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Review/Praca poglądowa

Crosstalk between BCR/ABL and RNAi

Interakcje pomiędzy BCR/ABL a RNAi

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ABSTRACT

Chronic myeloid leukemia (CML) is a malignant disease of progenitor myeloid cells caused by chromosomal translocation that results in the forming of diminutive Philadephia chromosome that harbors *BCR/ABL* fusion oncogene. The product of this oncogene, a tyrosine kinase, alters several important regulatory pathways related to cell growth and differentiation thus leading to cancer transformation. Major form of CML therapy is based on tyrosine kinase inhibitors, first of all imatinib (IM). Some patients develop resistance to IM in the course of treatment. In the process of leukemogenesis the activity of miRNAs – one of groups of RNAs involved in RNA interference (RNAi) – is altered. Signatures of altered miRNAs activity may serve as a prognostic factor in the development and therapy of several diseases. Moreover, other group of RNAs involved in RNAi – siRNA – might be valuable addition to array of specific therapeutics targeted the BCR/ABL kinase.

Introduction

Chronic myeloid leukemia (CML) is probably one of the most extensively studied human malignancies. It is associated with a consistent chromosomal abnormality – the Philadelphia (Ph) chromosome [1]. The Ph chromosome results from a reciprocal translocation t(9;22)(q34;q11) which involves the *ABL* proto-oncogene on chromosome 9 and *BCR* (for breakpoint cluster region) on chromosome 22 [2]. This translocation creates the *BCR/ABL* fusion gene which encodes the BCR/ABL protein, a tyrosine kinase protein that is believed to be the principal cause of the CML pathogenesis [1, 3]. Molecular signaling in CML affected by *BCR/ABL* is highly complex and activates multiple signal transduction pathways (see [1, 2] for more details). The activity of BCR/ABL

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leads to deregulated cellular proliferation, decreased adherence of leukemia cells to the bone marrow, and reduced apoptosis what results in the malignant expansion of pluripotent stem cells in bone marrow [3]. CML is subdivided into three phases: chronic phase (CP), which is the initial phase and disease, is usually diagnosed at this stage. Subsequently, it advances to an accelerated phase (AP). The last CML phase is called blast crisis (BC) [1]. Since CML is caused by BCR/ABL it was possible to design an effective targeted molecular therapy which selectively inhibits the aberrant BCR/ABL protein [4]. Imatinib mesylate (imatinib, IM, STI-571, Glivec®, Novartis Pharma) is a BCR/ABL tyrosine kinase inhibitor that is used for the treatment of CML. IM blocks the ATP-binding site and stabilizes the inactive form of BCR/ABL thus blocking downstream signal transduction pathways affected by this protein [4].

RNA interference (RNAi) is a process, in which small non-coding RNAs (ncRNAs) incorporated into multi-protein RNA-induced silencing complex (RISC) repress gene activity in a sequence-specific manner at transcriptional, post-transcriptional or translational level [5, 6]. The process of RNA-mediated gene silencing has an essential role in cell development, differentiation, proliferation, apoptosis, maintaining of chromatin structure, virus resistance and oncogenesis [7]. There are three types of small ncRNAs including micro RNA (miRNA), small interfering RNA (siRNA) and Piwi-interacting RNA (piRNA) [8]. In our review we will focus on siRNA and miRNAs as best understood groups of small ncRNAs, and their role in processes of leukemogenesis and interaction with signaling and regulatory pathways, in which BCR/ABL kinase is active.

