

miR-155 as a diagnostic and prognostic marker in hematological and solid malignancies

Minireview

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MicroRNAs (miRNAs) are small RNAs that have emerged as potent regulators of the target genes messenger RNAs expression in the response of cell to both physiological and pathophysiological conditions. Reflecting pathological processes today, miRNAs are widely validated for their potential role in diagnostic, prognostic and novel therapeutic targeting for cancerous and other diseases. miR-155 is considered as a typical multifunctional miRNA including its role as oncomiR (cancer-associated miRNA). Expression of miR-155 is upregulated in cells with high proliferative activity and decreased apoptotic capability. It belongs to cluster of well-characterized tumor associated miRNAs detectable also in the peripheral blood. In this review we summarize the principles of miR-155 host gene expressional regulation, as well as its role in regulation of the target genes mRNAs. Altered expression of miR-155 has been described in multiple cancerous and other diseases, reflecting staging, progress and treatment outcomes. Therefore, miR-155 became a potential biomarker and candidate for clinical utilization as predictor of the presence of cancer, its staging and prognosis.

Key words: miR-155, gene expression regulation, carcinogenesis, diagnosis, prognosis, therapy

MicroRNAs (miRNAs) are non-coding, small (20–25 nts) single-stranded RNAs that regulate the gene expression in cell development [1], proliferation [2] and differentiation [3], apoptosis [4], immune response, inflammation [5], viral infection [6], as well as in pathological states as autoimmune, cancerous [7] and other diseases [8].

miRNA post-transcriptionally regulate the gene expression [9] by binding to 3' untranslated regions (3'-UTRs) of the target mRNAs. They recruit the RNA-induced silencing complex (RISC) to repress their expression or degrade target mRNA [10]. miRNAs constitute only 1–3% of the human genome and they regulate the expression of approx. 60% of all protein coding genes in humans [11]. Each miRNA can affect numerous target mRNAs [9] and each mRNAs can be targeted by multiple miRNAs, enabling wide regulatory complexity of gene expression adjustment [12, 13].

miRNAs originate from independent miRNA genes or they are matured from the intronic regions of protein-coding mRNAs [14] along with their host genes. In the nucleus primary miRNA (pri-miRNA) is produced, capped and polyadenylated. After transcription, pri-miRNA is processed by Drosha and its cofactor DGCR8 (DiGeorge syndrome critical region gene 8) into precursor miRNA (pre-miRNA) losing 5' cap, the 3' poly (A) tail and the hairpin structure. Further, Exportin 5 exports pre-miRNA from nucleus to cytoplasm [13], where it is processed by Dicer-endoribonuclease, with its co-factor TRBP (trans-activating response RNA-binding protein 2, TARBP2), and RNA duplexes of ~ 22 nucleotides [14] are produced. Duplexes consist of the mature and its complementary miRNA strand. Afterwards, an Argonaute (Ago) protein binds to the short RNA duplexes and form complex – miRNA-induced silencing complex (miRNA/RISC) [15]. One of the two

strands, the “passenger miRNA” (complementary “miRNA*”), is released and degraded while the other strand, designated the “guide strand” or “mature miRNA,” is retained within miRNA/RISC [16]. But lately both arms of the pre-miRNA hairpin were shown to give rise to mature miRNAs [17]. According to nomenclature purposes, these products are now denoted with the suffix -5p: e.g. miR-155-5p (from the 5' arm) or -3p: miR-155-3p (from the 3' arm) [18]. Expression levels of miRNAs are tightly controlled by both transcriptional and post-transcriptional mechanisms [19].

The mature miRNA is assembled into the miRNA/RISC complex, recognizes its target mRNAs by base pairing miRNA seed region (nucleotides 2-8) and complementary nucleotides mostly in the 3'-UTR of the target mRNAs [11, 20]. A complex-bound mRNAs are subjected to translational repression or rather degradation [21, 22, 15].

miR-155 is a typical multifunctional miRNA involved in numerous biological processes including hematopoiesis, inflammation and immunity. Deregulation of miR-155 has been found associated with different kinds of cancer, cardiovascular diseases and viral infections.

