Furthermore, the AKT kinase family has an effect on hematopoietic stem cells (HSC). Juntilla et al that investigated the functionality of HSCs in AKT1 and AKT2 knockout mice. They also found that Ak1+Akt2 knockout had more effect, compared to the loss of only one isoform, which didn't have a significant effect on functioning of HSCs. HSCs from double-knockout mice couldn't compete sufficiently against wild-type HSCs in vivo. Also, these double-knockout cells stayed in the G0-phase of the cell cycle, causing long-term defects in function, and showed decreased reactive oxygen species homeostasis, which resulted in diminished hematopoiesis [20].

AKT2 is also an insulin signaling gene, among others like SORBS1 and CAV1. AKT2 is known to play a role in hypoglycemia. Also AKT2 loss causes insulin resistance in humans [21,22]. AKT2 has an important role in cancer. Upregulation of Akt is often found in human cancer. Therefore, AKT might be a promising therapy target. In a study done by Xu et al, the different Akt-types and their role in tumor development were investigated. Earlier research showed already that the occurrence of tumors in Pten+/- mice could be reduced by deactivating Akt1. Akt2 deficiency has no significant effect on tumor types like prostate-, endometrial- and intestinal malignancies, but an effect was found on thyroid tumors. This effect compared with Akt1 is limited, because Akt2-deficiency caused high insulin levels in the blood, and therefore the activity of Akt1 may increase, diminishing the total Akt activity reduction [23].

BCAP

BCAP is the B-cell adapter protein for phosphorinositide 3-kinase (PI3K). While the effect of miR-150 on BCAP is not yet fully clear, BCAP is involved in the PI3K/Akt/mTOR signaling pathway by binding to the SH2 domain of PI3K. The PI3K/Akt/mTOR pathway is involved in cell survival and apoptosis. When PI3K is activated, Akt is activated which in turn activates the serine/threonine protein kinase mTOR. mTOR regulates all kinds of cellular events, including apoptosis. Aberrant gene expression of BCAP leads to decreased PI3K recruitment, inhibited PIP3 formation and fails to activate Akt upon B-cell receptor ligation [24].

In contrast, BCAP has a role in mice B-cell activity via NF-kB. Yamazaki et al showed that mice with a deletion for BCAP did not have altered PI3K activity, but diminished expression of the

NF-kB subunits; Rel A and c-Rel. Therefore, activation of NF-kB was affected. Also, survival defects were seen in BCAP deficient cells. This was caused by inadequate expression of c-Rel [25].

c-myb

C-myb or Myb is an oncogenic transcription factor, and consists of three domains; a transcription regulating C-terminus, a N-terminus that binds DNA and a central domain that transactivates transcription. C-myb is mainly expressed in immature hematopoietic stem cells, where it regulates cell proliferation. As the cell differentiates, c-myb levels become lower until very low levels in terminally differentiated HSCs [26,27].

MiR-150 and c-myb are involved in Burkitt lymphoma. Chen et al analyzed miR-150 levels in Epstein-Barr positive and Epstein-Barr negative Burkitt cell lines. They discovered that Burkitt lymphoma cell lines had severely decreased expression levels of miR-150. Restoring these levels lead to reduced proliferation of EBV-positive cell lines, and these cells could differentiate into a terminal B-cell differentiation state. However, no significant changes were seen in the EBV-negative cell lines. In both cell lines re-expression of miR-150 was associated with lower c-myb levels. Therefore, the researchers knocked-out the gene for c-myb in EBV-positive cells, and found that the effect on these cells was similar to the earlier mentioned effect of miR-150 re-expression [7].

In acute lymphoblastic leukemia (ALL), which is a lymphocytic cancer, c-myb is highly expressed. Purvaba et al demonstrated that lowering the c-myb levels in a leukemic pre-B ALL cell line diminished cell viability. C-myb inhibition reduced cell proliferation and cells were in the G0/G1 cell cycle phase. These cells were more sensitive to cytotoxins in vitro, and in a leukemia mouse model disease onset was delayed [28].

