Review

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Review

The Role of Non-Coding RNAs in Myelodysplastic Neoplasms

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Simple Summary: Myelodysplastic neoplasms (MDS) are a group of hematologic malignancies with an increased risk of transformation to acute myeloid leukemia. Non-coding RNAs are RNA molecules of variable size which do not translate into proteins but regulate gene expression during multiple cellular processes. These RNAs have been found deregulated in several cancers, including MDS. In this review we aim to summarize research findings on the biological role of different non-coding RNAs in MDS development and progression, with emphasis on those molecules that have exhibited prognostic or predictive value and could, hence, guide decision making in clinical practice.

Abstract: Myelodysplastic syndromes or neoplasms (MDS) are a heterogeneous group of myeloid clonal disorders characterized by peripheral blood cytopenias, blood and marrow cell dysplasia and an increased risk for evolution to acute myeloid leukemia (AML). Non-coding RNAs, especially microRNAs and long non-coding RNAs serve as regulators of normal and malignant hematopoiesis and have been implicated in carcinogenesis. This review will present a comprehensive summary of the biology and role of non-coding RNAs, including the less studied circRNA, siRNA, piRNA, and snoRNA as potential prognostic and/or predictive biomarkers or therapeutic targets in MDS.

Keywords: myelodysplastic syndromes; non-coding RNA; microRNA; lnc-RNA; circ-RNA; piwi-RNA; t-RNA; sno-RNA

1. Introduction

Myelodysplastic neoplasms (MDS) are a group of myeloid neoplasms characterized by clonal proliferation of hematopoietic stem cells (HSC) and genetic and epigenetic abnormalities leading to ineffective hematopoiesis, peripheral cytopenias and a propensity to the development of acute myeloid leukemia (AML) [1,2]. Diagnosis is based on the full blood count parameters, the bone marrow morphology and blast count and the presence of cytogenetic and molecular abnormalities, mainly mutations [2]. The most recent World Health Organization (WHO) classification, 5th edition recognizes two main groups: a. MDS with defining genetic abnormalities and b. MDS, morphologically defined [3]. Following correct diagnosis and accurate classification, prognosis estimation and risk stratification is crucial to tailor therapy. The revised International Prognostic Scoring System (IPSS-R) is widely used for the risk stratification of MDS patients considering the number and depth of cytopenias and cytogenetic abnormalities [4]; while most recently the molecular IPSS (IPSS-M) combined genomic aberrations with hematologic and cytogenetic abnormalities and provided an improved risk stratification of patients with MDS [5]. In general, low risk patients are managed either expectantly or with recombinant human erythropoietin or luspatercept [6], whereas high risk patients are being offered hypomethylating agents (HMAs) and/or allogeneic hematopoetic stem transplantation (AlloSCT), that remains the only curative modality. Despite all this progress,

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there is currently no widely accepted predictive model nor a serviceable biomarker of response that can offer a timely and valid estimation of the expected benefit from these available treatment options.

In terms of pathophysiology, genes regulating epigenetic modifications seem to be the most commonly mutated in patients with MDS [7]. Among epigenetic modifiers, non-coding RNA molecules, especially micro-RNAs (miRNAs) and long non-coding RNAs (lncRNAs) have recently attracted research interest. Until fairly recently, it was believed that the molecules that are important for the function of a cell are those described by the "Central Dogma" of biology, namely messenger RNAs and proteins. However, almost three decades ago the discovery of microRNAs (miRNAs) in plants [8] and animals [9,10] changed this perception. Subsequent research efforts have demonstrated that large parts of an organism's genome will be transcribed at one time point or another into RNA, but will not be translated into an amino acid sequence. These RNA transcripts have been referred to as non-coding RNAs (ncRNAs). There are many recognizable classes of ncRNAs, each having a distinct function. These include the above-mentioned miRNAs, transfer RNAs (tRNAs) [11], ribosomal RNAs (rRNAs) [12], piwi-interacting (piRNAs) [13], small nucleolar RNAs (snoRNAs) [14], long intergenic ncRNAs (lincRNAs) [15] etc. The full extent of distinct classes of ncRNAs that are encoded within the human genome is currently unknown, but are believed to be numerous.

Functionally ncRNAs are divided into two main categories: housekeeping ncRNAs, which are involved in generic cellular functions and regulatory ncRNAs, which primarily regulate gene expression in multiple levels. Hence, their regulatory role in cellular physiology, including normal hematopoiesis, is important, as is their participation in initiation and progression of neoplasia. Indeed, several studies have demonstrated the role of ncRNAs in solid and hematological malignancies, either from a pathophysiologic point of view or as prognostic biomarkers [16,17].

In this review we present a comprehensive summary of findings regarding the emerging role of various ncRNAs in MDS biology, patients' prognosis and response to therapy.