In present minireview, we present evidence that miR-155 is over-expressed in a number of neoplastic diseases and that it plays a significant role in the process of carcinogenesis. Understanding biological activity of miR-155, its gene expression and target genes regulation provides wide platform for utilization of miR-155 (together with other miRNAs) as biomarker for diagnosis of cancerous disease, its staging, progression and prognosis.

Origin of miR-155

miR-155 originates from its host gene *BIC/MIR155HG*. It was originally identified as B-cell Integration Cluster (*BIC*) – the gene that was transcriptionally activated by promoter insertion at a common retroviral integration site in B-cell lymphomas. *BIC* shows strong sequence homology among human, mouse and chicken, and is highly expressed in lymphoid organs implying an evolutionary conserved function. The human homolog of the *BIC* gene consists of three exons that span a 13 kb region within human chromosome 21 (Hsa21) [23]. miR-155-5p and miR-155-3p strands are generated from *BIC* and they differently participate on post-transcriptional regulation of their target gene mRNAs. While miR-155-5p plays a major regulatory role, miR-155-5p-3p, a passenger strand, promotes the accurate processing of its partner miR-155-5p [24]. It is suggested that passenger strands are evolutionarily conserved and also have inhibitory activity [25, 26]. Its expression levels are usually very low and its biological relevance is questionable [27], with some studies suggesting that miR-155-3p can also be functional [28]. For example, in human plasmacytoid dendritic cells (PDCs) miR-155-3p expression is induced earlier in TLR7 (Toll-like receptor 7) stimulation than that of miR-155-5p, suggesting their co-operative involvement in PDC activation. In these cells, miR-155-3p positively

regulated IFN- α/β (interferon α/β) production by targeting interleukin-1 receptor-associated kinase 3 (IRAK3) mRNA for degradation, while miR-155-5p played a negative role in IFN- α/β production by targeting TGF- β (transforming growth factor β) activated kinase 1/MAP3K7 (mitogen-activated protein kinase kinase kinase 7) binding protein 2 (TAB2) mRNA for degradation [29]. In human primary astrocytes, cytokines and TLR ligands induced significantly higher expression of miR-155-3p compared to miR-155-5p. In contrast, adenoviral-mediated expression of the anti-inflammatory interferon regulatory factor 3 (IRF3) protein resulted in decreased levels of both miR-155-5p and -3p in astrocytes. This data points on potential novel pro-inflammatory role of miR-155-5p and miR-155-3p [28]. miR-155 is expressed in a number of tissues and cell types and plays a critical role in a variety of biological processes. In normal physiological conditions, miR-155 plays a role in hematopoiesis, inflammation and immune responses [30, 31], and it is also an essential molecule in the control of both myelopoiesis and erythropoiesis [32, 33]. Expression of miR-155 was detected for the first time in B-cells, T-cells, monocytes and granulocytes [34]. Using qPCR, the highest levels of miR-155 were demonstrated in thymus and the lowest levels in liver [35]. In pathophysiological conditions, the expression of miR-155 is usually increased. Stanczyk and co-workers [36] have shown that miR-155 is over-expressed in synovial fibroblasts and monocytes of rheumatoid joints. Elevated miR-155 levels have been described in a broad array of cancers including lymphomas, leukemia and solid tumors, suggesting significant role in development of carcinogenesis acting as an oncogenic miRNA – oncomiR.

MIR155HG transcriptional regulation in carcinogenesis

Transcriptional regulation of *MIR155HG* and its oncogenic properties depend on composition of the response elements present in the promoter region of *MIR155HG* (Fig. 1). Its regulation is ruled by multiple signaling pathways.

