Interplay between microRNAs and the STAT3 signaling pathway in human cancers

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Cao Q, Li YY, He WF, Zhang ZZ, Zhou Q, Liu X, Shen Y, Huang TT. Interplay between microRNAs and the STAT3 signaling pathway in human cancers. Physiol Genomics 45: 1206–1214, 2013. First published November 5, 2013; doi:10.1152/physiolgenomics.00122.2013.—MicroRNAs (miRNAs, also miR) are a class of noncoding endogenous RNAs that regulate gene expression through binding to protein-coding messenger RNA (mRNA) molecules, predominantly within the 3′-untranslated region (3′-UTR). Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that regulates a battery of genes involved in regulating a variety of biological processes. There is a growing body of evidence demonstrating that miRNAs are closely associated with the STAT3 signaling pathway. In this review, we focus on interactions between miRNAs and the STAT3 signaling pathway, focusing on their reciprocal regulation and roles in cancer. For instance, several papers independently support the existence of regulatory feedback loops between miRNAs and the STAT3 pathway in different cancer contexts including IL-6-STAT3-miR-24/miR-629-HNF4a-miR-124 and IL-6R-STAT3-NF-κB-Lin-28-let-7a. Furthermore, several miRNA components are reported to be involved in STAT3-mediated tumorigenesis, for example miR-21, miR-155, and miR-181b. Through binding to STAT3-binding sites within the promoters of these oncomiRs, STAT3 activates their transcription and mediates tumorigenesis. Some miRNAs directly modulate STAT3 activity through targeting the STAT3 3′-UTR; other miRNAs target SOCS, PIAS3, and EGFR genes, which encode proteins that regulate the STAT3 signaling pathway. Given that miRNAs represent a newly discovered class of regulatory molecules, investigating their biological functions and contribution to pathologies caused by STAT3 dysregulation is essential to improve our understanding of tumorigenesis and to develop novel anticancer therapeutics. The more we can learn about miRNAs-STAT3 interactions, the better able we will be to manipulate them for developing cancer therapeutics.

STAT3 signal pathway; miR-124; miR-17-92 cluster; let-7 family; miR-21

microRNAs (miRNAs, also miR) are a recently discovered class of bioactive cellular molecules with important functions in the regulation of gene expression in both normal physiological and disease processes (35). They are noncoding endogenous RNAs that bind to protein-coding messenger RNA (mRNA) molecules, predominantly within the 3′-untranslated region (3′-UTR). miRNA binding results in increased degradation and inhibition of translation of mRNAs involved in almost every biological process, including cell cycle regulation, proliferation, apoptosis, differentiation, and the immune response (26). For instance, ectopic expression of miR-21 promoted cell invasion by targeting the tissue inhibitor of metalloproteinase 3 (TIMP3) gene in human breast cancer cells (58); miR-31-5p modulates the cell cycle by targeting the MLH1 (mutL homolog 1) gene in several cancer cell lines (78); miR-497 induces neuroblastoma cell apoptosis through suppressing the key cell-cycle regulator WEE1 (13); and via targeting the Sp1 transcription factor, ectopic miR-22 expression inhibits cell migration and invasion (25); and, in particular, the designated oncomiR-1, miR-17-92 cluster, is reported to promote cell proliferation, inhibit differentiation, increase angiogenesis and sustain cell survival by regulating Bim, PTEN, p21, CTGF, and Tsp1, respectively (46). miRNAs are predicted to regulate the expression of almost 90% of human genes, which makes them likely to be important factors in controlling most cellular processes (48). Recently, several lines of evidence places miRNAs at a critical node in the progression and maintenance of cancer (32). Thus, understanding how miRNAs function will improve our understanding of the biological mechanisms involved in carcinogenesis.

