MicroRNA: master controllers of intracellular signalling pathways

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Abstract

Signaling pathways are essential intracellular networks that coordinate molecular outcomes to external stimuli. Tight regulation of these pathways is essential to ensure an appropriate response. microRNA (miRNA) is a class of small, non-coding RNA that regulates gene expression at a post-transcriptional level by binding to the complementary sequence on target mRNA, thus limiting protein translation. Intracellular pathways are controlled by protein regulators, such as Suppressor of Cytokine Signaling (SOCS) and A20. Until recently, expression of these classical protein regulators was thought to be controlled solely by transcriptional induction and proteasomal degradation; however, there is a growing body of evidence describing their regulation by miRNA. This new information has transformed our understanding of cell signaling by adding a

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previously unknown layer of regulatory control. This review outlines the miRNA regulation of these classical protein regulators and describes their broad effects at both cellular and disease levels. We review the regulation of three important signaling pathways, including the JAK/STAT, NF-κB and TGF-β pathways, and summarize an extensive catalogue of their regulating miRNAs. This information highlights that the importance of the miRNA regulon and reveals a previously unknown regulatory landscape that must be included in the identification and development of novel therapeutic targets for clinical disorders.

Keywords

microRNA; signal regulator; regulon; JAK-STAT; NF-κB; TGF-β

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Introduction

microRNA (miRNA) are a class of small, non-coding RNA of 19-25 nucleotides (nt) in length that regulate gene expression at a post-transcriptional level by binding to the 3'UTR of the target transcript [1]. They are transcribed in the form of a primary transcript (pri-miRNA), either under the control of their own promoter regions or processed from a coding gene [2]. pri-miRNA are subsequently processed in the nucleus by RNase III-type endonuclease, Drosha, in association with an accessory double-stranded RNA (dsRNA)-binding protein, DiGeorge Critical Region 8 (DGCR8), into a stem-loop dsRNA pre-miRNA (or precursor miRNA), of 60-70nt in length, with a 2nt overhang at the 3' end [3, 4]. This stem-loop dsRNA structure is transported from the nucleus to the cytoplasm by Exportin-5 [5, 6], where it is further processed by another RNase III-type endonuclease, Dicer, and, in some cases, also with the help of accessory dsRNA-binding proteins, PACT and TRBP, into a mature dsRNA duplex of 19-21nt base pairs each side and a 2nt overhang at each 3' end [7, 8]. This duplex is then loaded onto an effector complex called RNA-induced silencing complex (RISC). One strand from the duplex acts as a guide to direct RISC in binding the target mRNA complementary sequence that mediates gene silencing [9] (Fig.1). The discovery of microRNA in 1993 [10], revealed previously unknown layer of post-transcriptional control that revolutionized our concept of gene regulation. "Classical" protein regulators, such as SOCS and A20, are well known to quickly control signaling pathways through direct post-translational modification, such as phosphorylation or ubiquitination, of their target protein. miRNAs do not

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regulate the activity of existing proteins, but rather limit the synthesis of new proteins, providing an extra layer of control that is now accepted as being an essential component of pathway regulation in processes such as cell development or differentiation [11, 12]. However, as well as regulating key players within signaling pathways, miRNAs are increasingly being documented to regulate the "classical" regulators, thus providing additional control which we review in this manuscript. Here we describe this novel mechanism of molecular regulation of three major signaling pathways: Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and transforming growth factor beta (TGFβ). We also outline how each miRNA plays a role in different cellular, ranging from normal growth and development, and clinical contexts, such as autoimmunity and cancer. In fact, this review clearly highlights that regulators of the cellular signaling pathways are important targets of regulation by miRNAs, and are significant targets for future research.

JAK-STAT signaling pathway

The JAK-STAT signaling pathway is mainly adopted by cytokine receptors to effect their anti-viral, inflammatory and cell proliferative activity [13]. Upon binding of extracellular ligands, such as interferon (IFN)-α/β and interleukin (IL)-6, to their respective dimerized transmembrane receptors, the pre-associated JAK tyrosine kinases are brought into juxtaposition and activated by trans-autophosphorylation. Activated JAK kinases then mediate tyrosine

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phosphorylation on the conserved residue of the receptors, which causes the receptor recruitment of SH2-domain containing STAT proteins, which are in turn phosphorylated by JAKs, dissociate from the receptors, dimerize and translocate into the nucleus, where they act as transcriptional activators, driving expression of effector genes [14]. This pathway is central to the well being of our complex immune system, with dysregulation leading to serious lymphoproliferative and autoimmune diseases [13], and is therefore under tight regulation at multiple levels [reviewed in 15].

Regulation of SOCS by microRNAs

The best studied regulators of the JAK-STAT pathway are suppressor of cytokine signaling (SOCS) proteins, which constitute a family of 8 members, including SOCS1-7 and cytokine-inducible Src homology 2 protein (CIS) [16]. SOCS proteins bind phosphorylated tyrosines of JAKs and/or the receptor via their SH2-domains, thus blocking STAT recruitment [14]. Additionally, the SOCS box domain recruits elongin B and C-containing ubiquitin E3 ligase complexes and effectively mediates receptor degradation through the proteasome [14, 15]. Basal expression of SOCS is low, but can be up-regulated by cytokine stimulation, providing an essential and effective negative feedback loop for the activated pathway [16]. Recent publications have documented that miRNA regulation of SOCS expression is also key to optimal performance of the JAK/STAT pathway. The role of miR-155 in regulating SOCS1 protein expression has been implicated across a spectrum of cell types in nearly 20 publications. It was first described by

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Rudensky's group in the context of Foxp3 expression of regulatory T (Treg) cell homeostasis. The authors noticed that an up-regulation of SOCS1 protein level was detected in miR-155-deficient Treg cells, a phenotype which could be reverted by the reintroduction of miR-155 [17]. Not limited to T cell biology, the regulatory role of miR-155 on SOCS1 has also been implicated in NK cell development and functions. In this study NK cells from miR-155 knockout mice had elevated SOCS1 expression, and suffered from both impaired NK cell generation and response to viral infection [18]. The functional consequences of SOCS1 regulation by miR-155 are best illustrated by early work from Cao's group, showing that even though miR-155 did not alter IFN expression in virally infected macrophages, its suppression of SOCS1 levels increased STAT1 phosphorylation and downstream IFN stimulated gene (ISG) induction [19]. Interestingly, the miR-155-SOCS1 relationship has also been actively implicated in the field of cancer biology. An inverse correlation of miR-155 and SOCS1 expression was observed in breast cancer patients and cell lines, in which miR-155 conferred enhanced oncogenic properties [20]. In hepatocellular carcinoma (HCC), miR-155 regulation of SOCS1 increases STAT3 signaling, in turn stimulating matrix metalloproteinase (MMP)9 expression and increasing tumor invasion [21]. Other miRNAs that have been shown to regulate SOCS1 expression include miR-30d in prostate cancer [22], miR-122 in Huh-7 hepatocyte cells [23] and miR-150 in lupus nephritis pathogenesis model [24]. The miR-19a/b family was up-regulated in multiple myeloma (MM) and acted as an oncogenic regulator via suppression of SOCS1, an important inhibitor of IL-6-mediated growth in MM pathogenesis [25]. The miR-19a-SOCS1 relation has also been