First miR-155 oncogenic features were demonstrated in breast cancer cells. Up-regulation of *MIR155HG* was described as a consequence of activation of **TGF β /Smad signaling cascade** involving Smad response element (position -454 nt from transcription start site) and resulting in increased cell migration and invasion [37].

MIR155HG transcription is also modulated by transcription factors JunB, FosB and eventually c-Fos, that bind to conserved **AP-1 element** (activator protein 1 element, at position -40 nt) present in the *MIR155HG* promoter region. AP-1 element is important for B-cell activation, as shown in Epstein-Barr virus (EBV) – negative Burkitt lymphoma cell line [38]. In EBV-infected B-cells displaying type III latency, but not in EBV-infected cells displaying type I latency, over-expression of pri-miR-155 and miR-155-5p was described [39, 40]. miR-155-5p plays an important role during B-cell immortalization [41]. Inhibition of miR-155 decreased proliferation and increased apoptosis in EBV-positive B-cell lines [41].

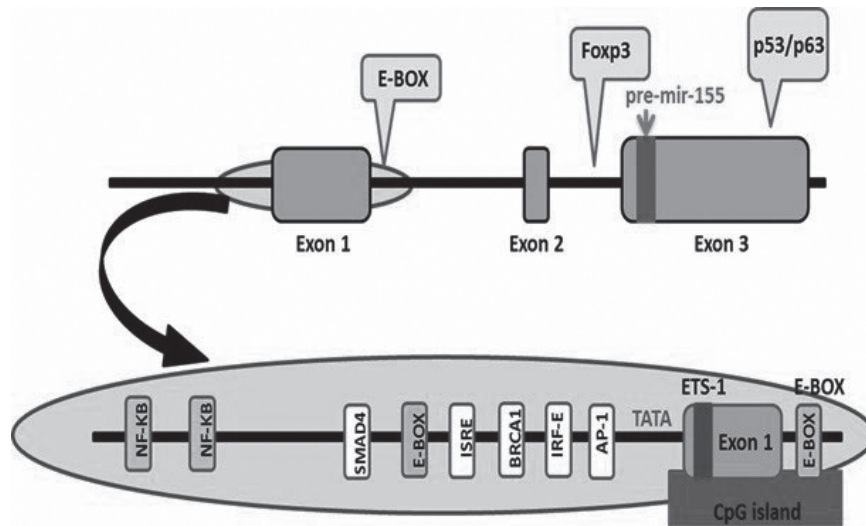


Figure 1. Transcription factor binding sites of the human *MIR155HG* localized upstream and just downstream of exon 1. The dark grey area represents a CpG island. TATA represents the location of the TATA box for *MIR155PHG*.

In addition, *MIR155HG* gene expression can be activated by transcription factors of the **NF-κB pathway** (nuclear factor κB pathway) [42]. As established by ChIP (chromatin immunoprecipitation) analysis, nuclear factor κB can alternatively bind to promoter sites at position -1150 and/or -1697 nt, (NF-κB p50/p65 complex) and activate gene transcription. P38/MAPK (p38 mitogen-activated protein kinase) signaling pathway can also stimulate *MIR155PHG* promoter. A critical role for the AP-1 site and a minor role for NF-κB have been established using the wild-type, NF-κB mutant, and AP-1 mutant *MIR155HG* reporter plasmids and JY cells (an EBV-immortalized B-cell lymphoblastoid cell line) [38].

Wang and colleagues [43] showed that **interferon regulatory factors (IRFs)** are also involved in *MIR155HG* induction by EBV-associated B-cell lymphomas displaying type III latency. Transcription factor binding site analyses of the human *MIR155HG* promoter region identified two putative IRF binding sites, both of which being functional in the reporter assays. ChIP experiments utilizing IB4 cells (EBV-transformed B-cell line) validated that endogenous IRF-4 and -7 can bind to the ISRE (interferon-sensitive response element) and IRFE (interferon regulatory factor element) harbored in the *MIR155HG* promoter *in vivo* [43]. These investigators demonstrated that IRF-4 negatively modulates endogenous INPP5D (inositol polyphosphate-5-phosphatase, *i.e.* SHIP1) levels (a miR-155-5p target) [44, 45] through the up-regulation of pri-miR-155 expression in EBV- or HTLV1-infected cells, providing evidence for a novel molecular mechanism underlying the IRF/*MIR155HG* pathway in viral oncogenesis.