CXCR4

CXCR4, the C-X-C chemokine receptor type 4, is an α-chemokine receptor that specifically binds to its ligand stromal-cell derived factor 1 (SDF-1). SDF-1 is a chemotactic protein for lymphocyte homing, a B-cell progenitor growth factor and has been identified in many human cancers [50]. MiR-150 targets CXCR4, as proven by Tano et al using bonemarrow-derived mononuclear cells [30].

Guo et al investigated the involvement of CXCR4 in diffuse large B-cell lymphoma (DLBCL). They saw increased CXCR4 levels in DLBCL patients. CXCR4 expression is correlated with VEGF expression, and this may have clinical significance for treating DLBCL by combined targeting [31].

Beider et al published on the role of CXCR4 in NHL. They treated NHL cell lines and NHL-patients bone marrow samples with a CXCR4 antagonist (BKT140). It resulted in diminished lymphoma cell growth and cell death activation. When BKT140 was given together with the monoclonal antibody drug Rituximab, apoptosis of lymphoma cells was enhanced. These results indicate that is CXCR4 involved in NHL, and could possibly serve as a new drug target in the future [32].

NOTCH3

NOTCH3 is the gene for neurogenic locus notch homolog protein 3. The Notch intracellular pathway plays a role in development of neurons [33], and also has tumor-suppressor or oncogenic functions, which is different per cell context. Early B-cell differentiation is inhibited by the tumor suppressor function of Notch signaling as it induces apoptosis and decreases cell growth. This mechanism is not fully clear yet [34].

NOTCH3 is a target gene for miR-150, as shown in a study done by Ghisi et al. They discovered that NOTCH3 levels were decreased in T-cell ALL cell lines where miR-150 was upregulated [35]. Kuang et al showed that NOTCH3 is hypermethylated in acute lymphoblastic leukemia of B-cells, so it probably is a tumor-suppressor gene that is silenced [34].

P2X7

This gene codes for the P2X purinoceptor 7, which is part of the ATP purinoceptor family. It is mainly found in the nervous system, retina, macrophages, microglia and endometrium. This gene has a role in cell growth by ATP-mediated apoptosis. P2X7 is a target for miR-150, as proven in a study by Zhou et al. In epithelial cancer cell lines, upregulation of miR-150 lowered P2X7 mRNA levels by activating instability target sites at the 3'-UTR region of P2X7 mRNA [36].

Wiley et al showed involvement of the P2X7 receptor with chronic lymphocytic leukemia (CLL). In CLL patients, lymphocytes go into apoptosis when the purinoceptor P2X7 was activated. Decrease of receptor function decreased apoptosis, and B-cells accumulated in the blood. They identified a loss-of-function variation in the gene for P2X7 which might have a role in the development of CLL. This mutation was a three-fold more often found in CLL patients compared to healthy controls [37].

ZEB1

ZEB1 is the gene that codes for zinc finger E-box-binding homeobox 1, which is a transcription factor. MiR-150 targets ZEB1 according to a study in endothelial cells by Luo et al [38]. Increased ZEB1 expression caused unfavorable disease progression and outcome in B-cell lymphomas [39]. Ellis et al investigated the role of ZEB1 in EBV positive cell lines. EBV plays a role in the pathogenesis of HL. Earlier studies proposed that expression of the EBV protein BZLF1 in B-cell lymphoma cells can be suppressed by ZEB1. BZLF1 activation causes reactivation of EBV, and repression of this gene causes a latent infection. ZEB1 and ZEB2 can both bind to the BZLF1 promotor Zp, and Ellis et al concluded that the maintenance of an Epstein-Barr infection by ZEB1 or ZEB2 in B-cells is dependent on cell type [40].

miR-26 targets

CCNE1

CCNE1 is a gene that codes for a G1/S-specific cyclin-E1, which is a protein involved in the cell cycle control. It is a subunit for the protein CDK2, and G1/S-specific cyclin-E1 regulates its activity. When CDK2 is activated, the cell proceeds from the G1 phase to the S phase, in which DNA is being replicated [41]. CCNE1 overexpression leads to chromosome instability and has been found in several types of tumors [42].

MiR-26a directly targets CCNE, as shown in a study by Zhu et al. They found that miR-26 binds to CCNE1 via a specific binding site in the 3'-UTR region of CCNE1 mRNA. When miR-26 was inhibited in liver cancer cells, more cells expressed cyclin E1 [43].