The seven members of the signal transducer and activator of transcription (STAT) protein family (STAT1, STAT2, STAT3,
STAT4, STAT5a, STAT5b, and STAT6) are important transducers of many cytokines and growth factors (50). STAT3 is the most common family member and is always constitutively activated in some cancers and immune diseases (24). Activation by inflammatory cytokines like interleukin-6 (IL-6), IL-11, and interferon (IFN), as well as by lipopolysaccharide (LPS), induces STAT3 tyrosine-705 phosphorylation and cytoplasmic-to-nuclear shuttling, recognition of STAT3-specific DNA-binding elements, and transcriptional activation of target genes (24). By modulating the transcription of many genes that regulate a wide variety of biological processes, including cell differentiation, proliferation, apoptosis, metastasis, and immune response, the STAT3 oncoprotein drives tumorigenesis in many tissue types (9, 57, 61). Moreover, elevated levels of constitutively activated STAT3 are associated with poor prognosis for some cancers (22, 36). Importantly, inhibition of constitutively activated STAT3 induces cell apoptosis, which makes it an effective target for cancer intervention (39). Recent studies have demonstrated that miRNAs can modulate the STAT3 signaling pathway; moreover, STAT3 also regulates the expression of several miRNAs. In this mini-review, we discuss recent reports of interactions between miRNAs and STAT3 signaling cascades and their roles in cancer. While providing a comprehensive overview, we focus on reciprocal regulation of miRNAs and the STAT3 signaling pathway and discuss how we can use this knowledge in novel cancer therapies. Both direct and indirect regulatory mechanisms mediate several positive and negative feedback loops between miRNAs and the STAT3 signaling pathway; it is therefore likely that the balance between miRNAs and the STAT3 signaling pathway activities contributes, at least partly, to cellular homeostasis. Therefore, more research is required to elucidate the contribution of miRNA/STAT3 interactions to this process. The more we learn about these interactions, the better we will be able to manipulate them for developing novel anticancer therapies. Moreover, to our knowledge, this is the first review to explicitly focus on the relationship between miRNAs and the STAT3 signaling cascades.

miR-124

miR-124 is a highly conserved miRNA that fine tunes the expression of many genes. It plays an important role in neural processes, from nervous system development to normal neuronal cell function (11). Recently, miR-124 has been reported to be a potential tumor suppressor, as well as an independent prognostic marker in many tumor types, including colorectal cancer (66), myelodysplastic syndrome (69), and prostate cancer (57). Strikingly, a great part of these reports indicate that the suppressive role of miR-124 in tumor is likely to be mediated by STAT3 signaling.

Hepatocyte nuclear factor 4α (HNF4α) is a member of the nuclear receptor family of ligand-dependent transcription factors and a key regulator of hepatocellular cancer initiation (77). HNF4α knockdown in immortalized human hepatocytes led to cellular transformation and increased tumor formation after transplantation into immunosuppressed mice. During an investigation into HNF4α-mediated cellular transformation, Hatzisapetolou et al. (27) validated the HNF4α gene as a target of both miR-24 and miR-629; overexpression of these miRNAs inhibits HNF4α 3'-UTR-dependent luciferase activity by >75%. Both miR-24 and miR-629 contain a STAT3-binding site in their promoters: the STAT3-binding site is highly conserved in the miR-24 promoter and moderately conserved in the miR-629 promoter. Chromatin immunoprecipitation analysis of SNU-449 hepatocellular carcinoma cells showed that STAT3 can bind to both the miR-24 and miR-629 promoters and that IL-6-induced STAT3 activation results in upregulation of both miRNAs (27). The authors initially assumed that this was a unidirectional regulation pathway; however, they later found that miR-124 also contains an HNF4α-binding site in its promoter and directly targets the IL-6 receptor (IL-6R), thus closing the loop of IL-6-mediated STAT3 activation. Thus, STAT3 phosphorylation can be induced by either inhibiting miR-124 expression or HNF4α knockdown (27). These data demonstrate the existence of a positive feedback loop comprising IL-6-STAT3-miR-24/miR-629-HNF4α-miR-124, which causes the inflammatory state to be maintained through multiple cell divisions following removal of the initial trigger. In response to the initial inflammatory signals, IL-6-mediated epigenetic circuits can drive cancer initiation in the absence of genetic alterations.

In addition to the lengthy regulatory feedback loop, Cai and colleagues (5) inferred that miR-124 regulation of STAT3 is also direct, i.e., not mediated by IL-6R. Remarkably, miR-124 downregulation occurs during cardiomyocyte differentiation from bone marrow mesenchymal stem cells (BMSCs). Ectopic miR-124 expression leads to a significant decrease in cardiac-specific markers (ANP, TNT, and α-MHC proteins), mediated by STAT3 (5). Both bioinformatics and luciferase assays demonstrated the STAT3 gene to be a direct target of miR-124; these researchers speculated that myogenic differentiation of BMSCs is mediated by STAT3 inhibition; thus, STAT3-regulated gene transcription may be crucial for cell differentiation (5). Moreover, Zhang et al. (74) found that by targeting STAT3, ectopically expressed miR-124 increased apoptosis in colorectal cancer cells and reduced cell growth in vitro and in vivo. Their experiments identified a direct role for miR-124 in regulating STAT3; this discovery may lead to the development of novel cancer therapies.