Recently, Vargova *et al.* [46] have demonstrated that the *MIR155HG* promoter harbors another response element modulating *MIR155HG* expression. Since miR-155-5p levels were significantly higher in **MYB** (v-Myb myeloblas-

tosis viral oncogene homolog) overexpressing B-cell chronic lymphocytic leukemia (B-CLL) patient cells, these authors hypothesized that this transcription factor may directly regulate the expression of *MIR155HG*. Three putative MYB binding sites (enhancer E-box elements) were identified. ChIP analyses established that this protein was consistently recruited to broad domains containing the multiple putative MYB binding sites only in primary B-CLLs. H3K9 (lysine 9 of histone H3) acetylation and H3K4 (lysine 4 of histone H3) trimethylation, both well-known markers of actively transcribed genes modifications were significantly elevated in the *MIR155HG* promoter regions in B-CLLs when compared to normal B cells. MYB stimulation of the *MIR155HG* promoter results in dysregulation of its epigenetic status and elevates levels of miR-155-5p. The overexpression of miR-155-5p results in the down-regulation of PU.1 and, as a consequence, the low levels of this protein may be implicated in leukemogenesis [46]. Biological activity of miR-155-5p regulation can be explained by several mechanisms.

Role of miR-155-5p in hematological malignancies development and progression

miR-155-5p is a well-studied miRNA that is closely related to the development of hematopoietic cell. Several studies have established that pri-miR-155 and miR-155-5p are highly elevated in human B- and T-cell acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia (B-CLL), acute myeloid leukemia (AML), diffuse large B-cell lymphoma (DLBCL), Hodgkin and non-Hodgkin lymphoma, primary mediastinal B-cell lymphoma (PMBL), and a number of leukemia and lymphoid cell lines [47, 48, 49, 34, 50, 51, 52, 53]. Important

regulatory role of miR-155-5p is presented not only in origin and development of malignancy but relates also to aggressiveness and therapeutically resistance in hematological malignancies, as it participates on expressional regulation of fundamental hematopoietic oncogenes as well as tumor suppressor genes. The targets of upregulated mir-155-5p include hematopoietic transcription factor PU.1 [42, 54]. The regulatory role of miR-155-5p has been shown mostly pronounced in pathogenesis of B-CLL and DLBCL

B-cell chronic lymphocytic leukemia (B-CLL). In B-CLL, the most common adult leukemia, several microRNAs deregulation was repeatedly described [55, 56]. B-CLL is characterized by clonal accumulation of B cell-like mature-appearing elements (> 5000/ μ L) [57] typically co-expressing the CD5, CD19, CD20, and CD23 surface markers. miR-155-5p appears to play a central role in B-cell function, and its up-regulation in lymphoproliferative disorders, including B-CLL, may lead to a block of differentiation and accumulation of lymphoid-like cells. Also, experiments on transgenic mice over-expressing miR-155-5p in B-cells showed that these mice developed pre-B-cell lymphoproliferative disorders with later progress to full B-cell lymphomas [2]. miR-155-5p is accounted for the most potent miRNA suppressing apoptosis in human T-cell leukemia of Jurkat cell line and in MDA-MB-453 human breast carcinoma cells. One explanation of its regulatory effect is blockade of Caspase-3 [58]. Overexpression of miR-155-5p is followed by down-regulation of p53-induced nuclear protein 1 (TP53INP1) that is capable of cell cycle arrest and induction of apoptosis [59]. This role in apoptosis is considered for oncogenic features of miR-155-5p in several carcinoma types.