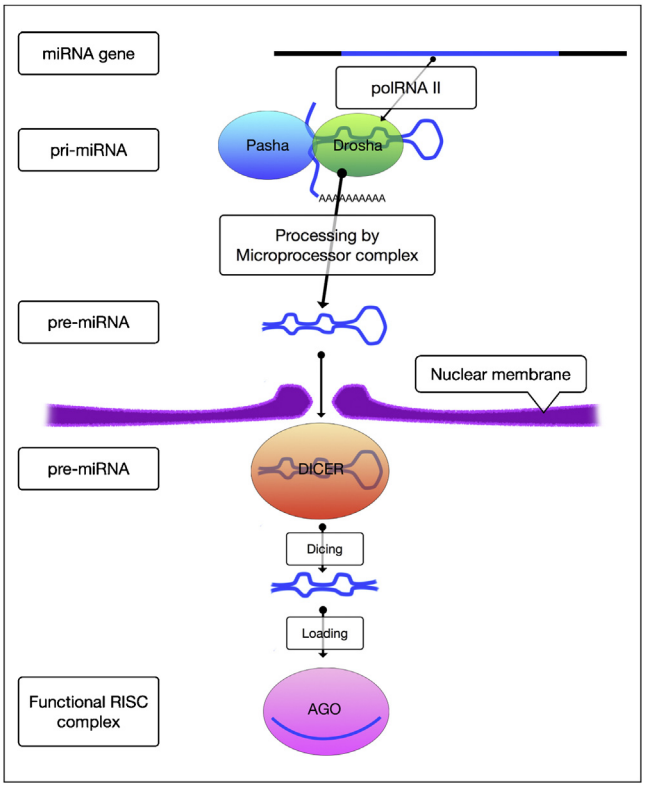

Fig. 1 – Biogenesis of miRNA. miRNAs are derived from endogenous precursors processed by the microprocessor complex, exported to cytoplasm, processed by DICER and loaded onto AGO protein forming mature RISC complexes

Biogenesis of miRNA and siRNA

Micro RNAs are small, approximately 22 nt long, RNAs that are derived from endogenous hairpin-shaped transcripts through the action of the Drosha and Dicer proteins, and subsequently bound by Ago-family proteins forming mature RISC complexes [9] (Fig. 1). Some of miRNA genes are located in introns of pre-mRNAs [10]. Other miRNA genes are clustered in the genome with an arrangement and expression pattern indicating polycistronic primary transcripts [11]. The primary transcripts of miRNA (pri-miRNAs) are usually generated by RNA polymerase II [12]. They are processed in the nucleus by Microprocessor complex, which contains Drosha and DGCR8, also known as Pasha, proteins [9, 13]. The pre-miRNAs are transported to the cytoplasm, where they undergo further processing by the Dicer protein, which cleaves dsRNA and releases a 22-nucleotide mature double-stranded miRNAs. One strand of the miRNA duplex is subsequently loaded onto the Ago protein, and then incorporated into fully functional RISC complex, while the other one, called passenger RNA, being degraded [9, 14, 15].

Small interfering RNAs are slightly shorter than miRNAs and are around 21 nt long. Originally siRNAs were believed to be exclusively derived from the exogenous RNA [6]. However, endogenous siRNA (endo-siRNA) were recently discovered in *Drosophila melanogaster*, mouse oocytes and some stem cells (reviewed in [9]). Similarly to miRNAs they form RISC complexes with Ago-family proteins [16], but they differ in their biogenesis. Their origin stems from long double-stranded RNAs are processed with Dicer but without Drosha activity (Fig. 2) [9]. Exogenous siRNAs may come from processed viral RNA [17], while endo-siRNA originates from precursors created through transcription of sense-antisense pairs of transposons, or from long, self-paired, double-stranded fragment of mRNAs [18].

RNA interference mediated by mi- and siRNAs

The core activity of mi- and siRNAs is similar: they allow for specific modulation of gene expression through guiding the activity of RISC complexes. The complex contains Ago, which is believed to be a core element of it with its ability to bind to single-stranded RNA (ssRNA) and dsRNA [19, 20]. After recognition of complementarity between targeted mRNA and mi- or siRNA template, RNase activity of RISC is induced, leading to degradation of mRNA [21].

The miRNAs can direct the RISC to downregulate gene expression by mRNA cleavage or translational repression. The pathway of silencing is determined by the kind of the target: the RISC will specify cleavage of the target mRNA if the mRNA has sufficient complementarity to the mRNA or it will repress productive translation if the mRNA does not