1.1. miRNAs in hematopoiesis and MDS pathogenesis

HSC are multipotent, self-renewing progenitors that generate all blood cells [18]. Many genetic and epigenetic regulatory mechanisms are involved in the homeostasis and differentiation of the normal hematopoietic system, including various miRNAs [19,20]. MiRNAs belong to a large family of naturally occurring, endogenous, single-stranded ~22-nucleotide-long noncoding RNAs that interact with their target RNA in a sequence-dependent manner, leading to their degradation or translational repression, rendering them significant regulators of posttranscriptional gene expression [21,22]. Each specific miRNA can target multiple mRNAs, while each mRNA may be targeted by several miRNAs. To date, more than 3700 human miRNAs have been identified [23]. MiRNAs are crucial regulators in normal and malignant hematopoiesis [24,25]. Chen et al were among the first researchers to identify three miRNAs namely miR-181, miR-223 and miR-142 that were specifically expressed in hematopoietic cells with a dynamic regulation during the early stages of hematopoiesis. MiRNAs implicated in the self-renewal of HSC in mouse models were miR-33 [26], miR-99 [27], and miR-125a [28]. In addition, at least 33 different miRNAs were found to be expressed in CD34+ HSC playing a role in many different cellular processes and blocking differentiation into mature cells [29]. On the other hand, oncogenic miRNAs (oncomiRs) negatively regulate the expression of tumor suppressor genes, whereas tumour suppressor miRNAs are negative regulators of oncogenes [30–32]. The first two oncomiRs that were found to be implicated in cancer were miR-15a and miR-16a in chronic lymphocytic leukemia with deletion 13q14 [33].

Abnormal expression of miRNAs has also been implicated in MDS in various differently prepared samples and using different techniques and statistical methods [34]. For instance, miR-150 plays an important role in the regulation of erythropoiesis and megakaryocytopoiesis and its deregulation has been linked to MDS development [35,36]. The main target of miR-150 is MYB. MYB or c-Myb is a regulatory transcription factor of the haematopoietic system and gastrointestinal tract preserving the balance between cell division, differentiation and survival [37]. Deregulation of MYB activity has been associated with several hematologic disorders [38]. In a zebrafish model, hyperactivity of MYB led to MDS [38]. In another study, investigators found that MYB was a direct

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target of miR-150-5p in MDS cells [36]. In these cells, MYB was increased and its knockdown significantly inhibited cellular proliferation and diminished the proliferation-promoting effect of the inhibitor miR-150-5p. [36].

Moreover, miR-145 affects megakaryocyte and erythroid differentiation by targeting Fli-1, a megakaryocyte and erythroid regulatory transcription factor [39]. The miR-17-92 is a polycistronic miR cluster, consisting of miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a, which is often overexpressed in certain malignancies. This cluster targets the tumor suppressor PTEN and the pro-apoptotic protein Bim by inhibiting their expression [40]. By targeting the pro-apoptotic protein Bim, miR-17-92 cluster ensures survival of haematopoietic stem and progenitor cells, playing a crucial role in hematopoiesis [41]. Moreover, two other members of miR-17-92 cluster namely miR-17-5p and miR-20a that down-regulate E2F1 were found to be under-expressed in high-risk MDS patients constituting favorable prognostic markers associated with increased overall survival (OS) [42]. In the same study, investigators found that let-7a that down-regulates KRAS was under-expressed in patients with intermediate- or high-risk karyotype [42].

MiR-143/145 differentially modulate HSC and progenitor activity via suppression of canonical tumor growth factor (TGF)-β signaling and loss of expression of these miRNAs can lead to MDS development [43]. The interaction between HSC, progenitor cells and bone marrow stromal cells is modulated by CXCL12, a chemokine that is regulated by several different miRNAs [44]. Among them, miR-23a may have a critical role in MDS pathogenesis by regulating the functional properties of the hematopoietic niche [44]. MiR-10a and miR-10b were found to be overexpressed in CD34+ cells leading to the up-regulation of TWIST-1 leading to reduced sensitivity to apoptosis [45]. High levels of miR-21 expression in MDS have been reported to mediate hematopoietic suppression by over-activation of TGF-β signaling [46]. Several tumor suppressor miRNAs, including several let-7 family members, miR-423, and miR-103a were down-regulated in MDS samples with *SF3B1*, *SRSF2*, and *U2AF1* (*U2AF35*) mutations compared to wild type samples, indicating their role in MDS development [47]. In another study, it was shown that up-regulation of miR-125a in MDS CD34+ cells modulates NF-kB activation and inhibits erythroid differentiation, rendering miR-125a a potential therapeutic target [48]. This miRNA is supposed to control the size of the stem cells pool by modulating their apoptosis [28]. Finally, mutations in the epigenetic modifier *TET2* are involved in the development of myeloid malignancies [49] and is a target of miR-22, a miRNA that is up-regulated in MDS [50].