Diffuse large B-cell lymphoma (DLBCL). In pathogenesis of DLBCL, miR-155-5p is deeply involved in multiple ways. DLBCL cells are characterized by overexpression of miR-155-5p [49]. It has recently been shown that mice carrying a mutation in the 3'-UTR binding sequence of miR-155-5p in the activation-induced cytidine deaminase (AICDA) gene, a key regulator of somatic hypermutation in the germinal center, resulted in an increase in AICDA protein and can increase the levels of *Myc-IgH* translocations, the characteristic genetic hallmark of B-cell lymphoma [60]. Mice that over-express AICDA do not develop lymphoma [61]. Interestingly, AICDA is also targeted by miR181 [62], an independent prognostic indicator of survival in R-CHOP (drug combination of rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone) treated DLBCL patients [63]. Rai *et al.* [64] demonstrated that miR-155-5p directly targets SMAD5 and its ectopic expression suppressed the *in vitro* growth inhibitory effects of TGF- β 1 and BMP2/4 (bone morphogenetic protein 2/4 heterodimer) in DLBCL cells. miR-155-5p can also regulate the PIK3CA-AKT1 (alpha serine/threonine specific protein kinase) pathway *via* direct targeting of the negative regulator PIK3R1 in DLBCL [65]. miR-155-5p has been shown by to target also Src homology-2 domain-containing inositol 5-phosphatase 1 (INPP5D) (also known as SHIP1) [45, 66].

Overexpression of miR-155-5p was found to promote TNF α (tumor necrosis factor α) -dependent growth of DLBCL cells both *in vitro* and *in vivo* xenotransplant models *via* direct modulation of INPP5D levels [66]. Interestingly, CD10, a well-known marker of germinal center B-cell (GCB)-like -type DLBCL, and constitutive expression of NF- κ B, a hallmark of activated B-cell (ABC)-type DLBCL [67], have been linked *via* a miR-155-5p/SPI1 (spleen focus forming virus proviral integration oncogene, PU.1) pathway [52]. In DLBCL pathogenesis miR-155 participates on expressional modulation of multiple target genes mostly related to cellular growth.

Role of miR-155-5p in solid tumors development and progression

Besides hematological malignancies, overexpression of miR-155-5p was also found in various solid tumors, including breast cancer [68, 69, 70], colon cancer [70] and pancreatic ductal adenocarcinoma [71, 72]. Upregulated mir-155-5p expression was described also in cervical [73], lung [74, 70] and thyroid carcinoma [75].

Breast cancer. Upregulated miR-155-5p expression in breast tumors associates with the promotion of tumorigenesis [76]. Recently, Changs *et al.* [77] established that overexpression of miR-155-5p was triggered by mutation of *BRCA1* (breast cancer 1, early onset). *BRCA1* is a well-known tumor suppressor that is involved in DNA damage response and repair and plays a role in regulating transcription through interactions with histone deacetylase complexes (HDACs) [78]. The R1699Q variant of *BRCA1* (arginine to glutamine amino acid change at position 1699 in the *BRCA1* protein) has been associated with predisposition to breast cancer, and mouse embryonic stem cells expressing this mutant protein were found to have high levels of miR-155-5p [77]. In human cell lines deficient in *BRCA1*, miR-155-5p levels were upregulated 50-fold compared to cells with functional *BRCA1*. Sequence analysis of the mouse *MIR155PHG* promoter identified two regions (the first site is conserved in humans) with putative *BRCA1*-binding sites, and ChIP experiments established that both sites were able to recruit *BRCA1*, suggesting that both sites played a role in repressing promoter activity. The binding of R1699Q *BRCA1* to the *MIR155HG* promoter was comparable to wild-type, signifying that this protein may recruit other transcriptional repressors. Cells expressing functional *BRCA1* treated with HDAC inhibitors upregulated the expression of miR-155-5p, suggesting that the *MIR155PHG* promoter is epigenetically controlled. ChIP analysis demonstrated that in *BRCA1*-deficient cells H2A and H3 were more acetylated, and HDAC2 was recruited to *MIR155HG* promoter in cells expressing wild-type *BRCA1* signifying that *BRCA1* negatively controls this promoter by decreasing the acetylation of H2A and H3. Finally, the knockdown of miR-155-5p in *BRCA1*-deficient cells by a miR-155-5p binding site-sponge led to a reduction in tumor growth, suggesting that miR-155-5p is