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implicated in gastric cancer [26]. To our surprise, when we analyzed the predicted targets of miR-19a within the JAK-STAT pathway by bioinformatics, we discovered that the miR-19a target sequence is conserved between SOCS1 and SOCS3, and found that SOCS3 is a putative target of miR-19a and modulates the activity of JAK-STAT signaling in response to IFN-α and IL-6 [27]. Together these results demonstrate a realization of how evolution has "chosen" a single miRNA species to regulate multiple cellular targets that converge onto the same signaling pathway to exert an amplified combinatory effect on the pathway [12]. This broad effect of miR-19a provides the cellular machinery with a very convenient switch to control the expression of a set of genes with powerful effect. While miR-19a regulates multiple targets of the JAK-STAT pathway its regulation also extends to the NF-κB signaling pathway [28]. Similarly, miR-155 inhibits SOCS1 and SOCS3 expression, which enhances IFN production during persistent viral infection, demonstrating its ability to also control several SOCSs that regulate more than one pathway [29].

Targeting of SOCS by multiple miRNA also seems to be a common strategy adopted by miRNA to regulate the JAK-STAT pathway. miR-203 was demonstrated by two independent groups to regulate SOCS3 expression in different cellular contexts. Ru and colleagues reported that miR-203 was up-regulated in breast cancer and its knock-down correlated with enhanced level of SOCS3 expression and improved chemosensitivity towards cisplatin [30]. Moffatt and Lamont demonstrated that gingival epithelial cells infected with *Porphyromonas gingivalis* had increased cellular miR-203 expression, resulting

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158 in SOCS3 down-regulation and enhanced STAT3 activation [31]. On top of this,
159 the latter showed that SOCS6 is also a putative target of miR-203 [31]. In another
160 example, although miR-221 was implicated in regulating SOCS3 expression level,
161 which conferred anti-tumorigenic effects in prostate cancer patients [32],
162 TargetScan also predicts that SOCS1 and SOCS7 are additional targets for this
163 miRNA [33], highlighting that our current knowledge and understanding of
164 SOCS-targeting microRNAs is in its infancy and that future investigations may
165 reveal an even more complex and intricate network of intracellular pathway
166 regulation.
167
168 **Regulation of PIAS by microRNAs**
169
170 While JAK kinase- and receptor-mediated signaling are directly regulated by
171 SOCSs, the downstream signaling protein, STAT, is regulated by protein inhibitor
172 of activated STAT (PIAS), which effectively fine-tunes the pathway activity. The
173 PIAS family in mammals is composed of 4 members: PIAS1, PIAS3, PIASx and
174 PIASy, recognized to target STAT1, STAT3, STAT4 and STAT1, respectively [15].
175 Each member of the PIAS family contains a RING-finger-like zinc-binding domain
176 (RLD), which confers small ubiquitin like modifier (SUMO) E3-ligase activity,
177 thus mediating SUMOylation and consequential deactivation of STATs [34]. PIAS
178 protein also regulates STAT independently of SUMOylation. Other mechanisms
179 include direct blockage of STAT DNA binding and recruitment of co-repressors,
180 such as histone deacetylase (HDAC) [reviewed in 34]. Although PIAS-STAT
181 interaction is believed to be cytokine-dependent [34], the recent discovery of

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their regulation by miRNAs could be crucial in understanding the maintenance of the pathway integrity and cellular homeostasis.

Since PIAS3 negatively regulates STAT3, a key player in the IL-6-mediated ISG induction (driven by the IFN-γ activated sequence [GAS]-containing promoter) [15], dysregulation of PIAS3 by miRNA could have devastating outcomes. Regulation of PIAS3 by miRNA was first proposed by Brock et al., who showed that miR-18a targeted the 3'UTR of PIAS3 mRNA, which suppressed its protein expression and resulted in IL-6-induced STAT3 activation in hepatocytes and triggered the acute-phase response [35]. Since the dysregulation of JAK-STAT signaling, via altered expression of SOCS by miRNA, is evident in many cancer models, it is no surprise to see it is equally true for PIAS. Indeed, regulation of PIAS3 by miR-18a has been implicated in gastric adenocarcinoma, in which a clinical correlation has been established between miR-18a, PIAS, JAK-STAT pathway activity and downstream anti-apoptotic and cell-proliferative genes [36]. Using proteomics PIAS3 was also identified as a cellular target of miR-21, which was highly expressed in MM [25], resulting in similar pathological outcomes to IL-6-induced JAK-STAT pathway activation [37]. PIAS3 is also a target for miR-125b in glioblastoma stem cells [38], further highlighting the multifaceted nature of its regulation and importance as a gatekeeper of oncogeneisis. Interestingly, microRNA regulation of PIAS3 even controls T cell development. In fact, inhibition of miR-301a expression in myelin auto-antigen exposed $CD4^+$ T helper cells altered their cytokine expression profile and

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hampered their differentiation into Th17 cells, which was thought to be controlled by PIAS3 regulation of IL-6-STAT3 signaling [39].

Although there are 4 mammal PIAS proteins, evidence on their expression regulated by miRNA has only been reported for PIAS3. This exclusivity may be partly explained by the length of 3'UTR of their mRNA, since only human PIAS3 mRNA carries 3' UTR that spans for nearly 1000nt long, while the others are just a few hundred. This speculation is supported by the study that some housekeeping genes which have strong preference to minimize miRNA regulation tend to evolve with a shorter 3' UTR, thus avoiding miRNA binding, which consequently minimizes the risk of their accidental and undesirable shutdown [40]. However, the physiological relevance of the PIAS's 3'UTR length in regulating the JAK-STAT pathway warrants further investigation. In summary, it is clear that the integrity of a functional JAK-STAT pathway is essential for cellular homeostasis. Dysregulation of this signaling by miRNA may attribute to many cancers and autoimmune diseases. However, it is important to remember that there are other signaling pathways, such as the NF-κB and TGFβ cascades, which also determine the outcome of effective cellular reactions and are now known to be under the regulation of miRNAs.

NF-κB signaling pathway

NF-κB mediates diverse biological processes at the cellular level, including growth, development and inflammatory responses [41]. The canonical NF-κB

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pathway mainly utilizes the RelA (also known as p65):p50 heterodimer as a transcription factor to activate downstream target genes. In unstimulated cells the RelA:p50 heterodimer is bound to inhibitor of NF-κB (IκB), making it inaccessible to the nucleus and thus blocking gene transcription [42]. To remove this suppressive constraint, IκB protein must be phosphorylated by IκB kinase (IKK) complex, which constitutes two catalytic subunits, IKKα and IKKβ, and one regulatory subunit, NF-κB essential modulator (NEMO) (also known as IKKγ). This initiates K48-polyubiquitination and subsequent proteosomal degradation of IκB protein [41]. In the non-canonical (or alternative) NF-κB pathway, which utilizes the RelB:p52 heterodimer, p100, the p52 predecessor, acts like IκB to suppress translocation and transcription activation of RelB when bound under unstimulated conditions [42]. Upon activation, NF-κB-inducing kinase (NIK), with the help of IKKα, induces phosphorylation of p100, which is then subjected to ubiquitination and processing into p52, that, with RelB, serves as a heterodimer transcription factor [reviewed in 43]. (Fig.2) Dysregulation of the NF-κB pathway accounts for many autoimmune, chronic inflammatory and cancerous diseases [41], therefore, as with the JAK/STAT pathway, multiple levels of regulation must be adopted to avoid disease [reviewed in 41].