1.2. miRNA deregulation and cytogenetic abnormalities in MDS

Cytogenetic abnormalities are very common in both de novo and secondary MDS [7,51]. The deregulation of several miRNAs has been associated with specific cytogenetic abnormalities. In particular, miR-595 is localized in chromosome 7 and targets RPL27A. It has been found to be down-regulated in MDS patients with monosomy 7/isolated loss of 7q (7q-) leading to RPL27A down-regulation, p53 activation, apoptosis, and inhibition of proliferation [52]. MiR-205-5p is encoded by chromosome 1 and its up-regulation contributes to MDS development via PTEN suppression causing MDS cells proliferation [53]. Another miRNA that is located in chromosome 1 and its deregulation is involved in MDS pathogenesis is miR-194-5p, in MDS patients with trisomy 1 [54].

MDS with isolated del(5q) is characterized by anemia and thrombocytosis [39]. Investigators examined the role of miRNAs that are in this region of chromosome 5 and found that the knockdown of miR-145 and miR-146a resulted in thrombocytosis, mild neutropenia and megakaryocytic dysplasia [55]. As discussed above, miR-145 affects megakaryocyte and erythroid differentiation by targeting Fli-1, a megakaryocyte and erythroid regulatory transcription factor [39]. Patients with del(5q) MDS were found to have decreased expression of miR-145 and increased expression of Fli-1 [39]. Overexpression of miR-150 was also associated with del(5q) MDS contributing to thrombocytosis [56,57]. In another study, investigators identified 21 different miRNAs that had aberrant expression in del(5q) MDS patients including miR-34a (up-regulated), miR-378 and miR-146a (downregulated) [58].

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The t(2;11)(p21;q23) translocation has been associated with the overexpression of miR-125b, while trisomy 8 was correlated to miR-383 overexpression in MDS patients [59,60]. Kang et al, reported increased expression of miR-661, which is encoded by chromosome 8, in MDS patients via p53 activation [61]. Another miRNA located on the same chromosome, miR-597, induces apoptosis through down-regulation of FOS Like 2 (FOSL2) and was found to be overexpressed in patients with MDS compared to controls, indicating a possible role in MDS pathogenesis [62].

1.3. miRNAs as potential prognostic biomarkers in MDS

Many studies have investigated the potential prognostic value of several miRNAs in MDS (Table 1). In one of the first relevant studies, Sokol et al identified a miRNA signature of ten different miRNAs that was associated with the IPSS risk category and noted the prognostic significance of miR-181 family members in lower-risk MDS patients [63]. Recently, miR-181a-2-3p was shown to be an independent prognostic biomarker in MDS patients in terms of OS [64]. Overexpression of miR-125a was associated with shorter OS and it was found to inhibit erythroid differentiation in leukemia and MDS cell lines [48]. Additionally, miR-22 targets the TET2 tumor suppressor gene and its overexpression, both in plasma and in CD34+ progenitor cells, was associated with high-risk subtypes of MDS and decreased OS [50,65].

Deregulation of many miRNAs is associated with the progression of MDS and transformation to AML, which is a synonym for poor prognosis [66]. Specifically, the up-regulation of miR-196b-5p and down-regulation of miR-29b have been associated with increased risk of AML transformation [67,68]. Similarly, Kirimura et al found that the down-regulation of miR-29b in MDS bone marrow cells could play a role in the transformation to AML via the up-regulation of the anti-apoptotic protein myeloid cell leukaemia 1 (MCL-1) [68]. Expression levels of miR-422a and miR-617 have also been correlated with disease progression in MDS patients [69]. All members of miR-320 family (miR-320a, miR-320b, miR-320c, miR-320d, and miR-320e) have been reported to be overexpressed in MDS patients and in a series of 82 patients high levels of miR-320c and miR-320d were related to shorter OS, while the up-regulation of miR-320d was found to be an independent prognostic factor [70].

Furthermore, low levels of miR-194-5p and miR-661 expression have been associated with decreased OS in MDS patients [54,61]. In a cohort of 41 patients, miR-125b-5p, miR-155-5p and miR-181a-2-3p bone marrow transcript levels were found elevated in higher-risk patients [71] and, likewise, low expression levels of miR-21, miR-126 and miR-146b-5p have been detected in lower-risk compared to higher-risk MDS patients. Among them, elevated levels of miR-126 and miR-155 were associated with shorter OS and leukemia-free survival (LFS), while elevated levels of miR-124a tended to be associated with reduced survival rates [72].

Peripheral blood circulating-microRNA profiles have also emerged as useful diagnostic and prognostic biomarkers for MDS patients [73,74]. In particular, the expression levels of miR-27a-3p, miR-150-5p, miR-199a-5p, miR-223-3p and miR-451a were found reduced in higher-risk MDS patients and the decreased levels of miR-451a and miR-223-3p were independently associated with a lower progression-free survival (PFS) and OS, respectively [74]. Zuo et al, identified and validated a 7-microRNA plasma signature (let-7a, miR-144, miR-16, miR-25, miR-451, miR-651, and miR-655) as an independent predictor of survival in patients with MDS and normal karyotype [73]. Finally, Hrustincova et al incorporated the expression levels of miR-1237-3p and miR-548av-5p from extracellular vesicles in a prognostic risk score, based on data from 42 patients, as they exhibited the strongest prognostic value in terms of OS [75].