LET-7 FAMILY

Let-7 miRNA family members are widely considered to be tumor suppressors. There are 13 different let-7 family members in humans: let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-98, and miR-202 (54). Decreased or completely deficient expression of these miRNAs is common in many tumors, including lung cancer, head and neck squamous cell carcinomas, and ovarian cancer (2). Treatment of MT-1 breast cancer and HeLa cervical cancer cells with an anti-let-7 sponge, which served as a decoy for the mature miRNAs of this family, abrogated endogenous let-7 function and increased cell survival, invasion, and adhesion (72). This finding indicates that restored expression of let-7 may become an important intervention strategy in the future treatment of cancer.

In another study, Iliopoulos et al. (28) identified a second regulatory loop between miRNAs and the STAT3 signaling pathway. They described an experimental model in which a nontransformed epithelial cell line (MCF-10A) could be induced to undergo malignant transformation (MCF-10A-ER-
S. Treatment of MCF-10A cells with tamoxifen resulted in phenotypic transformation, the formation of multiple foci, the ability to form colonies in soft agar, increased motility and invasive ability, and tumor formation upon injection into nude mice, which makes them very useful models for studying factors that influence epigenetic switch in cancer (28). When the authors profiled mRNA and miRNA expression patterns during cellular transformation, they found IL-6 to be upregulated at a very early stage and five members of the let-7 family (let-7a, -7b, -7c, -7d, and -7f) to be simultaneously strongly downregulated. To determine the relationship between let-7 family miRNAs and IL-6, they performed a series of experiments using let-7a. They showed that Src activation triggers a nuclear factor (NF)κB-mediated inflammatory response that directly activates the lin28 transcription, leading to a rapid reduction in let-7a levels (28). By activating STAT3, ectopically expressed let-7a induced cellular transformation. Moreover, a let-7-binding site is present in the IL-6 3′-UTR; thus, ectopic let-7a expression indirectly represses STAT3 transcription (28). Furthermore, STAT3 activation is necessary for both cellular transformation and NF-κB activation, as STAT3 knockdown inhibits let-7a-induced transformation and restoring STAT3 relieves the inhibition. These data indicate that inflammation activates another positive feedback loop that can maintain the epigenetic transformed state during multiple cell divisions in the absence of the inducing signal. Combined with the IL-6-STAT3-miR-24/miR-629-HNF4α-miR-124 positive feedback loop, these data indicate that miRNAs-STAT3 pathway-mediated epigenetic circuits can drive cancer initiation in the absence of genetic alterations, thus providing a solid foundation for chronic inflammation-mediated tumor initiation. Through these positive feedback circuits, miRNA/STAT3 interactions play an important role in the tumor progression in the absence of genetic alterations.

Coincidently, Guo et al. (25) found that the STAT3-coordinated lin28-let-7 circuit plays a crucial role in epithelial-mesenchymal transition mediated by oncostatin M (OSM, a member of the IL-6 family). Treatment of human breast cancer cells (MCF-7 and MDA-231) with OSM reduces the expression of four members of the let-7 family (let-7b, let-7d, let-7e, and let-7g) by approximately two- to fourfold in a JAK/STAT-dependent manner (45). Several lines of evidence support STAT3 regulation of let-7: 1) STAT3 knockdown completely prevents OSM-induced downregulation of let-7b/d/e/g; 2) overexpression of a constitutively active STAT3 mutant, STAT3C, significantly enhances 3′-UTR-dependent expression of HMG2 (high mobility group A), a known let-7 family target gene; and 3) STAT3C-induced HMG2 transcriptional activation leads to a concentration-dependent increase in HMG2 protein levels. Two consensus STAT3-binding sites have been identified upstream of the lin-28 5′-UTR (~2405 and ~369 bp), and protein binding to these sites blocks let-7 processing to form mature miRNA molecules (45). Therefore, STAT3 upregulation and subsequent lin-28 transactivation may contribute to transient let-7b/d/e/g downregulation. These data support the existence of a second positive feedback loop between STAT3 and let-7 (Fig. 1), which may play a crucial role in miRNA-mediated oncogenesis. The fact that the seed regions of all let-7 family members are identical suggests that all members of the family can target the same miRNAs and therefore have similar functions. However, we still have no idea whether the tiny difference in the seed sequences among family members may lead to competition for binding to specific mRNA targets.

miR-21

Arguably, miR-21/STAT3 is the most extensively studied miRNA/STAT3 interaction (34). miR-21 is known as an oncomiR (i.e., an miRNA that functions as an oncogene). miR-21 overexpression occurs in a variety of cancers, including lung cancer (1), breast cancer (63), and glioblastoma (68), as well as in cholesteatoma (20). The function of miR-21 in tumorgen-
esis and metastasis is associated with its ability to target genes encoding multiple tumor suppressors, such as PDCD4, MMP2, PTEN, Gata3, and sprouty1 (20, 55), that regulate multiple signaling pathways involved in apoptosis, proliferation, differentiation, and invasion. Effects on differentiation include inducing adipogenic differentiation (40) and promoting T helper 2 cell differentiation in nonpolarized T cells (32).