CCNE1 is involved in diffuse large B-cell lymphoma (DLBCL). Some B-cell cancers show translocations in the genome, where oncogenes juxtaposed to IGH genes. Nagel et al discovered a translocation in DLBCL where CCNE1 is juxtaposed to the IGH locus. As a result, CCNE1 is controlled by the IGH regulatory elements, which can lead to cancer. CCNE1 is hereby called a translocation partner and was found in t(8;14q24;q32)-IGH-MYC-positive DLBCL. CCNE1 might be an oncogene for DLBCL [44].

E2F7

The transcription factor E2F7 belongs to the family of E2F proteins. These are cell cycle regulators which have an effect on several target genes which influence for example DNA replication. E2F proteins are G1/S transition coordinators [45].

E2F7 is identified by Boni et al as a target for miR-26a with the commonly used microRNA target prediction software *TargetScan* [46].

There are 8 E2F genes that code for a total of 9 E2F subtypes. These subtypes can be further divided into transcriptional repressors and activators. E2F1, 2 and 3 are activators, E2F4 and 5 are repressors. There function is mainly determined by their binding to pocket protein family proteins, whereby the E2F protein, and with it its repressor or activator function, is inhibited. E2F7 can be considered a transcription repressor, however it does not possess a sequence to bind pocket proteins. E2F7 functions in a way similar to E2F6, namely it interacts with transcription-repressing Polycomb family proteins. However, the exact way in which E2F7 acts is yet not fully understood [45].

Salvatori et al proved that E2F7 is a target gene for microRNA 26a. Expression of E2F7 was increased in immature lymphocytes in acute myeloid leukemia (AML) [47].

EZH2

EZH2 is a gene that codes for the Polycomb-family enzyme histone-lysine N-methyl-transferase. The abbreviation stands for Enhancer of Zeste Homolog 2. EZH2 is a gene silencer by adding methyl groups to histones, and plays an important role in embryonic development by influencing cell differentiation. It was not clear if EZH2 was regulated by microRNAs until Sander et al established the effect of Myc on microRNAs. In most tumors, Myc is activated and thus affects cell growth, because Myc acts as an apoptosis and proliferation inducing gene. Sander et al used a mouse lymphoma model where Myc expression could be controlled by a Tet-off technique. A new tumor-suppressor microRNA was discovered, that could decrease proliferation in cells that were Myc-dependent. This

microRNA was miR-26a, whose expression turned out to be aberrant in human Burkitt lymphoma cells. The cell cycle was affected by miR-26a via EZH2. These results demonstrate that Myc not only directly targets genes, but also indirectly by targeting microRNAs [48].
Zhao et al showed that the Myc-miRNA-EZH2 loop is a feedforward system, which is upregulated in B-cell lymphomas. They inhibited c-Myc by using the BET bromodomain inhibitor JQ1, and inhibited EZH2 by DZNep, a histone methylation inhibitor. As a result, Myc expression was decreased and miR-26a expression was increased. Thereby a reduced aggressive lymphoma B-cell growth and cloning ability was seen. Zhao et al confirmed this effect by miR-26a as a tumor-suppressor miRNA, and substantiated a future prospect for the Myc-miRNA-EZH2 loop as a novel therapy target in B-cell lymphomas [49].

PLAG1

PLAG1, which is the gene that codes for pleomorphic adenoma gene 1, is a PLAG-family zinc finger transcription factor. The exact mechanism in which PLAG-proteins play a role in tumor genesis is yet not fully understood. PLAG1 appears to be an oncogene, while the PLAG1-like gene has a tumor-suppressor function. PLAG1 was originally discovered in pleomorphic salivary gland adenomas in humans. Later research demonstrated involvement of PLAG1 also in hepatoblastomas and lipoblastomas and in leukemogenesis. PLAG1 and PLAG2 expression was elevated in 20% of the AML cases [50].

PTEN

The gene PTEN codes for a phosphate and tensin homolog which acts as a tumor-suppressor protein. It binds to the cell membrane and regulates the signaling of PI3K [51]. As earlier mentioned in the subsection for BCAP, PI3K is involved in the PI3K/Akt/mTOR pathway which plays an important role in apoptosis and cell survival. Mavrakis et al studied the role of miR-26a (among others) in T-cell acute lymphoblastic leukemia (T-ALL). They discovered that miR-26a suppresses certain tumor-suppressor genes in a murine T-cell ALL model. This gene set included IKAROS, BIM, PHF6, NF1, FBXW7 and PTEN [52].