1.4. miRNAs as potential predictive biomarkers in MDS

Several studies have attempted to investigate the potential role of miRNAs as predictors of treatment response in patients with MDS (Table 2). Lenalidomide is an immunomodulatory agent that selectively suppresses the del(5q) clone and is used for the treatment of lower-risk MDS with del(5q) [6,76]. Down-regulation of miR-145 and miR-146, which are encoded by chromosome 5, plays a crucial role in the development of del(5q) MDS via increased expression of their target genes, TIRAP

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and TRAF6, respectively, leading to inappropriate activation of innate immune signaling [77]. In a phase II single arm study in lower-risk MDS patients with anemia, miR-145 and miR-146 were decreased at baseline in patients with del(5q) MDS and significantly up-regulated after 3 and 6 months of treatment with lenalidomide [78]. In another study investigators found that the expression levels of miR-143 and miR-145 were increased during treatment and lenalidomide selectively abrogated progenitor activity in cells depleted of miR-143 and miR-145 rendering them potential predictive biomarkers [79]. Similarly, expression of miRNAs clustering to the 14q32 region and pro-apoptotic miR-34a and miR-34a* was reduced following lenalidomide administration [80,81].

HMAs are nucleoside analogs used for the treatment of higher-risk MDS and the prediction of HMA responsiveness is deemed of critical importance [6]. In a study of 27 patients with higher-risk MDS or AML with myelodysplasia-related changes, the investigators examined the predictive value of specific miRNAs, expressed in bone marrow $CD34^+$ cells before and after the administration of azacytidine [82]. Up-regulation of miR-17-3p and down-regulation of miR-100-5p and miR-133b at baseline was associated with higher overall response rate (ORR) while increased levels of miR-100-5p were associated with shorter OS [82]. Furthermore, deregulation of 30 different miRNAs was observed after the administration of azacytidine in responders. Specifically, miR-10b-5p, miR-15a-5p/b-5p, miR-24-3p, and miR-148b-3p were down-regulated in responders after azacytidine treatment while they remained at the same levels in non-responders, thus rendering them potential predictive biomarkers [82]. Mongiorgi et al recently showed that miR-192-5p specifically targets and inhibits BCL2 and its overexpression in bone marrow mononuclear cells was correlated to increased OS and leukemia-free survival (LFS) in MDS patients responding to combination of azacytidine and lenalidomide [83]. In a recent study, investigators evaluated the predictive value of miR-22 in MDS patients after HMAs, however, they concluded that it is not an appropriate predictive biomarker [84].

Regarding circulating miRNAs in the peripheral blood, miR-21 is a potential predictive biomarker for response to HMA therapy in patients with MDS, since the baseline level of serum miR-21 was found significantly decreased in responders compared to non-responders [85]. MiR-124 is involved in MDS pathogenesis via targeting the cyclin-dependent kinase 6 (CDK6) gene and was up-regulated in response to epigenetic treatments, azacytidine or the histone deacetylase inhibitor panobinostat, in peripheral blood and bone marrow mononuclear cells [86,87]. In another study of 42 MDS patients, investigators identified five circulating miRNAs, namely miR-423-5p, miR-126-3p, miR-151a-3p, miR-125a-5p and miR-199a-3p whose combined expression levels in plasma could predict response to azacytidine therapy [75]. Finally, beyond HMAs, in a recent study investigators found that overexpression of exosomal miR-92a (member of miR17-92 cluster) in plasma promoted cytarabine resistance in MDS/AML by activating Wnt/β-catenin signaling pathway, rendering miR-92a both a potential predictive biomarker and a therapeutic target for patients with MDS [88].

2. Circular RNAs

Circular RNAs (cRNAs) are closed-loop single-stranded RNA molecules that have proved to be important regulators of gene expression at multiple levels, although initially considered as transcriptional by-products [89]. CircRNAs function as miRNA sponges or traps that indirectly modulate transcription, interact with intracellular proteins, regulate splicing and travel in extracellular vehicles called exosomes, enabling intercellular communication [90,91]. In the context of normal hematopoiesis, circRNAs show cell-type specificity and are considered as regulators of blood cells differentiation and maturation [92].