In 2007, Loffler and colleagues (40) identified two strictly conserved STAT3-binding sites in the enhancer sequence of miR-21. By activating STAT3, IL-6 induces miR-21 expression in multiple myeloma cells. A second group showed that IFN regulation of miR-21 expression is also mediated by STAT3. IFNs are important antiviral cytokines. By binding to their cognate cell surface receptors, IFNs activate the receptor-associated JAK/STAT signaling pathway, which in turn induces miR-21 expression. Both research groups completely inhibited STAT3 function by either siRNA or downregulated IL-6 expression, thus abrogating miR-21 induction (71). In addition, STAT3 activation of miR-21 and miR-181b-1, via inhibiting of PTEN and CYLD respectively, induces cells epigenetic switch, maintains the transformed state in diverse cell lines, and induces tumor growth in xenografts (29). Moreover, two potential BCL-6 binding sites overlapping with the STAT3 binding sites are located the promoter of miR-21. Because BCL-6 is a transcriptional repressor, BCL-6 competition for binding counteracts STAT3 activation of miR-21. The balance between BCL-6-mediated repression and STAT3-mediated activation thus determines the biology of regulatory T cells (32).

Furthermore, STAT3 plays crucial role in cancer cell chemosensitivity upon treatment with miR-21 inhibition. Suppressing miR-21 expression enhances glioma cell sensitivity to taxol and 5-fluorouracil. Moreover, the expression of STAT3 and phospho-STAT3 is decreased to relatively low levels after miR-21 inhibition (51); however, the mechanism for this is unclear. The same group next investigated the cytotoxic effects of coadministration of an miR-21 inhibitor and temozolomide (49): synergistic antiproliferative and proapoptotic effects were obtained. Strikingly, these effects were dependent on both the administration schedule and the cell lines used. In cells expressing PTEN, miR-21 inhibition and reduced PTEN expression synergistically block the PI3K/AKT pathway and stimulate apoptosis, thus having an antiproliferative effect. However, in cells with high STAT3 expression but low expression of PTEN, a synergistic effect was obtained only following sequential treatment of the two drugs (49). Maximal growth suppression was achieved by the greatest inhibition of STAT3 and phospho-STAT3 expression (49). As STAT3 is not a direct target of miR-21, the mechanism linking these observations was unclear. However, there was also a report showed that miR-21 enhanced the activity of STAT3-dependent signaling pathway through inhibiting protein inhibitor of activated STAT3 (PIAS3). PIAS3 is a negative regulator of STAT3 signaling pathway, and is now known to be a miR-21 target gene in multiple myeloma cells at the same time. Ectopic expression of miR-21 suppressed PIAS3, which in turn inhibited STAT3-dependent signaling pathway (70). Collectively, these data support the existence of another positive feedback loop consisting of miR-21/STAT3/PIAS3, in which STAT3 activation positively regulates its own function by upregulating miR-21 and downregulating PIAS3. This possibility may explain STAT3 hypoexpression and hypophosphorylation following miR-21 inhibition and reveal a new therapeutic route for treating patients with drug-resistant tumors.

miR-17-92 CLUSTER

The highly conserved miR-17-92 cluster is located on chromosome 13q31 and contains six miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1), including the first onco-miRNA to be identified; all are key regulators of various biological processes. For example, miR-17-5p is a key regulator of G/S phase cell-cycle transition (12); miR-18a enhances the IL-6-mediated acute-phase response (3); miR-20a reduces the suppressive potential of myeloid-derived suppressive cells (MDSCs) (75); and all miR-17-92 cluster members are all involved in regulating murine spermatogonial differentiation (65).

There are two seed sequences of miR-17-92 cluster in the STAT3 3’-UTR, and both sequences can regulate reporter gene expression (75). By targeting STAT3, miR-17-5p and miR-20a strongly reduce the production of reactive oxygen species, leading to a loss of suppressive function in MDSCs (75).