CHFR

CHFR is the gene coding for a RING finger domain protein, and it functions as an E3 ubiquitin ligase. Ubiquitin ligases play a role in protein ubiquitination, a key event for DNA damage-response activation to prevent genomic instability. Ubiquitination causes protein degradation in the proteasome [53]. When a double-strand DNA break (DSB) occurs, the protein ubiquitination loop is activated, where CHFR acts as the first activated ubiquitinating protein. It binds ubiquitin to chromatin-associated PARP1, which is a PAR polymerase, after the CHFR gene product is transported to the DSB location by poly-ADP-ribose (PAR). So, CHFR is important for repairing DNA damage in an early phase, and its action is based on the interaction between ubiquitination and poly-ADP-ribosylation [54].
Yan-Lei et al found that miR-26b targets CHFR, using a colon cancer cell line. In this study, miR-26b overexpression lead to decreased cell invasion and proliferation and increased apoptosis in vitro [55].

Discussion/Conclusion

The most promising targets for miR-150 are c-myb and CXCR4. C-myb is important in the pathogenesis of Burkitt lymphoma, because miR-150 targets c-myb and causes terminal differentiation of lymphoma B-cells. C-myb levels are increased in acute lymphoblastic leukemia, and lowering of these levels induced several effects that are advantageous in this type of cancer [7,26,27,28]. CXCR4 is involved in B-cell growth, and CXCR4 levels are elevated in DLBCL. It is proven to enhance NHL cell growth and diminish apoptosis in NHL cells. CXCR4 is a potential therapeutic target in DLBCL and NHL [29,30,31,32].

Furthermore, NOTCH3, BCAP and AKT2 can also be potential targets in B-cell lymphomas. NOTCH3 may have an effect on B-cell growth and differentiation and the development of B-cell leukemia. In B-cell ALL NOTCH3 is hypermethylated and acts as a tumor-suppressor gene. When a tumor-suppressor gene product is inhibited by a miRNA as for example miR-150, this also has a silencing effect. Because miR-150 is proven to target NOTCH3 in T-cells, it is probably also a target in B-cells. In this manner, miR-150 can decrease NOTCH3 expression and play a role in the pathogenesis of B-cell leukemia's and lymphomas [33,34,35].
BCAP is involved in numerous B-cell specific events, for example the PI3K/Akt/mTOR pathway which has its influence in cell survival and apoptosis, and the survival of B-cells via expression of NF-kB subunits. If miR-150 inhibits the expression of BCAP, this could lead to cancer due to diminished cell apoptosis and anomalous cell survival [24,25].
Akt2 plays an important role in the development and survival of peripheral B-cells. Akt2 is often upregulated in cancer, but Akt2 deficiency only had a significant effect on thyroid tumors. Akt2 in combination with Akt1 ensures hematopoietic stem cell functioning [18,19,20,21,22,23].

Because BCAP and Akt are part of the same pathway, namely the PI3K/Akt/mTOR pathway (*figure 2*), more research should be done for investigating which part of the pathway is the most effective to target.

At last, P2X7 and ZEB1 may play a role in the pathogenesis of B-cell lymphomas. While little is known about the nature of P2X7, Wiley et al showed that a mutation in the P2X7 gene causes less receptor functioning, which in turn causes less apoptosis and proliferating B-cells, which can lead to leukemia. Therefore, P2X7 might be a tumor-suppressor gene in B-cells [36,37] ZEB1 may indicate involvement in HL by maintaining or reactivating EBV infection [38,39,40].