The hypothesis of circRNAs interfering with MDS pathophysiology was supported by the observation that exogenous inhibition of the spliceosome components, commonly affected by MDS mutated genes, can cause an imbalance between circular and linear RNA concentrations within affected cells, towards overexpression of the circular molecules [93,94]. Wedge et al recently reported that specific cancer-associated circRNAs, such as circZNF609 and circCSNK1G3, are up-regulated in MDS patients with U2AF1 mutations compared to unmutated controls [95]. Additionally, global circRNA expression has been found to be up-regulated in the continuum from normal hematopoiesis to clonal cytopenias of undetermined significance (CCUS) and further to MDS. Even among MDS

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patients, a higher risk group was correlated with increased global circRNA expression and a "Myeloid Circ Score" was developed based on 14 specific circRNAs with potential prognostic value, to stratify patients in terms of risk and disease outcomes [96]. Another research group detected 145 circRNAs to be up-regulated and 224 down-regulated in MDS patients compared to healthy controls. Researchers also suggested that of all these circRNAs, hsa_circRNA_100352, hsa_circRNA_104056 and hsa_circRNA_102817 could be used as MDS prognostic biomarkers, since their increased expression was significantly correlated with poorer OS. Bioinformatics network analysis indicated that these 3 circRNAs are probably associated with multiple cancer-related molecular pathways, including Wnt/β-catenin and PTEN/Akt/mTOR [97,98]. Additionally, the circ-ANAPC7 might be another promising circRNA biomarker as its expression in MDS patients has recently been shown to be up-regulated along with increasing risk group, by IPSS-R [99]. Finally, several circRNAs are differentially expressed between responders and non-responders to azacytidine, although only one circRNA, hsa_circ_0006595, is considered a potential predictor for response to azacytidine treatment [100]. Whether circRNAs will soon be used in clinical practice for diagnostic, prognostic or predictive purposes remains to be answered, given the need for bone marrow sampling, since the reproducibility of findings in peripheral blood has not been proven yet.

3. Long non-coding RNAs

Long non-coding RNAs (lncRNAs) are a functionally heterogeneous class of thousands of RNA molecules, each containing more than 200 nucleotides, which are not translated into functional proteins. They are produced through DNA transcription, either from genes or intergenic regions (lincRNAs), and have multiple functions including epigenetic chromatin modifications, regulation of neighboring and distant gene transcription, RNA splicing, response to DNA damage, sponging miRNAs and participation in signaling pathways [101,102]. In the field of normal hematopoiesis, from murine models to humans, it is known that lncRNAs are expressed in a stage-specific and lineage-specific pattern from hematopoietic stem cells (HSCs) to mature blood cells in a way that they enable self-renewal of HSCs, such as H19 lncRNA, but also determine lineage commitment of progenitor cells e.g. example EGOT lncRNA for eosinophil maturation, in co-operation with transcription factors [103–109].

After the identification of MEG3 (Maternally Expressed Gene 3) lncRNA hypermethylation in many MDS patients, evidence that linked aberrant expression of lncRNAs with multiple hematological malignancies, including MDS, began to accumulate. The aforementioned lncRNA is considered a tumor suppressor, whose down-regulation has been associated with poor OS in several solid neoplasms [110–115]. While the scientific interest on lncRNAs was increasing, researchers identified a positive feedback loop in MDS cells, involving lncRNA bc200-miR-150-5P-MYB, which resulted in sustained cell proliferation. On the other hand, the inhibition of this axis seemed to suppress neoplastic growth of bone marrow MDS cells, implying potential therapeutic targeting of BC200 [36]. Additionally, increased expression of the lncRNAs KCNQ10T1 and HOXB-AS3 has been associated with adverse prognosis in MDS, with the latter pertaining to only lower-risk patients [116,117]. Further basic research and computational analysis revealed a vast number of differentially expressed lncRNAs between MDS patients and healthy controls, with functions including cell adhesion, differentiation and chromatin modifications, mainly through functional interaction with DNA methylation processes [118,119]. Of these lncRNAs, H19 emerged as one of the most promising prognostic biomarkers in MDS patients. Interestingly, a set of 14 lncRNAs were considered as reliable predictive biomarkers to inform about potential patients' response to azacytidine [100,119,120]. To improve MDS risk stratification by connecting laboratory research with clinical practice, Yao et al developed a scoring system based on the expression of 4 lncRNAs with the highest prognostic potential (TC07000551.hg.1, TC08000489.hg.1, TC02004770.hg.1, TC03000701.hg.1). A higher lncRNA score was significantly associated with higher bone marrow blast percentage, higher-risk subtypes by WHO, complex karyotypes, high-risk gene mutations (RUNX1, ASXL1, TP53, SRSF2, and ZRSR2) as well as shorter OS [121]. Consequently lncRNAs, overall, appear to be promising prognostic and predictive biomarkers for patients with MDS,

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probably awaiting their future incorporation in widely accepted prognostic scoring systems to assist in decision making.