a potential therapeutic target for BRCA1-deficient tumors [77].

Neilsen and co-workers [79] suggested that *MIR155HG* may be a downstream target of the mutant p53, given that mutant p53 and miR-155-5p expression are both associated with invasive properties *in vivo* [80]. miR-155-5p expression levels were significantly higher in breast tumors with p53 mutations compared to those of the wild-type p53 [79]. Since mutant p53 can sequester p63 [81, 82], transcription of *MIR155HG* is no longer repressed and the levels of miR-155-5p would be aberrantly high and would drive invasion and metastasis. In p53 mutants, ZNF652 (a novel zinc-finger protein 652 transcriptional repressor) expression is decreased in primary breast tumors and correlates with increased invasion and metastasis, in part due to the ability of exogenous TGF- β to activate SMAD proteins. In support of this observation, Kong *et al.* found that TGF- β induced *MIR155HG* expression after the binding of Smad4 to the promoter region. These data suggest that miR-155-5p may play an important role in TGF- β mediated epithelial-mesenchymal transition and cell migration and invasion by directly targeting RhoA (ras homolog family member A) [37].

In contrast, FOXP3 (forkhead box P3) activation of *MIR155HG* expression in normal epithelia may actually prevent the transformation of the healthy breast epithelium to a cancerous phenotype [83]. FOXP3 has been identified as a tumor suppressor in the breast and prostate epithelia [84] and this transcription factor can directly repress the expression of the ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2) and the SKP2 (S-phase kinase-associated protein

2) oncogenes [85]. FOXP3 directly suppressed the expression of the oncogene SATB1 (Special AT-rich binding protein-1) and activated *MIR155HG* levels. Further, the SATB1 3'-UTR harbors miR-155-5p binding site and this miRNA could down-regulate the expression of this oncogene [83] (Fig. 2).

Colorectal carcinoma (CRC). Overexpression or down-regulation of a subset of miRNAs have also been described in colorectal cancer and potential role of miR-155 was suggested too [86, 87]. In CRC, mir-135b is the most abundantly over-expressed miRNA. Shibuya and colleagues [88] presented that miR-155-5p and miR-21 also importantly relate to diagnostics and prognostics of CRC. Their targets, PDCD4 (programmed cell death protein 4) and TP53INP1 genes, were analyzed by qRT-PCR. High levels of miR-21 were associated by vein invasion of CRC, metastasis in liver and clinical tumor stage. Increased levels of miR-155-5p were associated with metastasis in lymphatic system. Patients with higher level of miR-21 or miR-155-5p showed shorter overall survival and disease free survival. The expression of target mRNAs negatively correlated with the level of miR-21 and miR-155-5p, suggesting their potential role as prognostic biomarkers.

Pancreatic carcinoma (PaC). Singh *et al.* [89] described the role of miR-155-5p and its deregulation in PaC as important point of ROS generation (reactive oxygen species) and responsiveness to hypoxia sensitization *via* regulation of O₂ sensor and transcription factor HIF1a (hypoxia inducible factor 1).

There is growing evidence and accumulating data proving important regulatory involvement of miR-155-5p in the development and progression of tumorigenesis of many human tissues.