Regulation of PP2A/C by microRNAs

Although the canonical and non-canonical pathways mobilize different cell modulators, they are regulated using a similar mode of action: phosphorylation, ubiquitination, and then proteosomal processing of the inhibitory binding

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253 partner of NF-κB. Due to the importance of NF-κB regulation, it is not surprising
254 to see that some of these steps are also controlled by miRNA. In both pathways,
255 IKKα/β is the major protein kinase, engaging in the initial phosphorylation and
256 subsequent processing or degradation of the inhibitory binding partner [42]. In
257 order to become activated, IKKα/β complex requires trans-autophosphorylation
258 and phosphorylation from another upstream kinase, such as TGF-β activated
259 kinase-1 (TAK1) or NIK. These phosphorylation sites are subjected to
260 dephosphorylation by a group of protein phosphatases called PP2A/C [44].
261 Regulation of PP2A/C by miRNA is evident in cancer models. Two papers
262 recently reported that miR-520h targets PP2A/C and promotes NF-κB-driven
263 tumorigenic gene expression in breast cancer and ovarian cancer cell lines, as
264 well as in lung cancer patient samples [45, 46]. The significance of regulating
265 PP2A/C is evident in the broad spectrum of miRNAs that control its expression,
266 including miR-1, miR-19, miR-31 and miR-133. While these miRNAs have not
267 been shown to impact the NF-κB pathway in disease models, they will most
268 likely affect responses to the pathway and thus identify an area of research that
269 warrants investigation [47-49].
270
271 **Regulation of CYLD by microRNAs**
272
273 Ubiquitination of target proteins is arguably one of the most important and
274 influential molecular events within a cell and is thereby controlled by a series of
275 processes. Polyubiquitnation does not only enable IκB degradation or p100
276 processing, but is implicated throughout the NF-κB pathway [reviewed in 50]. In

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277 the canonical pathway, NEMO undergoes K63-polyubiquitination in response to
278 TNF stimulation, which facilitates the recruitment of upstream activating factors
279 and, in turn, the activation of IKK complex [51]. To regulate IKK activation, the
280 tumor suppressor deubiquitinase, CYLD (cylindromatosis), removes
281 polyubiquitin chains from NEMO [42]. But the regulation of CYLD is now known
282 to involve miRNA, which has been shown to play an important role in cancer
283 pathogenesis. For example, miR-181b-1 was found to be up-regulated in an
284 oncogenic Src kinase transformed model and manipulation of cellular miR-181b-
285 1 levels altered CYLD expression, NF-κB activity and mammary epithelial cell
286 line transformation [52]. The targeting of CYLD by miR-181b-1 was later
287 implicated in pancreatic cancer, in which increasing miR-181b-1 levels confer
288 cell line chemoresistance to gemcitabine, via the down-regulation of CYLD and
289 up-regulation of NF-κB activity [53]. In gastric cancer patients miR-362 was up-
290 regulated in tumor tissue samples, which inversely correlated to CYLD
291 expression, suggesting that miR-362 regulation of CYLD promoted NF-κB activity
292 and subsequently enhanced cell proliferation and apoptotic resistance [54]. In
293 addition, CYLD mRNA has been shown to be directly targeted by miR-182 and
294 miR-486, which promoted tumor aggressiveness of gliomas, again through NF-
295 κB dysregulation [55, 56]. The broad inhibitory remit of both these miRNAs is
296 clearly demonstrated in their spectrum of targets, with miR-486 also regulating
297 Cezanne (A20 family deubiquitinase) and A20-interacting partners, TNF-α-
298 induced protein 3 (TNFAIP3) interacting protein (TNIP)1/2/3; and miR-182
299 regulating TNIP1, optineurin ubiquitin-binding protein (OPTN), and the
300 deubiquitinase ubiquitin-specific protease 15 (USP15) [55, 56].

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Regulation of A20 by microRNAs

Another deubiquitination enzyme that has received much attention is A20 (also known as TNFAIP3,). A20 contains an N-terminal ovarian tumor (OTU) deubiquintation domain and a C-terminal zinc finger (ZnF) E3 ligase domain, which are thought to have dual functions in K63-linked deubiquitination and K48-linked polyubiquitination of substrates, such as receptor-interacting protein (RIP)1 kinase [57]. RIP1 is an upstream activating kinase of TAK1, and its K63-linked polyubiquitination is indispensible for IKK activation in the TNF-induced NF-κB pathway [41]. Indeed, A20 mediates deubiquination of K63-linked polyubiquitin chain on RIP1, but K48-linked polyubiquitination is actually mediated by A20-binding partner, ITCH (also known as itchy E3 ubiquitin protein ligase), which targets RIP1 for proteosomal degradation, thus terminating the transduced signal [42]. (Fig.2) With its ability to negatively regulate NF-κB activity, A20 is regarded as a tumor suppressor and its inactivation is frequently observed in various cancer models [57]. miRNA have also been documented to manipulate A20 expression levels with obvious consequences for NF-κB activity in tumor cells.

Gantier and colleagues reported that global depletion of miRNA expression, through conditional Dicer knock-out, impaired pro-inflammatory cytokine induction [28]. Initially using TargetScan they predicted that negative regulators of NF-κB, including A20 and other related proteins, such as its binding partners

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(Ring Finger Protein 11 (RNF11) and ITCH), A20 regulator (TNIP1) and other deubiquitinases (CYLD and Cezanne), were targets of an oncogenic miRNA cluster, miR-17-92 [28, 33]. Among the four miRNA families expressed in this cluster, miR-19 was demonstrated to have a significant impact on NF-κB activity [28]. In this study, A20 and RNF11, as well as two other regulators of NF-κB (KDM2A and ZBTB16), were shown to be validated targets of miR-19, whereas the suppressive effect on other predicted targets, including CYLD, was not observed [28]. However, in T-cell acute lymphoblastic leukemia (T-ALL) patient samples and cell lines, up-regulation of miR-19 inhibited CYLD expression, leading to sustained NF-κB activity [58], clearly demonstrating that miRNAs regulate multiple targets from the same pathway in a cell- and disease-type dependent fashion and highlighting the vast chasm of knowledge still to be explored. In addition, apart from miR-19, miR-146a was also able to regulate the expression of RNF11, which facilitated Hendra virus replication in NF-κB-dependent manner [59]. miR-18a, from the miR-17-92 cluster, also reduced A20 in a model of rheumatoid arthritis (RA) and enhanced NF-κB-dependent expression of the matrix degrading enzyme, MMP1, and inflammatory cytokines, such as IL-6, in synovial fibroblasts [60]. Similar establishment of NF-κB dysregulation by miRNA-targeting of A20 was observed during Japanese encephalitis virus infection. This virus induced cellular miR-29b expression, which regulated A20 expression in a microglial cell line, thus enhancing NF-κB activity [61]. In stark contrast, another miRNA from the same miR-29 family, miR-29c, was found to be down-regulated in a Hepatitis B Virus (HBV)-related HCC cell line and patient samples. This loss of miR-29 expression up-regulated

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A20 expression, resulting in restricted cell proliferation and enhanced apoptosis [62]. While miRNA are thought to predominately suppress target gene expression, in a sarcoma model, miR-29 bound the A20 3'UTR and prevented physical association of an RNA-binding protein HuR, thus protecting A20 mRNA from destabilization and degradation. In the same study, the authors showed that miR-125 could also regulate A20 expression [63], an observation mirrored in a macrophage polarization and diffuse large B-cell lymphoma model [64, 65].