4. PIWI-interacting RNAs

PIWI-interacting RNAs (piRNAs), the third major class of small non-coding RNAs, are single-strand 26-31 nucleotide-long RNA molecules. Their main function, apart from epigenetic modifications, was first believed to be the maintenance of germ line DNA integrity through the guidance of PIWI proteins (P-element Induced Wimpy testis proteins) towards silencing transposons, which are mobile parasitic genomic elements [122,123]. Further research indicated that aberrant expression of specific piRNAs is associated with the development and progression of several solid and hematological cancers, as these molecules are considered to play a role in continuous proliferative signaling, resistance to apoptosis, tumor invasion and angiogenesis of malignant tissues, and even resistance to antineoplastic treatment [124,125]. On the other hand, though, there has been increasing evidence that aberrant expression of piRNA-pathway genes alone, might not be adequate for the formation of piRNA-PIWI silencing complexes with biological impact on tumorigenesis [126].

Although the importance of piRNAs in other hematological malignancies such as multiple myeloma and classic Hodgkin lymphoma has gathered research interest, data in MDS have been scarce. The first study of piRNAs in bone marrow cells of patients with MDS demonstrated a higher expression (9%) of piRNAs in patients with MDS with refractory anemia (low-risk MDS) compared to patients with MDS with refractory anemia and excess of blasts-2 (high-risk MDS) and healthy controls (2% and 1% respectively), assuming a DNA-protective role of piRNAs in lower risk MDS [127,128]. Small non-coding RNA analysis from plasma and extracellular vesicles also showed an up-regulation of specific piRNAs (hsa_piR_019914/gb/DQ597347 and hsa_piR_020450/gb/DQ598104) in MDS patients compared to controls. Two other piRNAs, hsa_piR_000805/gb/DQ571003 and hsa_piR_019420/gb/DQ596670, were differentially expressed between patients with low- and increased blasts-MDS. The latter piRNA was also shown to be correlated with OS with a protective role, but no piRNAs were found to have predictive value about patients' response to azacytidine [75]. The biologic interpretation of these findings as well as the extent to which they can be incorporated in everyday clinical practice, remain to be further elucidated.

5. Ribosomal RNAs

Ribosomal RNAs (rRNAs) are indispensable components of ribosomes, the cell's protein-producing machinery. Ribosomes in human cells are comprised of 4 rRNAs (28S, 5S, 5.8S and 18S) and approximately 80 proteins which are assembled into a small (40S) and a large (60S) subunit through a multilevel process which mainly takes place in the nucleolus [129–132].

The dependence of highly proliferative cells, such as the hematopoietic cells, upon protein synthesis has given the rationale for extensive research on the role of aberrant ribosomal synthesis in several human diseases including hematopoietic neoplasms. In this context, mutation of Nol9 a ribosomal-biogenesis protein required for 28S rRNA processing, was found to affect hematopoiesis in animal models by reducing proliferation of hematopoietic stem and progenitor cells [133]. Moreover, *DNAJC21* mutations were associated with bone marrow failure with increased tendency to malignancy, attributed to impaired biosynthesis and cytoplasmic maturation of the 60S ribosomal subunit [134]. Similarly, a whole group of diseases termed as "ribosomopathies" arising from congenital or acquired genetic abnormalities that lead to impaired ribosomal construction and function have been associated with bone marrow failure and increased risk of hematological malignancies, such as Shwachman-Diamond syndrome or congenital dyskeratosis [135,136]. Further data supporting the correlation of rRNA deregulation with myeloid neoplasms indicate the potential role of *DDX41*, whose germ-line mutations predispose to myeloid malignancies, in the processing of pre-ribosomal rRNA to mature rRNA [137]. *U2AF1* somatic mutations, commonly detected in MDS patients, apart from altered splicing, are also believed to cause aberrant ribosomal synthesis, mediated by NPM1, which is considered a ribosomal biogenesis factor [138]. Finally, bone marrow CD34+ cells from patients with MDS show decreased rRNA expression compared to controls, which

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is probably driven by increased promoters' methylation of DNA loci coding for these rRNAs (rDNA). Interestingly, this hypermethylation can be reversed by hypomethylating agents such as azacytidine and it is therefore implied that methylation status of rDNA could be used as a predictor of response to treatment with such agents, instead of genome-wide methylation status, although this hypothesis has yet to be proved [139,140]. Researchers have recently focused on the study of short RNA fragments cleaved from rRNA, called rRNA-derived fragments (rRFs), as they are believed to regulate cellular functions and show sequence overlap with miRNAs and piRNAs [141,142].

6. Small nuclear and small nucleolar RNAs

Small nucleolar (snoRNAs) RNAs are 60-300 nucleotide long RNA molecules derived from coding and non-coding genes and they are in the nucleolus of eukaryotic cells. Their main function is processing of other RNA molecules such as ribosomal RNAs and small nuclear RNAs (snRNAs) via pseudouridylation and 2'-O-methylation. In turn, snRNAs are vital components of the spliceosome, the cell machinery that catalyzes pre-mRNA splicing through intron excision and joining of exons, to form functional mature mRNAs [143]. Additionally, snoRNAs are involved in regulation of alternate splicing and also act like miRNAs to selectively suppress gene expression [144,145].