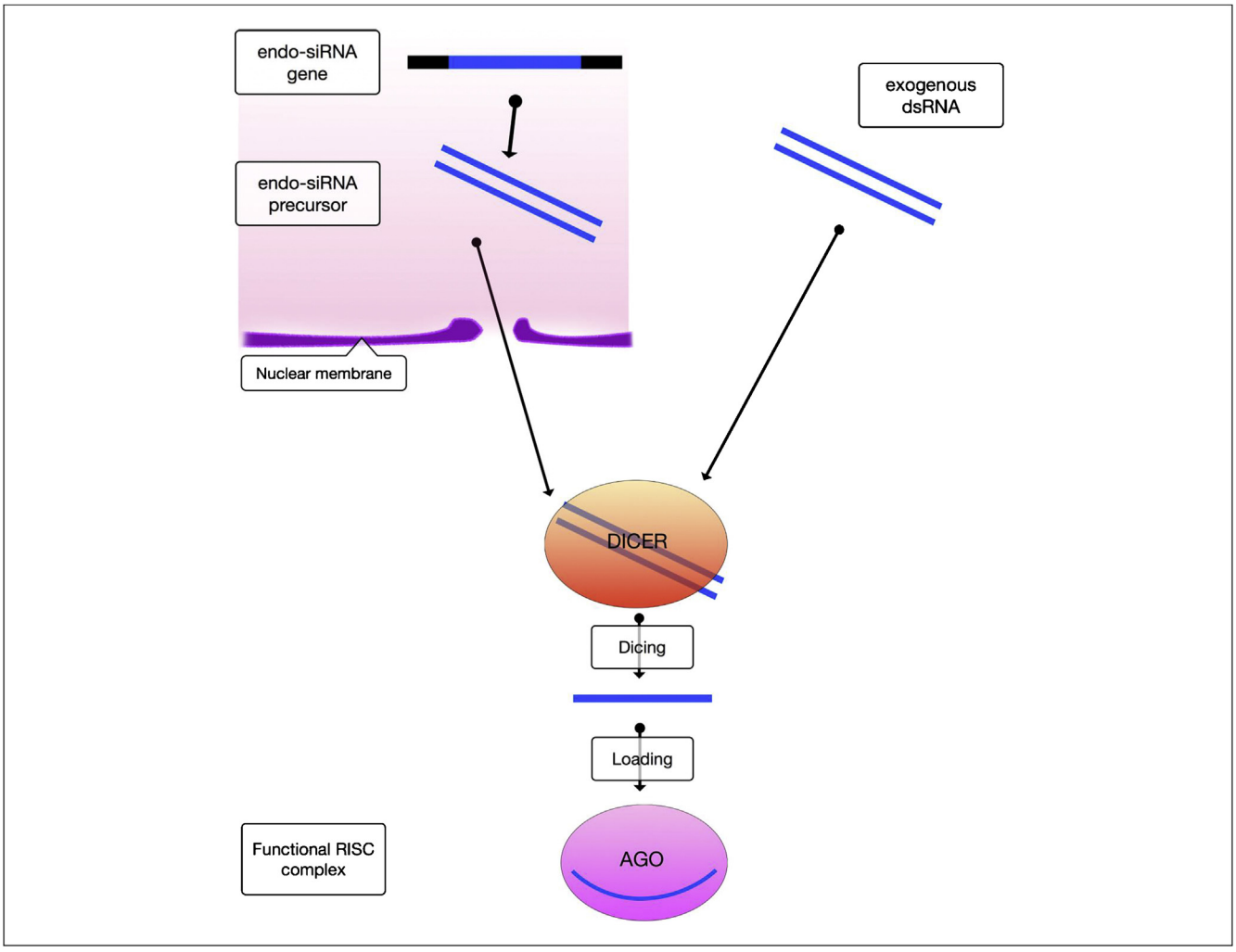

Fig. 2 – Biogenesis of siRNA. siRNAs can be derived both from endogenous precursors encoded in the genome as well as from exogenous double-stranded RNAs. Processing of endogenous precursors occurs without Drosha activity. After being exported into cytoplasm double stranded miRNA precursors are processed by DICER complex and loaded onto AGO protein

have sufficient complementarity to be cleaved. [19]. Recently, it was also shown that miRNAs could induce transcriptional silencing in human cells, directing Ago1, Polycomb group (PcG) and histone methyltransferase enhancer of zeste homolog1 (EZH2) recruitment to specific DNA sequences, altering histone methylation status [22]. Over the several past years it was shown that miRNAs are able to repress the expression of important cancer-related genes and expression of miRNA genes contribute to the pathogenesis of human malignances [23].

Small interfering RNAs can repress gene activity through transcriptional gene silencing in human cells [5]. This mechanism needs an mRNA transcribed with 5′ extended untranslated region overlapping with gene promoter, and probably involves some modification in methylation status [24, 25]. For example, after exposure to the siRNA, the targeted promoter exhibits higher levels of the silencing histone methyl marks H3 lysine-9 di-methylation (H3K9me2) and histone H3 lysine-27 tri-methylation (H3K27me3) [26]. However, the role that DNA methylation plays in this transcriptional silencing remains uncertain, as siRNA mediated transcriptional gene silencing was observed in the absence of increased DNA methylation at the targeted promoter, but these observations were not confirmed in other studies [27, 28]. In addition, the question how exactly siRNAs recognize and modulate transcriptional gene silencing through histone methylation remains unanswered [24]. Presumably, transcriptional gene silencing involves DNA methyltransferase 3a (DNMT3a), Ago1 as well as Ago2, HDAC-1 and EZH2 and/or G9a [24, 26, 29–31]. Despite the role of siRNA in transcriptional gene silencing, they were also reported to act in gene activation in human cells [29, 32].