In general, aberrant NF-κB activity resulting from the dysregulation of its regulator by miRNA drives the expression of numerous pro-inflammatory cytokines and chemoattractants at the site of injury, and confers aggressiveness and apoptotic tolerance to tumors at the cellular level. These events have been evident in many of the aforementioned examples and believed to be the center of many inflammatory diseases and cancers. Therefore, further elucidating the control of NF-κB regulators by miRNA will help us better understand the development and progression of these diseases and reveal much needed therapeutic targets.

TGFβ signaling pathway

From the beginning of life the TGFβ pathway is indispensable in coordinating cell development and differentiation and is essential for sustaining a functioning immune response [66]. The signal begins when functional, mature TGFβ is freed by an endoprotease from a latent complex held within the extracellular matrix

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373 [67]. The binding of TGFβ to its cognate receptor complex, consisting of two
374 type-I receptors and two type-II receptors, triggers serine/threonine kinase
375 activity on the type-II receptor molecule, which subsequently activates and
376 phosphorylates the type-I receptor at its cytoplasmic domain [68]. Smad2 and
377 Smad3 (Receptor (R)-Smad proteins) are recruited to the phosphorylation site
378 and themselves phosphorylated by the activated type-I receptor complex.
379 Phosphorylated Smad2/3 can then form a trimeric complex with other
380 coactivators, such as Smad4 and TIFIγ, to regulate gene expression in nucleus
381 [67]. Alternatively, the receptor complex can activate a Smad-independent
382 pathway through modulating Rho GTPase, MAP kinase and PI3K signaling
383 pathway activity, which regulates a different sets of target genes [66]. (Fig.2) To
384 achieve optimized signaling activity, the TGFβ signaling pathway output is tightly
385 regulated at different stages.
386
387 **Regulation of Smad7 by microRNAs**
388
389 While R-Smad proteins convey activating downstream signals, inhibitory Smad
390 (I-Smad) proteins regulate this intracellular transduction. Smad7, for example, is
391 expressed in response to TGFβ pathway activation and provides efficient
392 negative feedback through several mechanisms [67]. It can physically bind to the
393 type-I TGFβ receptor, acting as a direct competitor to R-Smad [67], or it can
394 further recruit other regulatory proteins, including PP1 phosphatase and the
395 Smad ubiquitin regulatory factor (Smurf) E3 ligase, which inactivate and
396 promote degradation of the receptor molecule, respectively [67, 68]. We now

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know that expression of Smad7 is regulated by multiple miRNAs. The up-regulation of miR-21 expression has been shown to suppress Smad7 expression in HCV-infected liver biopsies. Interestingly, these findings correlated with patient HCV load and fibrosis stage, indicating a role for miR-21 in accelerating fibrogenesis [69]. Others observed that miR-21-mediated reduction of Smad7 correlated with expression of TGFβ-induced fibrotic markers, such as alpha smooth muscle actin (α-SMA) and fibronectin (Fn), which promoted epithelial–mesenchymal transition (EMT) in lung fibrosis [70, 71], renal fibrosis [72, 73], and systemic sclerosis [74, 75]. EMT also plays an important role in the initiation of cancer metastasis. Complementary to this notion, miR-21 was up-regulated in the invasive ductal carcinoma region of breast cancer and knock-down of miR-21 restored Smad7 levels in a breast cancer cell line [76]. miR-21-mediated reduction of Smad7 expression has also been implicated in the generation of carcinoma-associated fibroblasts (CAFs), which confer tumorigenesis, proliferation and invasiveness characteristics of tumors [77]. However, elevated miR-21 levels are not always associated with enhanced proliferation and differentiation. In the case of myelodysplastic syndromes (characterized by ineffective hematopoiesis), suppression of Smad7 by miR-21 was found to decrease erythroid colony formation of CD34+ cells, while inhibiting miR-21 could rescue red blood cell count and stimulate erythropoiesis in transgenic mice [78]. As seen with miR-21, suppression of endogenous Smad7 is a common cancinogenic mechanism that promotes EMT. Other examples include the miR-216a/217 cluster in HCC [79], miR-20a in gall bladder carcinoma [80], miR-181a in ovarian cancer [81], and miR-106b-25 in breast

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421 cancer [82]. While the oncogenic role of these microRNAs has been described,
422 the tumor suppressive role of a selection has also been reported, with reduced
423 miR-25 in colon cancer [83] and miR-181c in metastatic neuroblastoma patients
424 [84]. Interestingly, both reports reasoned that the tumor suppressive roles of
425 miR-25 and miR-181c were accounted for by reduced Smad7 protein expression
426 and TGFβ activity, which would otherwise stimulate tumor growth and
427 migration [83, 84].
428
429 **Regulation of Smurf by microRNAs**
430
431 Reduced Smad7 expression in bronchial epithelial cells was also associated with
432 overexpressed miR-15b in a chronic obstructive pulmonary disease model [85].
433 In their cell-based assays, the authors also found that, apart from Smad7, Smurf2
434 expression was affected by miR-15b [85]. Smurf proteins are E3 ubiquitin ligase
435 proteins, recruited by Smad7 to the type-I TGFβ receptor complex. Their
436 recruitment promotes proteosomal degradation of the receptor complex, thus
437 restricting signal transduction [86]. Antagonizing miR-322 and miR-503 action
438 on Smurf2 regulation was shown to inhibit the phosphorylation of Smad2 [87].
439 As miRNA-suppression of Smad7 was observed in many cancers, it is not
440 surprising to see in an aggressive breast cancer model, Smurf2 was down-
441 regulated by miR-15, miR-16 and miR-128 [88]. miR-15b also targets Smurf1
442 during osteoblast differentiation, as a way to activate the expression of a master
443 transcription factor, Runx2 [89]. Smurf1 is also a target of miR-17 and miR-497;