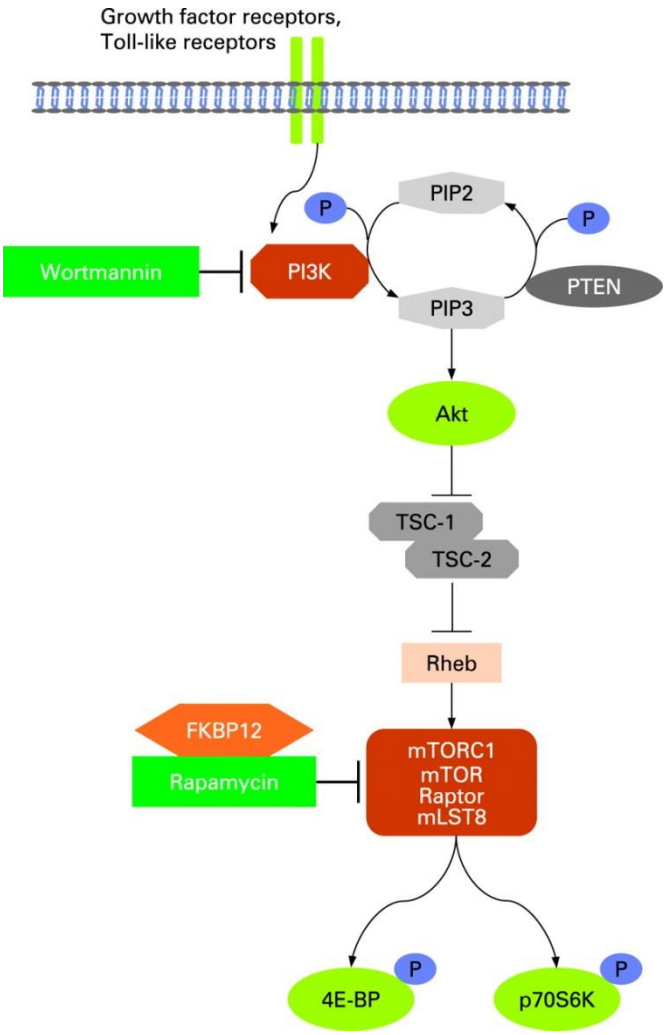

Figure 2: The PI3K/Akt/mTOR pathway. PTEN dephosphorylates PIP3, which produced PIP2. PIP2 can in turn be phosphorylated by PI3K. PIP3 activates Akt, and Akt inhibits the dimer TSC-1/TSC-2. By inhibiting this dimer, active Rheb can activate mTOR, a kinase involved in e.g. cell proliferation and apoptosis, which forms a complex with Raptor (mTORC1) [56].

For miR-26, the most important targets are EZH2 and CCNE1.
The oncogene Myc represses miR-26a resulting in an increased expression of EZH2. Increased expression of EZH2 leads to increased histone methylation, by which tumor-suppressor genes are silenced. In this manner, the Myc-miRNA-EZH2 loop might be a novel therapy target in B-cell lymphomas [48,49].
CCNE1 has oncogenic properties in DLBCL, and certain IGH-associated translocations can lead to an overexpression of CCNE1, causing B-cell lymphomas. CCNE1 is a direct target for miR-26a, which represses cyclin E1 expression [42,43,44].

CHFR and E2F7 are also promising targets for miR-26 in B-cell lymphomas. When CHFR expression is inhibited by miR-26b, this leads to an aberrant DNA damage-response. A possible consequence might be accumulation of mutations or deletions in the DNA, which in turn can result in tumor genesis. miR-26b overexpression caused diminished cell proliferation and more apoptotic activity in vitro, so CHFR is possibly an oncogene. As DNA

damage systems are comparable in most cell types, CHFR can be a target gene in B-cell lymphomas. More research will be needed to confirm the role of CHFR in B-cell cancer [53,54,55]. E2F7 may be an oncogene in myeloid leukemia. As cell cycle control systems work comparable in most types of dividing cells, E2F7 might be involved in the development of B-cell lymphomas [45,46,47].

Last, PLAG1 and PTEN potentially play a role in B-cell lymphoma pathogenesis. PLAG1 is proven to be involved in leukemia, where the expression of this gene is increased. As its nature is oncogenic, increased expression is responsible for tumor genesis. If PLAG1 expression is influenced by miR-26, this could be a promising future target in cancer treatment [50]. By inhibiting the tumor-suppressor function of PTEN with miR-26a, cancer can arise in T-cells. T- and B-cell both posess the PI3K/Akt/mTOR pathway, where PTEN regulates signaling of PI3K, so PTEN could possibly play a role in B-cell cancers [52].

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