In HSC, snoRNAs expression is supposed to be cell-typic specific and play an important role in cell homeostasis, self-renewal and stress response, while their aberrant expression has been linked to several hematological malignancies, MDS included [146–148]. For example, *DDX41* regulates snoRNA processing, ribosomal biogenesis and protein synthesis in hematopoietic stem and progenitor cells (HSPCs) and its germline mutation is known to confer predisposition to clonal myeloid disorders. More specifically, mono-allelic DDX41 mutations, as in germline predisposition, increase the risk for age-dependent hematopoietic defects and confer competitive proliferation advantage to HSPCs. On the other hand, biallelic DDX41 mutations deregulate snoRNA processing causing intracellular accumulation of inappropriately processed snoRNAs, impair protein synthesis and finally result in cell cycle arrest. Most of the affected snoRNAs belong to the SNORA family and are typically involved in RNA pseudouridylation [149]. Similarly, snoRNA U33, which is a mediator of cell metabolic stress, has been found to be up-regulated in MDS patients. More importantly, this snoRNA was shown to be significantly associated with OS of patients, albeit no relevant biologic explanation is provided [75,150].

7. Transfer RNAs and their derived fragments

Transfer RNAs (tRNAs), with their unique stem-loop pattern formed by internal base pairing, are essentially the carriers of amino acids to the growing polypeptide chain at the ribosome, during translation, but are also believed to have additional functions such as modulation of gene expression and control of cell death. Cleavage of pre- or mature tRNAs produces the tRNA-derived fragments (tRFs) or tRNA-derived small RNAs (tsRNAs) or tRNA-derived RNAs (tDRs) which are involved in multiple biological processes including translational regulation with gene silencing, intercellular communication, cellular stress response and immune cells activation, rather than being useless by-products of tRNA degradation [151–154].

Specific tRNAs (chr2.tRNA27-GlyCCC, chr.18Trna4-LysCTT) as well as overall tRNA to rRNA ratio have been found up-regulated in marrow cells from MDS patients compared to controls and it was assumed that this increase might contribute to decreased programmed cell death and increased leukemic transformation, since tRNAs are known to inhibit cytochrome c activated apoptosis [75,106,127,155]. On the other hand, the commonly seen in MDS $SF3B1^{K700E}$ mutation seems to reduce translational machinery components, primarily tRNA synthetases [156]. Another somatic mutation in the mitochondrial tRNA repertoire, $MtRNA^{Leu(UUR)}$, in bone marrow cells is suspected to contribute to ineffective hematopoiesis [157].

When it comes to tRFs, some of them show enhanced expression while others are down-regulated in MDS cells. Interestingly, the combined expression of 4 tRFs (chr6.tRNA157.ValCAC, chr11.tRNA17.ValTAC, chrM.tRNA12.TS1 and chrX.tRNA4.ValTAC) in treatment naïve patients was

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found to have predictive value as of the likelihood of response to treatment and this is also the case with one mitochondrial tRNA (MT-TSI), while it is suggested that tDR-Asp family members could be used as predictors for progression to AML [158,159].

Even post-transcriptional modifications of these non-coding RNAs are suspected to interfere with MDS pathophysiology. Pseudouridylation by PUS enzymes, for instance, of mini tRFs containing 5- terminal oligoguanine, was found to regulate the renewal of human embryonic stem cells and also promote the differentiation of impaired HSPCs in MDS, indicating a potential therapeutic approach [160–162].

8. Short interfering RNAs

Short or small interfering RNAs (siRNAs) are 21-25 nucleotide long RNA molecules with crucial role in gene silencing, primarily through mRNA degradation and by promoting heterochromatin formation. These interfering RNAs are produced via the procession of long double stranded RNAs or short hairpin RNAs by the DICER endoribonuclease. The produced double stranded siRNA is then packed with proteins to form the RNA- induced silencing complex (RISC). One strand of the RNA is discarded and the remaining strand guides the RISC towards the targeted mRNA, which is recognized with perfect complementarity with the siRNA and is finally cleaved by Ago2 protein of the RISC [163–165].

The well-established way of action of RNA interference has made it possible for researchers to not only better understand its implications in cancer pathogenesis, but also gave the possibility to utilize siRNAs towards gene expression knockdown with research and therapeutic purposes. For instance, siRNAs have been used in basic research as tools to knockdown expression of genes that are commonly mutated in MDS patients, such as ZRSR2 and anti-apoptotic "survivin", so as to better investigate their role in MDS pathophysiology [166,167]. Additionally, Mackin et al showed that compared with azacytidine which is a hypomethylating pharmacologic agent, siRNAs targeting *DNMT* expression (DNA methyltransferase) proved more efficient at overall demethylation within the genomic transcription units [168]. Another clue of the potential therapeutic role of siRNAs came when the siRNA-mediated inhibition of p38*a* MAP kinase, a mediator of apoptosis which is constitutively activated in low-risk MDS bone marrow cells, led to in vitro improvement of hematopoiesis from MDS myeloid and erythroid progenitors [169]. It is therefore implied that siRNAs could provide a means of therapeutically targeting multiple genes that are aberrantly expressed in MDS patients, although no such agents have been tested in MDS patients to date.