Interaction between miRNAs and *BCR/ABL*

There is growing evidence that miRNAs may play an important role in BCR/ABL-dependent malignancies (see Table I and Fig. 3). For example, miR-203 controls the expression of the *ABL1* gene as well as the fusion oncogene

Table I – Micro RNAs (miRNAs) expressed in cells harboring BCR/ABL kinase

miRNA	Feature	Reference
miR-7	Down regulated in IM-responding CML patients	[44]
miR-10a	Down regulation in CML, not BCR/ABL-dependent. Target of this miRNA is a growth factor	[36]
polycistronic miR17-92 cluster	Down regulated by IM action and siRNA targeted at BCR/ABL.	[37]
miR-23a/26a/29a/29c	Down regulated in IM-responding CML patients	[44]
miR-30a	Inhibited by IM, acts as inhibitor of autophagy	[45]
miR-30b/30c	Down regulated in IM-responding CML patients	[44]
miR-31	BCR/ABL-dependent down regulation in CML cells	[39]
miR-96	BCR/ABL-dependent down regulation in CML cells	[36]
miR-100/126#	Down regulated in IM-responding CML patients	[44]
miR-130a/b	Down regulated after BCR/ABL knockdown, negatively regulates anti-growth factor CCN3	[40]
miR-134/141	Down regulated in IM-responding CML patients	[44]
miR-138	Decreases activity of BCR/ABL, sensitizes to IM action	[38]
miR-150	BCR/ABL-dependent down regulation in CML cells	[36, 41, 42]
miR-151	BCR/ABL-dependent down regulation in CML cells	[36]
miR-155	BCR/ABL-dependent down regulation in CML cells	[39]
miR-183/191/196b/199a	Down regulated in IM-responding CML patients	[44]
miR-203	Lost through deletion in some hematopoetic malignancies. This miRNA down regulates ABL1, including infusion with BCR.	[33]
miR-224/326/422b/520a	Down regulated in IM-responding CML patients	[44]
miR-564	BCR/ABL-dependent down regulation in CML cells	[39]

BCR/ABL. It was demonstrated that increased levels of miR-203 expression resulted in a decreased expression of BCR/ABL [33]. Moreover, an inhibition of *BCR/ABL* expression with miR-203 sensitized BCR/ABL-positive cells to IM action [34]. Another study suggests the presence of a reciprocal regulatory loop between *BCR/ABL* and miR-451 inducing the inhibition of miR-451 by theBCR/ABL activity. Because miR-451 is BCR/ABL negative regulator, this results in the increased expression of *BCR/ABL*. Moreover, this process may lead to synergy in the activity of miRNA and BCR/ABL tyrosine inhibitor IM [35]. Other interactions may include synergy in oncogenic activity between some miRNAs and BCR/ABL kinase itself. One such case is miR-125b that was found over-expressed up to 90 times in acute myeloid leukemia cells with chromosomal translocation t(2;11)(p21;q23), and could accelerate leukemia development even when co-expressed with BCR/ABL [36].

Recent findings show that specific miRNAs may play a fundamental role in the activation of oncogenic signal transduction pathways in CML cells. A decreased expression of miR-326 was reported to lead to unrestricted activity of Smo transducer, a key player in the Hedgehog pathway, in CML cells [37]. Similarly, miR-138 acts in mini circuitry with BCR/ABL and GATA1, an important factor in regulation of erythroid and megakaryocytic development. Overexpression of miR-138 decreases BCR/ABL activity. Conversely, IM treatment increases miR-138 activity, which in turn activates GATA1 [38].