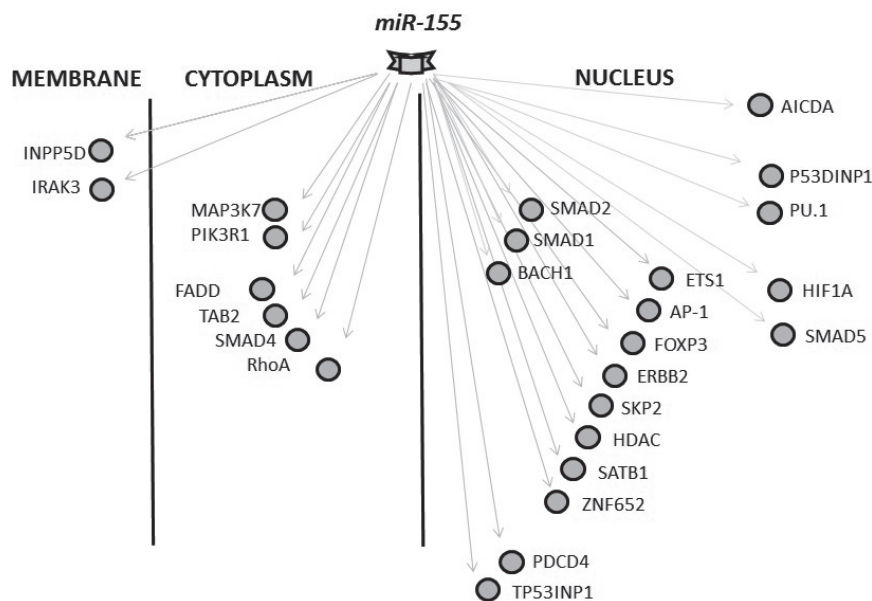


Figure 2. Schematic view of miR-155 targets. Target genes modulated by miR-155 in malignant development and progression are included in group of transcription factors located in nucleus (e.g. PU.1), kinases in cytoplasm (e.g. MAP3K7), binding proteins (e.g. SATB1), membrane bound proteases (e.g. INPP5D), and proteases, kinases and enzymes in cytoplasm.

miR-155 – controlled target genes

Computational predicted miR-155/mRNA targets are not necessarily restricted to a particular functional category or biological pathway [9, 90]. It is important to note that the total number of miR-155-5p putative binding sites is greater than the presumed number miR-155-5p/mRNA targets since some of the mRNAs harbor multiple miR-155 binding sites. mRNA target validation remains a critical step in defining the functions of individual miRNAs [91]. Recently, Nielsen and co-workers [92] assembled a comprehensive list of miR-155-5p/mRNA targets from the literature which were experimentally authenticated by both the demonstration of endogenous transcript regulation by miR-155-5p and validation of the miR-155-5p seed sequence through a reporter assay. This list was comprised of 140 genes and includes regulatory proteins for myelopoiesis and inflammation and known tumor suppressors [92, 93]. From point of view of functional diversity, miR-155 target genes constitute groups of transcriptional factors, receptor ligands, G – protein coupled receptors, receptors with kinase activity, proteases, kinases and enzymes, *etc.* (Fig. 2). Given that miR-155-5p plays an important role in many biological pathways, further studies are needed to fully define the *in vivo* mRNA targets of this miRNA.

Conclusion

Dysfunctional miRNA-mediated regulation has been implicated in the pathogenesis of many diseases. Selected miRNAs has been shown to be associated with lymph node positivity, the development of distant metastases and advanced tumor, node, metastasis staging. Increased miRNAs expression is

also correlated with shorter disease-free interval and poor therapeutic outcome. Understanding the expressional patterns of miRNAs enables their utilization therapeutically, *i.e.* for diagnosis, prognosis and target therapy [94, 95]. Prognostic and biological roles of miR-155 have been evaluated in individual studies and meta-analyses. Relevant works are listed in Tab. 1, including selected adopted information from meta-analysis of He and coworkers [96]. These authors summarize eligible studies and show that higher expression of miR-155 is associated with poor survival in patients with diverse types of cancer. However, more clinical studies are needed prior introducing of miR-155 in prognosis of cancer. In targeted therapy under-expressed miRNAs may be replenished by the administration of miRNA mimetics, whereas overexpressed miRNAs can be targeted by a novel class of synthetic oligonucleotides known as antagomirs.