During embryonic development, miR-17-92 cluster members are upregulated in differentiating cells, and changes in their expression alter cell fate during embryonic stem cell differentiation. Coincidently, STAT3 is also a key regulator of cell fate decisions during differentiation. Carraro et al. (8) reported that STAT3 dysregulation in differentiating cells is probably indirect, due to dysregulation of miRNAs, especially the miR-17-92 cluster. By specifically targeting STAT3 and MAPK14, the miR-17-92 cluster regulates the FGF10/FGFR2b downstream signaling pathway and, hence, E-cadherin expression, which in turn modulates epithelial bud morphogenesis (18). These findings raise the question of whether miRNAs are the first point of regulation for cell differentiation, while STAT3 may simply be a downstream effector. To our question, the answer may be no. However, the interaction between miR-17-92 cluster and STAT3 is not unidirectional. Previous studies identified a highly conserved STAT3-binding site in the promoter of the EFCAB12 gene, which encodes the entire miR-17-92 cluster (4). Ectopic expression of STAT3 upregulates the expression of the entire miR-17-92 cluster. Bone morphogenic protein receptor type II (BMPR-2), which has a crucial role in the pathogenesis of pulmonary hypertension, is reported to be a miR-17-92 cluster target. Persistent STAT3 activation induces miR-20a overexpression and downregulates BMPR2, thereby contributing to the development of pulmonary hypertension (4). Bim is another miR-17-92 cluster target. Moreover, by upregulating miR-17, STAT3 blocks both Bim expression and PARP cleavage, which induce resistance to MEK inhibitors in lung cancer cells. Thus, MEK inhibition combined with a small molecule-based inhibitor of STAT3 or miR-17-92 cluster may provide a new and effective approach for lung cancer therapy (14). At the same time, it is not difficult to imagine that STAT3-mediated upregulation of the miR-17-92 cluster, through activating the EFCAB12 gene, could reversibly suppress STAT3 expression, thus forming the first reported negative regulatory loop between miRNAs and the STAT3 signaling pathway.
OTHER miRNAs TARGETING STAT3 SIGNALING PATHWAY

A number of other miRNAs also interact with the STAT3 signaling pathway. For example, through downregulating STAT3, miR-125b suppresses the proliferation and migration of osteosarcoma cells (37). Moreover, by targeting both STAT3 and Bcl-2 homologous antagonist/killer (BAK1), miR-125b plays an important role in granulocyte-colony stimulating factor (G-CSF)-induced granulocytic differentiation. Specific RNAs for STAT3 and BAK1 are insufficient to interfere with G-CSF functions; however, gene-specific silencing of Jun family member D (JUND) mimics miR-125b downregulation, suggesting that miR-125b-mediated differentiation involves several signaling pathways and that STAT3 is just one of these (62).

Macrophages are important mediators of the immune response, inflammation, and many other homeostatic processes (43). MiR-223 levels are significantly decreased in LPS- or poly (I:C)-stimulated primary bone marrow-derived macrophages in mice. Moreover, miR-223 downregulation results in the activation of STAT3, which is a direct target of miR-223. On the other hand, it has been found that STAT3 activation could promote production of the proinflammatory cytokines IL-6 and IL-1β, and treatment with recombinant murine IL-6 significantly decreases miR-223 expression. Thus, IL-6/miR-223/STAT3 form a newly identified negative regulatory loop in the Toll-like receptor (TLR)-triggered inflammation response (10). However, this loop was identified in mice and their role in humans has not been identified yet.

Furthermore, by targeting STAT3, ectopic miR-337-3p sensitizes NCi-HI5 nonsmall-cell lung cancer cells to paclitaxel (16), and miR-106a overexpression is associated with cognitive impairment (76). MiR-106a is upregulated in the hippocampus of ovariec tomized mice relative to both age-matched controls (after 6 and 8 wk) (76); in addition, both STAT3 and phospho-STAT3 were downregulated. Furthermore, prediction algorithms and luciferase assays both identified STAT3 as a target of miR-106a; therefore, miR-106a promoted cognitive impairment might be abolished by STAT3 inhibition (76). However, in SUM159 breast cancer cells, targeting STAT3 is not the only way in which miR-93 regulates cell cycle progression. Ectopic miR-93 expression induces mesenchymal-epithelial transition in SUM159 cells, which is associated with downregulation of multiple stem cell regulatory genes, including JAK1, STAT3, AKT3, SOX4, EZH1, and HMGA2. Suppressing these genes resulted in cancer stem cell depletion. However, it is unknown whether all or only some of these genes are involved in the process (38). Direct binding between these miRNAs and STAT3 is shown in Fig. 2. In summary, STAT3 is involved in a large number of miRNA-mediated carcinogenesis pathways, and improving our understanding of these interactions will improve our knowledge of the mechanistic basis of carcinogenesis.