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444 both of these studies showed reduced miRNA and up-regulated Smurf1
445 expression in periodontitis and metastatic ovarian cancer, respectively [90, 91].
446
447 **Regulation of GARP by microRNAs**
448
449 TGFβ is secreted and stored in the extracellular matrix inside a large latency
450 complex. The cytokine remains inactive and bound to the latency-associated
451 peptide until positive regulators increase the efficiency of its dissociation from
452 the large latency complex and it is processed into a mature form. Glycoprotein A
453 repetitions predominant protein (GARP), is expressed by T regulatory (Treg)
454 cells and tightly associated with the latency-associated peptide bound to
455 immature TGFβ [67]. GARP is essential for TGFβ activation [92], as it frees
456 immature TGFβ molecules from the latency complex [67]. miR-142-3p regulates
457 GARP expression and thus controls Treg cell proliferation [93]. Specifically, the
458 authors observed decreased expression of miR-142-3p in CD25+ CD4 T cells and
459 manipulation of miR-142-3p levels resulted in altered proliferation of these cells
460 [93], which is consistent with the concept of TGFβ-mediated Treg cell
461 proliferation. No matter whether a miRNA is targeting the positive or negative
462 regulators of the TGFβ signaling pathway, any upset in the homeostatic balance
463 could lead to serious pathological consequences, like fibrosis or oncogenesis.
464
465 **Regulation of microRNA levels**
466

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So far this review has discussed how miRNAs participate in signaling pathway regulation and provide an additional layer of supervision on pathway regulators, but, in order to fully understand how these “master regulators” control our signaling networks it is important to note how miRNAs themselves are regulated. Endogenous levels of miRNA are significantly linked to the final output of signalling pathways and are under the control of several factors. The majority of miRNAs is located in either intragenic or intergenic regions and is transcribed together with its host gene or from its own promoter [2]. The miR-106b-25 cluster is an example of intragenic miRNA, which sits itself within intron 13 of the miniature chromosome maintenance 7 (MCM7) gene [94]. This miRNA cluster encodes three miRNAs, namely miR-106b, miR-93, and miR-25, two of which, miR-106b and miR-25, we described above as regulators of Smad7. These miRNA were found to be frequently co-expressed and probably co-regulated with their host mRNA [95] and amplification of the MCM7 gene locus and its elevated expression with miR-106b-25 cluster have been associated with human malignancies [94, 96]. The miR-17-92 cluster (also known as oncomir-1) is an example of intergenic miRNA that is expressed and processed from the C13orf25 transcript [96]. This miRNA cluster encodes six mature miRNAs, namely miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1, which have broad effects on multiple pathway regulators, including SOCS, PIAS, A20, Smad7 and Smurf1.

It is interesting to note that the expression of both MCM7/miR-106b-25 and miR-17-92 genes are induced by common transcription factors, E2F1 and MYC [96]. E2F1 and MYC are involved in a positive feedback loop making both

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proteins capable of regulating one another [97]. The entire regulatory network may be further complicated by the negative regulation of E2F1 expression by miR-106b and miR-20a [96]. These constitute an important regulatory mechanism that allows these miRNAs to be expressed at optimized level. It is foreseeable that in the event of sub-optimal miRNA levels, the negative feedback constrain is lenient, so the positive feedback loop of the transcription factors encourages the expression of these miRNAs. Alternative, when miRNA expression becomes excessive, it places a heavy negative feedback constrain on the transcription factors, so that the continuous expression of miRNA can be eventually shut off. Therefore, it is evident that the sequence of molecular events responsible for miRNA expression can be more complex than their simple regulation of target genes and networks, and hence should be analysed case by case. In fact, when considering the mechanisms that alter the steady-state level of any miRNA, we should also take into account the cell type, its half-life and transient intracellular turnover [reviewed in 98].

Insights derived from regulator-targeting miRNAs

Although the role played by each miRNA appears to be context-dependent in individual studies, some have collectively demonstrated its versatility in regulating multiple regulators of a signaling pathway. Examples include miR-15 (targeting Smad7, Smurf1 and Smurf2 in TGFβ pathway) [85, 88, 89], miR-19 (targeting SOCS1 and SOCS3 in JAK-STAT pathway; A20, CYLD and RNF11 in NF-κB pathway) [25-28, 58], miR-155 (targeting SOCS1 and SOCS3 in JAK-STAT

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pathway) [29], miR-203 (targeting SOCS3 and SOCS6 in JAK-STAT pathway) [30, 31]. This versatility is indeed conferred by the ability of miRNA to regulate gene expression at translation level by binding to and targeting the complementary sequence present on 3' UTR of any gene, irrespective of their actual protein coding sequence, such that multiple genes that share similar gene function and acquire the same complementary sequence can be regulated simultaneously by a common miRNA. The miRNA-target relationship is now thought to be under tight natural selection and is believed to have co-evolved with one another, as well as the whole regulatory network [99].

To date, vast majority of publications have focused on the validation of a single gene targeted by miRNA that has implications in different biological models. While this has been limited by both our lack of knowledge and experimental capabilities, it has certainly led to an under-estimation of miRNA capacity in modulating the entire regulatory network as a "master regulon". Fortunately, the recent advancement in next-generation sequencing and other molecular biological techniques, such as photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP), have already improved our understanding and knowledge in the transcriptome-wide regulation of miRNA in many cellular contexts. Bioinformatic database analysis remains a cornerstone for the predictive analysis of miRNAs and their targets and will continue to be used to understand how miRNA can act beyond a single gene to regulate an entire network [100]. Careful data-mining procedures and the use of a systems biology approach will conserve efforts from validating all of the predicted targets

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539 and focusing on the pathway predicted to be affected by the specific miRNA and
540 the corresponding regulators involved. Additionally, when regarding the
541 timeframe and strength of miRNA regulation, our current knowledge is greatly
542 limited by the use of existing non-physiological methodology that manipulates
543 endogenous miRNA expression in cell-based systems. Furthermore, it is
544 important to note, that in most cases, owing to direct gene amplification of the
545 miRNA region or altered expression of the transcription factors responsible for
546 regulating miRNA expression, miRNAs levels during disease pathogenesis are
547 aberrantly expressed [96].
548
549 The plethora of current evidence outlined in this review identifies miRNAs as
550 “master controllers” of intracellular signaling pathways in many disease models
551 and in the era of new therapies against miRNA, this evidence highlights them as
552 powerful targets for therapeutic development with highly significant clinical
553 applications. In many of the studies covered in this review, manipulation of
554 endogenous miRNA levels by chemically synthesized analog or inhibitor could
555 revert the phenotype caused by the dysregulated miRNA, and therefore provide
556 the proof-of-principle for potential drug development. While therapeutic
557 development, from “bench to bedside” is a long, arduous and expensive process,
558 recent advances with the development of the first miRNA-targeting drug,
559 miravirsen, (miR-122 targeting locked-nucleic acid (LNA)-modified inhibitor for
560 treatment of hepatitis C virus infection, currently in phase 2 clinical trial), have
561 brought the entire miRNA research community closer to therapeutic solutions
562 than ever before [101]. Another miRNA-based drug, MRX34, is the first miRNA

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mimic for miR-34 and has entered phase 1 trial for treatment of HCC [102]. These outstanding advances demonstrate that miRNA are promising targets for therapeutic intervention and with our advanced understanding of their regulation of different cellular pathways and disease pathogenesis, it is expected more pre-clinically validated drugs will enter clinical trials and be used in our actual daily clinical practice.