As shown above miRNAs can directly modulate the expression of *BCR/ABL*. However, the tyrosine kinase activity of BCR/ABL was also shown to contribute to the expression of distinct miRNAs. Although changes in activity of many miRNAs are observed in CML cells, not all are BCR/ABL-dependent. It was shown that hsa-miR-10a, hsa-miR-150, and hsa-miR-151 were down-regulated, whereas hsa-miR-96 was up-regulated in CML $CD34^+$ cells. Among these miRNAs, only activity of hsa-miR-150 and hsa-miR-151 was related to BCR/ABL kinase function, which was assessed by inactivation of BCR/ABL by IM [39]. It was reported that miR-17-92 cluster was expressed in a BCR/ABL-dependent manner, and expression of pri-miR-17-92 was also mediated by myelocytomatosis oncogene (c-MYC). In addition, miR-17-92 miRNAs were overexpressed in CML cells in CP, but not in BC [40]. Another study showed that miR-31, miR-155, miR-564 and, miR-328 despite possessing distinct targets, were downregulated and this effect was dependent on BCR/ABL tyrosine kinase activity [41, 42]. Moreover, the downregulation of miR-328 was also dependent on *BCR/ABL* in dose-dependent manner [41]. BCR/ABL was reported to upregulate some miRNAs and in consequence inhibit negative growth regulators and promoting cell growth. As an example of these may be miR-130a and -130b. Expression of miR-130a and miR-130b was observed to be BCR/ABL-dependent, but the exact mechanism by which BCR/ABL affects the expression of these miRNAs remains unknown. However, apparently after stimulation of the activity of these miRNAs by BCR/ABL inhibition of CCN3 negative growth regulator occurs promoting cell proliferation. [43].

Micro RNAs and therapy of BCR/ABL dependent leukemias

The use of IM revolutionized treatment of CML [4]. Despite the clinical success obtained with IM, resistance to this drug is an emerging problem [3]. Since abnormal expression of miRNAs was described in leukemias, miRNAs may modulate response to IM treatment in CML patients. It was reported that lentivirus-mediated over-expression of polycistronic miRNAs of miR-17-92 cluster enhanced response to IM [40]. It was also shown that miR-150 and miR-146a were upregulated, while miR-142-3p and miR-199b-5p were downregulated after

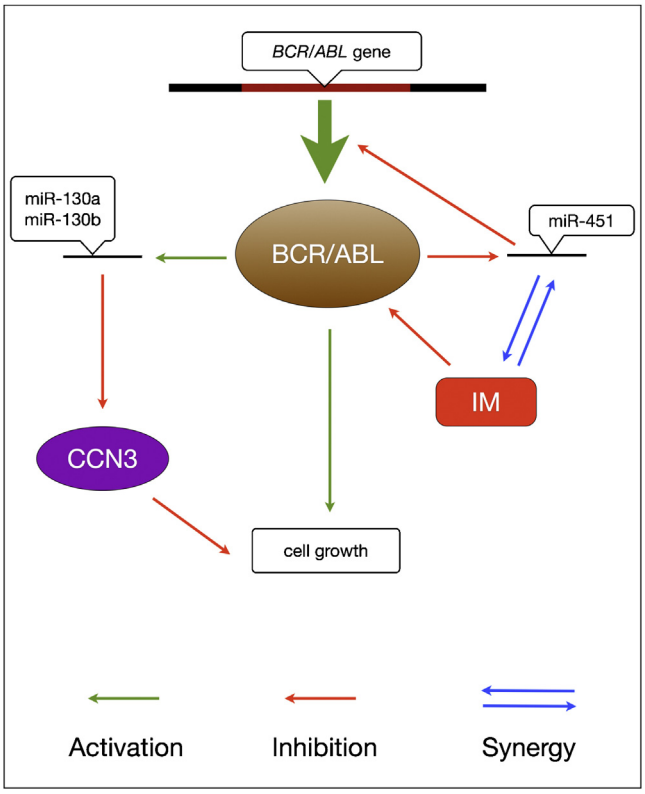

Fig. 3 – Interaction between BCR/ABL and miRNAs. miRNAs may inhibit activity of BCR/ABL ormay be inhibited by this kinase. This may lead to synergistic interactions between some miRNAs and kinase inhibitors such as imatinib (IM). Moreover, as it is in the case of miR-451 negative interactions between BCR/ABL and miRNAs may form reciprocal interactions resulting in mutual downregulation. BCR/ABL may also act with synergy on other miRNAs, as in the case of promoting cell growth with miR-130, a negative regulator of CCN3, which functions as a cell growth inhibitor