OTHER miRNAs REGULATING STAT3 SIGNALING PATHWAY BY INDIRECT MECHANISMS

In addition to direct interactions between miRNAs and the STAT3 gene, miRNAs may regulate the STAT3 signaling pathway by indirect mechanisms. MiR-200c expression is notably increased during monocyte-derived MCP-1-induced transformation of breast epithelial cells; in contrast, IL-6 expression is lower in estrogen receptor alpha (ERα)-positive human breast cancer cell lines than in three ERα-negative cell lines. IL-6 treatment of MCF-10a breast cancer cells leads to ERα downregulation, mediated by STAT3 binding to the ERα promoter (52). Furthermore, ERα silencing suppresses miR-200c. ZEB1 (zinc finger E-box binding homeobox 1) is another miR-200c target gene. MiR-200c suppression induces ZEB1 expression followed by transcriptional activation of spleen tyrosine kinase and protein tyrosine phosphatase Z1 (PTPRZ1) (63), which have key roles in the activation of JNK1 and the JNK2/STAT3 signaling pathway. Therefore, through this positive feedback loop IL-6 mediates the constitutive activation of inflammatory signals, as well as Neu-driven mouse mammary cell transformation and tumorigenesis (52).

The suppressor of cytokine signaling (SOCS) family contains eight members (SOCS-1–7 and CIS), named for their conserved central SH2 domain and COOH-terminal SOCS box. The most active members of this family are SOCS-1 and SOCS-3, which act as negative regulators of several intracellular pathways, in particular the JAK/STAT pathway (15). Using computational prediction methods and luciferase reporter assays, Jiang et al. (31) demonstrated SOCS1 to be an evolutionarily conserved miR-155 target gene. Expression of miR-155 leads to SOCS1 downregulation, which in turn relieves STAT3 pathway inhibition and promotes cell proliferation, colony formation, and xenograft tumor growth (40), as well as increasing cell migration and the rate of invasion (60). There is evidence that several miRNAs, including miR-122 (73), miR-30d (33), and miR-301a (44), function upstream of SOCS1 and SOCS3. Through decreasing expression of a
SOCS3 suppressor, miR-122 overexpression significantly inhibits IFN-stimulated response element activity, leading to an improved response to IFN (the current standard therapy for hepatitis C) and enhanced improved response to IFN (the current standard therapy for hepatitis C). MiR-30d overexpression promotes cell proliferation and invasion, and miR-30d (high)/SOCS1 (low) mice have an increased risk of early biochemical recurrence. MiR-19a also augments STAT3 activity by targeting SOCS1 (47).

PIAS3 is another suppressor of JAK/STAT signaling pathway through affecting the DNA-binding activity of STAT3. Interestingly, PIAS3 is a target gene of both miR-21 and miR-18a (3). There is an miR-18a binding site in the PIAS3 3′-UTR. Ectopic miR-18a expression suppresses PIAS3 expression at both the mRNA and protein levels. By targeting PIAS3, miR-18a activates the STAT3 signaling pathway, induces cell proliferation, and inhibits apoptosis. Moreover, gain/loss-of-function experiments have revealed that miR-103a targeting of PIAS3 also contributes to the development of T helper-17 cells via regulating the IL-6/23-STAT3 pathway (3, 44). In addition, miR-7 decreases phospho-STAT3 levels by inhibiting the expression of epidermal growth factor receptor (EGFR), another upstream regulator of STAT3. However, there is no direct evidence that EGFR is a direct target of miR-7 (67). Collectively, miRNAs can regulate the STAT3 signaling pathway by not only targeting STAT3 directly but also through indirect mechanisms involving the SOCS, PIAS3, and EGFR proteins. These mechanisms are summarized in Fig. 1. Although these findings demonstrated merely indirect STAT3 regulation by miRNAs, such indirect regulations through direct targets can mediate important biological mechanisms.