Conclusion

This article has reviewed how miRNAs potently act as novel regulators of classically known inhibitors of the JAK-STAT, NF-κB and TGFβ signaling pathways. We now have a much deeper understanding of the way in which miRNAs regulate many pathological diseases and normal developmental processes. More importantly, we have identified a reiterating concept, whereby miRNAs bind 3'UTRs of their target irrespective of the protein coding sequence, and regulate multiple targets, which usually work at different levels of the signaling cascade, within the same signaling pathway. This allows miRNA to provide another layer of signaling regulation, in order to achieve maximal effect and avoid detrimental responses to stimuli. With this concept in mind, it is essential for future research of miRNA-target identification to consider the regulation network (or regulon) of specific miRNA, in order to achieve total understanding of the mechanism of any cellular process. More advanced techniques can reveal the transcriptome-wide regulation of miRNAs should be considered a standard and essential approach. This not only takes the concept of

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587 a single miRNA regulating different targets within the same pathway into
588 account, but also provides a bigger picture how miRNA can regulate different
589 targets among different signaling pathways.

590

591 **References**

592

593 1. P. D. Zamore, B. Haley, Ribo-gnome: the big world of small RNAs. *Science* **309**,
594 1519-1524 (2005).

595

596 2. D. P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*
597 **116**, 281-297 (2004).

598

599 3. Y. Lee, C. Ahn, J. Han, H. Choi, J. Kim, J. Yim, J. Lee, P. Provost, O. Radmark, S.
600 Kim, V. N. Kim, The nuclear RNase III Drosha initiates microRNA processing.
601 *Nature* **425**, 415-419 (2003).

602

603 4. J. Han, Y. Lee, K. H. Yeom, Y. K. Kim, H. Jin, V. N. Kim, The Drosha-DGCR8
604 complex in primary microRNA processing. *Genes Dev.* **18**, 3016-3027 (2004).

605

606 5. C. Gwizdek, B. Ossareh-Nazari, A. M. Brownawell, A. Doglio, E. Bertrand, I. G.
607 Macara, C. Dargemont, Exportin-5 mediates nuclear export of minihelix-
608 containing RNAs. *J. Biol. Chem.* **278**, 5505-5508 (2003).

609

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

610 6. R. Yi, Y. Qin, I.G. Macara, B. R. Cullen, Exportin-5 mediates the nuclear export of
611 pre-microRNAs and short hairpin RNAs. *Genes Dev.* **17**, 3011-3016 (2003).
612
613 7. R. F. Ketting, S. E. Fischer, E. Bernstein, T. Sijen, G. J. Hannon, R. H. Plasterk,
614 Dicer functions in RNA interference and in synthesis of small RNA involved in
615 developmental timing in C. elegans. *Genes Dev.* **15**, 2654-2659 (2001).
616
617 8. K. H. Kok, M. H. Ng, Y. P. Ching, D. Y. Jin, Human TRBP and PACT directly
618 interact with each other and associate with dicer to facilitate the production of
619 small interfering RNA. *J. Biol. Chem.* **282**, 17649-17657 (2007).
620
621 9. S. L. Ameres, J. Martinez, R. Schroeder, Molecular basis for target RNA
622 recognition and cleavage by human RISC. *Cell* **130**, 101-112 (2007).
623
624 10. R. C. Lee, R. L. Feinbaum, V. Ambros, The C. elegans heterochronic gene lin-4
625 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75**, 843–854
626 (1993).
627
628 11. G. Hutvagner, J. McLachlan, A. E. Pasquinelli, E. Balint, T. Tuschl, P. D. Zamore,
629 A cellular function for the RNA-interference enzyme Dicer in the maturation of
630 the let-7 small temporal RNA. *Science* **293**, 834-838 (2001).
631
632 12. M. Inui, G. Martello, S. Piccolo, MicroRNA control of signal transduction. *Nat.*
633 *Rev. Mol. Cell Biol.* **11**, 252-263 (2010).

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

634

635 13. J .J. O'Shea, R. Plenge, JAK and STAT signaling molecules in immunoregulation
636 and immune-mediated disease. *Immunity* **36**, 542–550 (2012).

637

638 14. W. M. Schneider, M. D. Chevillotte, C. M. Rice, Interferon-stimulated genes: a
639 complex web of host defenses*. Annu. Rev. Immunol.* **32**, 513–545 (2014).

640

641 15. K. Shuai, B. Liu, Regulation of JAK-STAT signalling in the immune system. *Nat.*
642 *Rev. Immunol.* **3**, 900–911 (2003).

643

644 16. B. A. Croker, H. Kiu, S. E. Nicholson, SOCS regulation of the JAK/STAT
645 signalling pathway. *Sem. Cell Dev. Biol.* **19**, 414-422 (2008).

646

647 17. L. F. Lu, T. H. Thai, D. P. Calado, A. Chaudhry, M. Kubo, K. Tanaka, G. B. Loeb,
648 H. Lee, A. Yoshimura, K. Rajewsky, A. Y. Rudensky, Foxp3-dependent
649 microRNA155 confers competitive fitness to regulatory T cells by targeting
650 SOCS1 protein*. Immunity* **30**, 80-91 (2009).

651

652 18. C. L. Zawislak, A. M. Beaulieu, G.B. Loeb, J. Karo, D. Canner, N. A. Bezman, L. L.
653 Lanier, A. Y. Rudensky, J. C. Sun, Stage-specific regulation of natural killer cell
654 homeostasis and response against viral infection by microRNA-155. *Proc. Natl.*
655 *Acad. Sci. U.S.A.* **110**, 6967-6972 (2013).

656

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

657 19. P. Wang, J. Hou, L. Lin, C. Wang, X. Liu, D. Li, F. Ma, Z. Wang, X. Cao, Inducible
658 microRNA-155 feedback promotes type I IFN signaling in antiviral innate
659 immunity by targeting suppressor of cytokine signaling 1. *J. Immunol*. **185**,
660 6226-6233 (2010).
661
662 20. S. Jiang, H. W. Zhang, M. H. Lu, X. H. He, Y. Li, H. Gu, M. F. Liu, E. D. Wang,
663 MicroRNA-155 functions as an OncomiR in breast cancer by targeting the
664 suppressor of cytokine signaling 1 gene. *Cancer Res*. **70**, 3119-3127 (2010).
665
666 21. X. L. Yan, Y. L. Jia, L. Chen, Q. Zeng, J. N. Zhou, C. J. Fu, H. X. Chen, H. F. Yuan, Z.
667 W. Li, L. Shi, Y. C. Xu, J. X. Wang, X. M. Zhang, L. J. He, C. Zhai, W. Yue, X. T. Pei,
668 Hepatocellular carcinoma-associated mesenchymal stem cells promote
669 hepatocarcinoma progression: role of the S100A4-miR155-SOCS1-MMP9 axis.
670 *Hepatology* **57**, 2274-2286 (2013).
671
672 22. N. Kobayashi, H. Uemura, K. Nagahama, K. Okudela, M. Furuya, Y. Ino, Y. Ito, H.
673 Hirano, Y. Inayama, I. Aoki, Y. Nagashima, Y. Kubota, H. Ishiguro, Identification of
674 miR-30d as a novel prognostic maker of prostate cancer. *Oncotarget* **3**, 1455-
675 1471 (2012).
676
677 23. A. Li, W. Song, J. Qian, Y. Li, J. He, Q. Zhang, W. Li, A. Zhai, W. Kao, Y. Hu, H. Li, J.
678 Wu, H. Ling, Z. Zhong, F. Zhang, MiR-122 modulates type I interferon expression
679 through blocking suppressor of cytokine signaling 1. *Int. J. Biochem. Cell Biol.* **45**,
680 858-865 (2013).