Table 1. ncRNAs with prognostic value in MDS.

Class of ncRNAs	ncRNA	Sample	Reference
miRNAs	miR-125a	BM	Gañán-Gómez 2014 [48]
	miR-22	BM and PB (plasma)	Ma 2020 [65]
	miR-196b-5p	BM	Wen 2017 [67]
	miR-29b	BM	Kirimura 2016 [68]
	miR-320c, miR-320d	BM	Wan 2021 [70]
	miR-194-5p	BM	Choi 2015 [54]
	miR-661	BM	Kang 2019 [61]
	miR-126, miR-155, miR-124a	BM	Choi 2019 [72]
	miR-181a-2-3p	BM	Liang 2022 [64] Kontandreopoulou 2022 [71]
	miR-125b-5p, miR-155-5p	BM	Kontandreopoulou 2022 [71]
	miR-451a, miR-223-3p	PB (plasma)	Dostalova-Merkerova 2017 [74]

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	let-7a, miR-144, miR-16, miR-25, miR-451, miR-651, and miR-655	PB (plasma)	Zuo 2015 [73]
	miR-1237-3p, miR-548av-5p	PB (extracellular vesicles)	Hrustincova 2020
circRNAs	hsa_circRNA_100352 hsa_circRNA_104056 hsa_circRNA_102817	BM and PB (MNCs)	Wu 2020 [98]
	KCNQ10T1	PB (serum)	Zhang 2020 [116]
	HOXB-AS3	BM	Huang 2019 [117]
lncRNAs	H19, WT1-AS, LEF1-AS, TCL6	BM	Szikszai 2020 [120]
	TC07000551.hg.1 TC08000489.hg.1 TC02004770.hg.1 TC03000701.hg.1	BM	Yao 2017 [121]
piRNAs	hsa_piR_019420	PB (EVs)	Hrustincova 2020 [75]
snoRNAs	U33	PB (EVs)	Hrustincova 2020 [75]
tDRs	tDR-Asp family	FFPE preparations	Guo 2017 [159]

BM: bone marrow, PB: peripheral blood, MNCs: mononuclear cells, EVs: extracellular vesicles, FFPE: formalin-fixed paraffin-embedded.

Table 2. ncRNAs with predictive value of treatment response in MDS.

Class of ncRNAs	ncRNA/ gene	Sample	Reference
	miR-143, miR-145	BM	Venner 2013 [79]
	miR-145, miR-146	BM	Oliva 2013 [78]
	miR-34a and miR-34a*	PB	Merkerova 2015 [81]
	miR-17-3p, miR-100-5p, miR-133b miR-10b-5p, miR-15a-5p/b-5p, miR-24-3p, miR-148b-3p	BM	Krejcik 2018 [82]
miRNAs	miR-124	BM	Wang 2017 [86]
	miR-21	PB (serum)	Kim, 2014 [85]
	miR-423-5p, miR-126-3p, miR-151a-3p, miR-125a-5p, miR-199a-3p	PB (plasma)	Hrustincova 2020 [75]
	miR-192-5p	BM and PB	Mongiorgi 2023 [83]
	miR-92a	PB(plasma)	Li 2022 [88]
circRNAs	hsa_circ_0006595	BM	Merkerova 2022 [100]
lncRNAs	AC010127.5, CTC-482H14.5, RP11-557C18.3, RP4-580N22.1, RP11-419K12.2, MIR4512, MIR3164, RF00019, RPS6P16, RP11-478C6.2, RP11-177A2.5, RP4-740C4.7, AC097382.5, RP11-736I24.4	BM	Merkerova 2022 [100]
tRNA/tDRs	chr6.tRNA157.ValCAC chr11.tRNA17.ValTAC chrM.tRNA12.TS1 chrX.tRNA4.ValTAC MT-TS1 chr1.tRNA35.GlyGCC chr21.tRNA2.GlyGCC chr19.tRNA9.PseudoTTT	BM	Guo 2015 [158]

BM: bone marrow, PB: peripheral blood.

9. Conclusions

Myelodysplastic neoplasms are very heterogenous in terms of genetic and epigenetic background, clinical presentation, and prognosis. Treatment decisions are mainly based on the risk stratification of the patients with the use of validated prognostic scoring systems such as IPSS-R and most recently IPSS-M. Yet, more biomarkers are needed not only to assess prognosis but also to predict response to therapy. Non-coding RNAs and mostly miRNAs have been found to be implicated in normal and malignant hematopoiesis including MDS. Their role as prognostic and predictive biomarkers is beginning to emerge and deserves to be further evaluated in large number of patients. Moreover, it is important that experiments are performed in well preserved and well-defined samples so that reliable data are generated, and safe conclusions drawn.

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