OTHER miRNAs REGULATED BY STAT3 DIRECTLY

Because of its involvement in a wide variety of cell processes, it is critical that STAT3 activity is tightly regulated. Recently, Rozovski et al. (53) identified miRNAs whose promoters contain putative STAT3-binding sites and showed that these miRNAs were downregulated upon transfection with STAT3 shRNA. A total of 132 promoters of single pre-miR genes and 28 promoters of pre-miR clusters were identified from the ChIP-seq ENCODE’s database (53), among of which only 38 miRNAs were repressed following transfection with STAT3 shRNA. Unfortunately, the authors only verified five of these miRNAs in further experiments; the other miRNAs await verification, including miR-15 and miR-16, the first miRNAs shown to be involved in human cancer development. MiR-15/16 have been shown to be underexpressed in ~69% of chronic lymphocytic leukemias (7), and reintroduction of miR-15a/16–1 induced apoptosis in MEG01 leukemic cells and inhibited tumor growth in a xenograft model (6). Despite the importance of MiR-15/16 and the high likelihood of cross talk with other signaling pathways, few reports have demonstrated the role of MiR-15/16 in regulating the STAT3 signaling pathway; Moreover, the experiments described above identified only SOCS1 as a direct target of miR-155 (31), while no effect of STAT3 on miR-155 expression has yet been shown. Rozovski et al. (53) found that STAT3-deficient cells exhibit significantly decreased miR-155 expression, indicating the possible existence of another positive circuit of miR-155-SOCS1-STAT3. Therefore, further research is required to validate this feedback loop and also to elucidate how many miRNAs are regulated by STAT3 and how many regulatory circuits exist among them. As we learn more about miRNA/STAT3 interactions, we will gain the ability to manipulate them in cancer therapy.

Interestingly, not only repressed miRNAs were reported; nine upregulated miRNAs were also identified after STAT3 shRNA transfection (Table 1). These may result from STAT3-shRNA-mediated epigenetic silencing of a variety of genes, such as histone deacetylases, or inhibitory effect of direct binding of unphosphorylated STAT3 on gene transcription (64); however, further experiments are required to identify the exact mechanisms involved.

CONCLUSION

The role of miRNAs in promoting human cancer is supported by an increasing body of experimental evidence, from

Table 1. miRNAs regulated by STAT3 directly

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Chromosome</th>
<th>Tissue/Cell Type</th>
<th>Target Processes</th>
<th>Oncogene/Tumor Suppressor</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>17 q23.1</td>
<td>ubiquitous</td>
<td>cell apoptosis, tumor growth</td>
<td>oncogene</td>
<td>29, 40, 52, 67, 70</td>
</tr>
<tr>
<td>miR-181b-1</td>
<td>9 q33.3</td>
<td>ubiquitous</td>
<td>cell transformation, tumorigenicity in vivo</td>
<td>oncogene</td>
<td>29, 52</td>
</tr>
<tr>
<td>miR-24</td>
<td>9 q22.32/19p13.13</td>
<td>hepatocellular carcinoma</td>
<td>hepatocellular transformation, tumor growth</td>
<td>oncogene</td>
<td>27, 52</td>
</tr>
<tr>
<td>miR-629</td>
<td>15 q23</td>
<td>hepatocellular carcinoma</td>
<td>hepatocellular transformation, tumor growth</td>
<td>oncogene</td>
<td>27, 52</td>
</tr>
<tr>
<td>miR-7*</td>
<td>15 q26.1</td>
<td>malignant glioma</td>
<td>cell growth, metastasis, EGFR signaling</td>
<td>suppressor</td>
<td>52, 6</td>
</tr>
<tr>
<td>miR-155*</td>
<td>21 q21.3</td>
<td>breast cancer, hepatoma cell</td>
<td>antiviral immunity, cell proliferation, tumor growth</td>
<td>oncogene</td>
<td>31, 40, 52</td>
</tr>
<tr>
<td>miR15a/16-1†</td>
<td>13 q14.2</td>
<td>ubiquitous/enriched in CLL</td>
<td>apoptosis, cell cycle, tumor growth development, cell invasion, organism growth, metastasis, cell proliferation,</td>
<td>suppressor</td>
<td>6, 7, 52</td>
</tr>
<tr>
<td>let-7a-3′-7b*</td>
<td>22</td>
<td>ubiquitous</td>
<td></td>
<td>suppressor</td>
<td>28, 44</td>
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<tr>
<td>let-7a-1/-7f-1/7d*</td>
<td>9</td>
<td>ubiquitous</td>
<td></td>
<td>suppressor</td>
<td>52</td>
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</table>

We summarized the microRNAs (miRNAs) whose promoters harbor putative signal transducer and activator of transcription 3 (STAT3) binding sites according to the ENCODE ChIP-seq database and were downregulated by transfection with STAT3-shRNA in the experiment of Hatziapostolou et al. (27). MiR-21, miR-181b-1, miR-24, and miR-629 have been reported to be targeted by STAT3 in other studies, which are shown in the references. *MiR-7, miR-155, and the let-7 family have been reported to regulate the expression of STAT3 through suppressing of VEGF, PIAS1, and IL-6, respectively. However, the effects of STAT3 on these miRNAs have not been identified yet. †Moreover, although miR-15a and miR-16-1 have been reported to be downregulated in cells transfection with STAT3-shRNA, the interplay between miR-15a/16-1 and STAT3 signaling pathway is less known.