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

24. H. Zhou, S. A. Hasni, P. Perez, M. Tandon, S.I. Jang, C. Zheng, J. B. Kopp, H. 3rd Austin, J. E. Balow, I. Alevizos, G. G. Illei, miR-150 promotes renal fibrosis in lupus nephritis by downregulating SOCS1. *J. Am. Soc. Nephrol*. **24**, 1073-1087 (2013).

25. F. Pichiorri, S. S. Suh, M. Ladetto, M. Kuehl, T. Palumbo, D. Drandi, C. Taccioli, N. Zanesi, H. Alder, J. P. Hagan, R. Munker, S. Volinia, M. Boccadoro, R. Garzon, A. Palumbo, R. I. Aqeilan, C. M. Croce, MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 12885-12890 (2008).

26. S. Qin, F. Ai, W. F. Ji, W. Rao, H. C. Zhang, W. J. Yao, miR-19a promotes cell growth and tumorigenesis through targeting SOCS1 in gastric cancer. *Asian Pac. J. Cancer Prev.* **14**, 835-840 (2013).

27. A. S. Collins, C. E. McCoy, A. T. Lloyd, C. O'Farrelly, N. J. Stevenson, miR-19a: an effective regulator of SOCS3 and enhancer of JAK-STAT signalling. *PLoS One* **8**, e69090 (2013).

28. M. P. Gantier, H. J. Stunden, C. E. McCoy, M. A. Behlke, D. Wang, M. Kaparakis-Liaskos, S. T. Sarvestani, Y. H. Yang, D. Xu, S. C. Corr, E. F. Morand, B. R. Williams, A miR-19 regulon that controls NF-κB signaling. *Nucleic Acids Res.* **40**, 8048-8058 (2012).

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

705 29. A. Zhai, J. Qian, W. Kao, A. Li, Y. Li, J. He, Q. Zhang, W. Song, Y. Fu, J. Wu, X. Chen,
706 H. Li, Z. Zhong, H. Ling, F. Zhang, Borna disease virus encoded phosphoprotein
707 inhibits host innate immunity by regulating miR-155. *Antiviral Res.* **98**, 66-75
708 (2013).
709
710 30. P. Ru, R. Steele, E. C. Hsueh, R. B. Ray, Anti-miR-203 Upregulates SOCS3
711 Expression in Breast Cancer Cells and Enhances Cisplatin Chemosensitivity.
712 *Genes Cancer* **2**, 720-727 (2011).
713
714 31. C. E. Moffatt, R. J. Lamont, Porphyromonas gingivalis induction of microRNA-
715 203 expression controls suppressor of cytokine signaling 3 in gingival epithelial
716 cells. *Infect Immun*. **79**, 2632-2637 (2011).
717
718 32. B. Kneitz, M. Krebs, C. Kalogirou, M. Schubert, S. Joniau, H. van Poppel, E.
719 Lerut, S. Kneitz, C. J. Scholz, P. Ströbel, M. Gessler, H. Riedmiller, M. Spahn,
720 Survival in Patients with High-Risk Prostate Cancer Is Predicted by miR-221,
721 Which Regulates Proliferation, Apoptosis, and Invasion of Prostate Cancer Cells
722 by Inhibiting IRF2 and SOCS3. *Cancer Res*. **74**, 2591-2603 (2014).
723
724 33. B. P. Lewis, C. B. Burge, D. P. Bartel, Conserved seed pairing, often flanked by
725 adenosines, indicates that thousands of human genes are microRNA targets. *Cell*
726 **120**, 15-20 (2005).
727

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

728 34. K. Shuai, B. Liu, Regulation of gene-activation pathways by PIAS proteins in
729 the immune system. *Nat. Rev. Immunol.* **5**, 593-605 (2005).
730
731 35. M. Brock, M. Trenkmann, R. E. Gay, S. Gay, R. Speich, L. C. Huber, MicroRNA-
732 18a enhances the interleukin-6-mediated production of the acute-phase proteins
733 fibrinogen and haptoglobin in human hepatocytes. *J. Biol. Chem.* **286**, 40142-
734 40150 (2011).
735
736 36. W. Wu, M. Takanashi, N. Borjigin, S. I. Ohno, K. Fujita, S. Hoshino, Y. Osaka, A.
737 Tsuchida, M. Kuroda, MicroRNA-18a modulates STAT3 activity through negative
738 regulation of PIAS3 during gastric adenocarcinogenesis. *Br. J. Cancer* **108**, 653-
739 661 (2013).
740
741 37. Q. Xiong, Q. Zhong, J. Zhang, M. Yang, C. Li, P. Zheng, L. J. Bi, F. Ge,
742 Identification of novel miR-21 target proteins in multiple myeloma cells by
743 quantitative proteomics. *J. Proteome Res.* **11**, 2078-2090 (2012).
744
745 38. L. Shi, Y. Wan, G. Sun, S. Zhang, Z. Wang, Y. Zeng, miR-125b inhibitor may
746 enhance the invasion-prevention activity of temozolomide in glioblastoma stem
747 cells by targeting PIAS3. *BioDrugs* **28**, 41-54 (2014).
748
749 39. M. P. Mycko, M. Cichalewska, A. Machlanska, H. Cwiklinska, M. Mariasiewicz,
750 K. W. Selmaj, MicroRNA-301a regulation of a T-helper 17 immune response

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

751 controls autoimmune demyelination. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E1248-
752 1257 (2012).
753
754 40. A. Stark, J. Brennecke, N. Bushati, R. B. Russell, S. M. Cohen, Animal
755 MicroRNAs confer robustness to gene expression and have a significant impact
756 on 3'UTR evolution. *Cell* **123**, 1133-1146 (2005).
757
758 41. J. Napetschnig, H. Wu, Molecular basis of NF-κB signaling. *Annu. Rev. Biophys.*
759 **42**, 443-468 (2013).
760
761 42. S. Vallabhapurapu, M. Karin, Regulation and function of NF-kappaB
762 transcription factors in the immune system. *Annu. Rev. Immunol.* **27**, 693-733
763 (2009).
764
765 43. S. C. Sun, Non-canonical NF-κB signaling pathway. *Cell Res.* **21**, 71-85 (2011).
766
767 44. M. S. Hayden, S. Ghosh, Shared principles in NF-kappaB signaling. *Cell* **132**,
768 344-362 (2008).
769
770 45. J. L. Su, P. B. Chen, Y. H. Chen, S. C. Chen, Y. W. Chang, Y. H. Jan, X. Cheng, M.
771 Hsiao, M. C. Hung, Downregulation of microRNA miR-520h by E1A contributes to
772 anticancer activity. *Cancer Res.* **70**, 5096-5108 (2010).
773