Given that miR-155-5p overexpression appears to play a pathophysiological role in a number of disease processes, a decrease in the *in vivo* concentration of this miRNA would thereby derepress expression of its targets. The levels of the aberrantly over-expressed miRNAs can be reduced through the use of antimiRs (miRNA inhibitors) harboring the full or partial complementary reverse sequence of a mature miRNA [97]. Several recent studies have demonstrated utilization of miR-155-5p antimiR inhibitors to efficiently mediate *in vivo* miR-155-5p attenuation and target de-repression in mice [98, 99, 100, 101, 102, 103, 104, 105]. However, systemic delivery of these modulators may not be a preferred approach due to the pleiotropic effect of miRNA on gene expression. Therefore, organ specific delivery of miRNA inhibitors/modulators remains a major challenge. With the advancement of RNA delivery technology, it is anticipated that miRNA-based therapeutics will become the latest challenge in human cancer treatment.

Table 1. List of 11 selected relevant studies on hematological malignancies and solid tumors focused on hazard ratio (HR) and prognostic value of miR-155. Of these, 5 lack of HR data. PDAC, pancreatic ductal adenocarcinoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B cell lymphoma; qRT-PCR, quantitative real-time PCR; OS, overall survival; DFS, disease-free survival; TFS, treatment-free survival; SC, survival curve. (Adopted and modified from He *et al.*, 2013.)

Author	Population origin	Disease	N	miR-155 assay	Survival analysis	HR statistics	Follow-up, months
Chen 2011	China	breast cancer	92	RT-PCR	OS,DFS	SC	60
Greither 2010	Germany	PDAC	55	qRT-PCR	OS	reported	15.9 (1-61)
Ishihara 2012	Japan	leukemia	35	qRT-PCR	OS	SC	33
Papaconstantinou 2012	Greece	PDAC	88	qRT-PCR	OS	SC	78
Rossi 2010	USA	CLL	104	qRT-PCR	OS, TFS	reported	160
Shibuya 2010	Japan	colorectal cancer	156	qRT-PCR	OS,DFS	reported	60
Kong 2011	China	PDAC	35	qRT-PCR		-	
Lawrie 2007	UK	DLBCL	76	qRT-PCR		-	
Lawrie 2008	UK	DLBCL	60	qRT-PCR, serum		-	
Sun 2012	China	breast cancer	103	qRT-PCR, serum		-	
Wang 2010	China	acute leukemia	85	qRT-PCR		-	

Babar *et al.* [98] in their miR-155-5p anti-miR inhibitor studies encapsulated a peptide nucleic acid anti-miR-155-5p inhibitor in poly(lactic-co-glycolic acid) (PLGA) nanoparticles that were decorated with the cell-penetrating peptide, penetratin. These nanoparticles uniquely favored the delivery of their anti-miR-155-5p cargo into pre-B cells. In a nanoparticle treated transgenic lymphoma mouse model, an *in vivo* anti-miR-155-5p delivery lead to lymphoid tumor growth delay [98]. This strategy offers a promising therapeutic approach for the treatment of lymphoma/leukemia. anti-miR inhibitor therapy may be useful in treating diseases mediated by the overexpression of miR-155-5p.

Zhang and coworkers summarized novel technical approaches to gain-of-function and loss-of-function manipulation of miRNA expression *in vitro* and *in vivo* and its targeted delivery and highlight their advantages and disadvantages [106]. In common, miRNAs hold a promise to be developed into novel biomarkers with clinical implications as diagnostic, treatment monitoring and prognostic markers as well as very promising therapeutically targets.

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