14 days of IM therapy [46]. Moreover, this study also found that aberrant expression level of these miRNAs was returning toward normal levels as seen in healthy donors, suggesting that miRNAs, by regulating distinct genes, may be involved in *BCR/ABL*-driven leukemogenesis [46]. Another study, which was aimed at identifying miRNAs and their related targets associated with CML pathogenesis, revealed that miR-150, miR-20a, miR-17, miR-19a, miR-103, miR-144, miR-155, miR-181a, miR-221 and miR-222 were deregulated in CML [47]. However, stating if they function in dependence or independence of BCR/ABL needs further investigation [47]. Micro RNAs may also play a role in the development of IM-resistance. It was shown that IM inhibits expression of miR-30a, which functions as inhibitor of autophagy through downregulation of Beclin 1 and ATG5 expression. Autophagy, in turn, is one of ways of avoiding cell death induced by IM treatment. Thus this miRNA may serve as a basis for mechanism of IM-resistance. It was shown that decreased levels of miR-30a correlated with increased levels of *ABL1* and *BCR/ABL* expression [48].

Since growing numbers of patients are suffering from resistance to IM it is necessary to find markers which will enable identification of patients who may develop such resistance. One kind of such markers might be specific signatures of altered miRNA activity. It was revealed that a group of 19 miRNAs may function as predictors for IM resistance in newly diagnosed CML patients [44]. Among them 18 were downregulated and only one was upregulated [44]. Another study showed that miR-451 was downregulated in IM-resistant patient as compared to IM-responding patients suggesting that miR-451 expression levels are correlated with response to IM therapy [49]. Nevertheless, further studies are required to confirm and validate the role of miRNAs as novel markers in predicting IM resistance.

In recent years a new area of potential drug development emerged, that aims synthetic siRNAs targeted at specific oncogenes as a means of suppressing their activity. Several attempts were performed for evaluation of validity of that method for treatment of BCR/ABL-induced leukemias. It was demonstrated that delivery of double-stranded RNA targeted at *BCR/ABL* oncogene resulted in RNAi that caused BCR/ABL inhibition and death of leukemic cells [50]. Activity of RNAi-based inhibitors appears to be even more specific than classical tyrosine inhibitors. snRNA designed for targeting specific variants of BCR/ABL – formed by b3a2 breakpoint – induced cell death only in cells carrying this specific variant of that gene [51]. Successes in inducing cell death in leukemic cells with siRNA targeted at BCR/ABL led to evaluation of combined treatments with classical tyrosine inhibitors and siRNAs. Delivery of IM to K-562 cells carrying BCR/ABL together with siRNA targeted at this oncogene resulted in 3-fold decrease in IM EC_{50} [52]. Synergism was also observed between action of siRNAs targeted at *BCR/ABL* and other oncogenes [53]. Synthetic siRNAs were also used for overcoming IM resistance in patient with CML recurring after IM therapy. These results suggest that siRNAs may be feasible in CML therapy [54]. Similar results were obtained *in vitro* for leukemic cells derived from patient with relapsed acute myeloid leukemia, who exhibited Ph+ chromosome *de novo* during clinical treatment. Delivery of siRNA resulted in cell-death induction in cells resistant to IM [55]. Moreover, it was demonstrated that siRNAs could overcome IM-resistance based both on BCR/ABL overexpression and its point mutations [56].

Future perspectives

Current findings indicate that many intertwined pathways involving BCR/ABL kinase activity and miRNA epigenetic modulation of gene expression are present in leukemic cells. Those mutual influences may be positive or negative in nature. Because recent advances in understanding the role of small non-coding RNAs provide a plenty of newly identified miRNAs and siRNAs this area of research may create a new opportunities for studying of mechanisms of cancer transformation. Moreover, this may lead to development of new therapies, or to reinforcement of treatment potential of currently used drugs. However, there are some obstacles. The sole task of annotating snRNAs involved in modulating

cellular metabolism is prone to error and many wrongly annotated snRNAs without any real functional meaning are present in databases [9]. This may make finding of new interactions between BCR/ABL and snRNAs complicated.

Nevertheless, there is no doubt that epigenetics in general, and snRNAs specifically, will be one of major area of interests in coming years. In case of many leukemias, especially CML, genetic components of cancer transformation are relatively well understood. Yet still many differences in disease development as well as therapy response are observed between different patients. It is apparent that mechanisms of epigenetic nature are one of major factor responsible for that differences. Thus unraveling the complex web of mutual interactions between genetic and epigenetic factors involved in determining the way in which normal cells undergo cancer transformation is essential for future development of diagnosing and treating of leukemias.

Authors' contributions/Wkład autorów

According to order.

Conflict of interest/Konflikt interesu

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Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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