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early profiling studies to more recent demonstrations of the causal role of these molecules in the tumorigenic process and their possible use as biomarkers or therapeutic tools (30). We have reviewed the interactions between miRNAs and STAT3 and their effects on STAT3 signaling pathway, including the main regulatory feedback loops. It is likely that both direct and indirect mechanisms contribute to miRNA interplay with the STAT3 signaling pathway, although details of these mechanisms await further elucidation. Indirect mechanisms may involve interactions between miRNAs and SOCS, PIAS3, and EGFR, which all regulate activity of the STAT3 pathway. As well as being STAT3 downstream effectors, miRNAs may also directly repress STAT3 transcriptional activators. Although our understanding of the critical miRNA/STAT3 interactions in cells is far from complete, the pathways described so far provide us with a foundation to build upon.

Epidemiological studies suggest that as many as 25% of all cancers may be caused by chronic inflammation (42). However, the mechanisms linking inflammation to cancer are unclear. We have described several papers that independently support the presence of positive circuits between miRNAs and the STAT3 pathway in different cancer contexts, such as IL-6-STAT3-miR-24/miR-629-HNF4α-miR-124, IL-6-STAT3-NF-κB-Lin-28let-7a, and miR-220c-ZEB1-SYK/PTPRZ1-STAT3-Erk; we suggest that epigenetic circuits modulated by miRNAs can lead to permanent alterations in cell phenotypes and drive cancer initiation with a similar potency to that attained by genetic alterations in oncogenes or tumor suppressors (56). In addition to their regulatory roles in the STAT3 pathway, miRNAs also modulate NF-κB signaling (41); cooperation between the NF-κB and STAT3 signaling pathways thus links inflammation to cancer (17). Moreover, different miRNA phenotypes may result in differentially dysregulated signaling in different cell types. An improved understanding of the mechanisms underlying these observations may allow us to optimize the use of anticancer drug to prevent or limit inflammation-related tumor progression.

Physiological homeostasis is maintained by numerous regulatory feedback loops. The huge regulatory network composed of positive and negative miRNA/STAT3 regulatory circuits may be involved in maintaining cellular homeostasis. Disturbances to the normal balance can lead to the emergence of cancer and related immune diseases, which in turn contributes to either overexpression of or deficiencies in specific miRNAs in different cancer cells. Through conducting a comprehensive study into miRNAs and the STAT3 signaling pathway, we are obtaining a clearer understanding of their regulatory interactions. However, one problem is that the interactions described in this review were all identified in different cancer cell lines. Because of the different cancer cell microenvironments, we cannot be sure that these interactions occur in all cancers. Therefore, the biological consequences of miRNA-STAT3 pathway interactions in specific cancer cell lines await further characterization.

Recent data described in this review this will undoubtedly open the door to developing cancer therapies through exogenous miRNA dysregulation. Many different strategies for the pharmacological manipulation of miRNAs are now being carried out in preclinical animal models (23, 59). For example, local injection of anti-miR-21 reduced the growth of breast cancer xenografts and restored trastuzumab sensitivity by increasing PTEN expression (23); As the first miRNA in phase I clinical trials, miR-34a functions as a novel predictive in serum biomarker for pemetrexed-based chemotherapy in advanced nonsmall-cell lung cancer (19); and miR-34a overexpression induced apoptosis and inhibited tumorigenesis in lung cancer cells (21). However, as a particular 3’-UTR may contain binding sites for several different miRNAs and a single miRNA may have several target genes, there will be a critical need for cell-specific miRNA delivery. Moreover, further similar investigations should help us to overcome current problems associated with chemotheraphy resistance.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: Q.C. conception and design of research; Q.C., Y.-Y.L., and Z.-Z.Z., edited and revised manuscript; Q.C. approved final version of manuscript; Y.-Y.L., W.-F.H., Z.-Z.Z., X.L., and Y.S. prepared figures; Y.-Y.L. drafted manuscript; W.-F.H., Q.Z., Y.S., and T.-T.H. analyzed data.

REFERENCES

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