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

774 46. Y. H. Yu, H. A. Chen, P. S. Chen, Y. J. Cheng, W. H. Hsu, Y. W. Chang, Y. H. Chen, Y.
775 Jan, M. Hsiao, T. Y. Chang, Y. H. Liu, Y. M. Jeng, C. H. Wu, M. T. Huang, Y. H. Su, M. C.
776 Hung, M. H. Chien, C. Y. Chen, M. L. Kuo, J. L. Su, MiR-520h-mediated FOXC2
777 regulation is critical for inhibition of lung cancer progression by resveratrol.
778 *Oncogene* **32**, 431-443 (2013).
779
780 47. X. Liu, L. F. Sempere, H. Ouyang, V. A. Memoli, A. S. Andrew, Y. Luo, E.
781 Demidenko, M. Korc, W. Shi, M. Preis, K. H. Dragnev, H. Li, J. Direnzo, M. Bak, S. J.
782 Freemantle, S. Kauppinen, E. Dmitrovsky, MicroRNA-31 functions as an
783 oncogenic microRNA in mouse and human lung cancer cells by repressing
784 specific tumor suppressors. *J Clin. Invest*. **120**, 1298-1309 (2010).
785
786 48. K. J. Mavrakis, A. L. Wolfe, E. Oricchio, T. Palomero, K. de Keersmaecker, K.
787 McJunkin, J. Zuber, T. James, A. A. Khan, C. S. Leslie, J. S. Parker, P. J. Paddison, W.
788 Tam, A. Ferrando, H. G. Wendel, Genome-wide RNA-mediated interference
789 screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic
790 leukaemia. *Nat. Cell Biol.* **12**, 372-379 (2010).
791
792 49. A. E. Belevych, S. E. Sansom, R. Terentyeva, H. T. Ho, Y. Nishijima, M. M. Martin,
793 H. K. Jindal, J. A. Rochira, Y. Kunitomo, M. Abdellatif, C. A. Carnes, T. S. Elton, S.
794 Györke, D. Terentyev, MicroRNA-1 and -133 increase arrhythmogenesis in heart
795 failure by dissociating phosphatase activity from RyR2 complex. *PLoS One* **6**,
796 e28324 (2011).
797

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

798 50. J. Chen, Z. J. Chen, Regulation of NF-κB by ubiquitination. *Curr. Opin. Immunol.*
799 **25**, 4-12 (2013).
800
801 51. H. Sebban, S. Yamaoka, G. Courtois, Posttranslational modifications of NEMO
802 and its partners in NF-kappaB signaling. *Trends Cell Biol*. **16**, 569-577 (2006).
803
804
805 52. D. Iliopoulos, S. A. Jaeger, H. A. Hirsch, M. L. Bulyk, K. Struhl, STAT3 activation
806 of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch
807 linking inflammation to cancer. *Mol. Cell* **39**, 493-506 (2010).
808
809 53. D. Takiuchi, H. Eguchi, H. Nagano, Y. Iwagami, Y. Tomimaru, H. Wada, K.
810 Kawamoto, S. Kobayashi, S. Marubashi, M. Tanemura, M. Mori, Y. Doki,
811 Involvement of microRNA-181b in the gemcitabine resistance of pancreatic
812 cancer cells. *Pancreatology* **13**, 517-523 (2013).
813
814 54. J. T. Xia, L. Z. Chen, W. H. Jian, K. B. Wang, Y. Z. Yang, W. L. He, Y. L. He, D. Chen,
815 W. Li, MicroRNA-362 induces cell proliferation and apoptosis resistance in
816 gastric cancer by activation of NF-κB signaling. *J. Transl. Med.* **12**, 33 (2014).
817
818 55. L. Song, L. Liu, Z. Wu, Y. Li, Z. Ying, C. Lin, J. Wu, B. Hu, S. Y. Cheng, M. Li, J. Li,
819 TGF-β induces miR-182 to sustain NF-κB activation in glioma subsets. *J. Clin.*
820 *Invest.* **122**, 3563-3578 (2012).
821

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

822 56. L. Song, C. Lin, H. Gong, C. Wang, L. Liu, J. Wu, S. Tao, B. Hu, S. Y. Cheng, M. Li, J.
823 Li, miR-486 sustains NF-κB activity by disrupting multiple NF-κB-negative
824 feedback loops. *Cell Res.* **23**, 274-289 (2013).
825
826 57. S. G. Hymowitz, I. E. Wertz, A20: from ubiquitin editing to tumour
827 suppression. *Nat. Rev. Cancer* **10**, 332-341 (2010).
828
829 58. H. Ye, X. Liu, M. Lv, Y. Wu, S. Kuang, J. Gong, P. Yuan, Z. Zhong, Q. Li, H. Jia, J.
830 Sun, Z. Chen, A. Y. Guo, MicroRNA and transcription factor co-regulatory network
831 analysis reveals miR-19 inhibits CYLD in T-cell acute lymphoblastic leukemia.
832 *Nucleic Acids Res.* **40**, 5201-5214 (2012).
833
834 59. C. R. Stewart, G. A. Marsh, K. A. Jenkins, M. P. Gantier, M. L. Tizard, D.
835 Middleton, J. W. Lowenthal, J. Haining, L. Izzard, T. J. Gough, C. Deffrasnes, J.
836 Stambas, R. Robinson, H. G. Heine, J. A. Pallister, A. J. Foord, A. G. Bean, L. F. Wang,
837 Promotion of Hendra virus replication by microRNA 146a. *J. Virol.* **87**, 3782-3791
838 (2013).
839
840 60. M. Trenkmann, M. Brock, R. E. Gay, B. A. Michel, S. Gay, L. C. Huber, Tumor
841 necrosis factor α-induced microRNA-18a activates rheumatoid arthritis synovial
842 fibroblasts through a feedback loop in NF-κB signaling. *Arthritis Rheum.* **65**, 916-
843 927 (2013).
844

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

845 61. M. C. Thounaojam, D. K. Kaushik, K. Kundu, A. Basu, MicroRNA-29b modulates
846 Japanese encephalitis virus-induced microglia activation by targeting tumor
847 necrosis factor alpha-induced protein 3. *J. Neurochem.* **129**, 143-154 (2014).
848
849 62. C. M. Wang, Y. Wang, C. G. Fan, F. F. Xu, W. S. Sun, Y. G. Liu, J. H. Jia, miR-29c
850 targets TNFAIP3, inhibits cell proliferation and induces apoptosis in hepatitis B
851 virus-related hepatocellular carcinoma. *Biochem. Biophys. Res. Commun.* **411**,
852 586-592 (2011).
853
854
855 63. M. Y. Balkhi, O. H. Iwenofu, N. Bakkar, K. J. Ladner, D. S. Chandler, P. J.
856 Houghton, C. A. London, W. Kraybill, D. Perrotti, C. M. Croce, C. Keller, D. C.
857 Guttridge, miR-29 acts as a decoy in sarcomas to protect the tumor suppressor
858 A20 mRNA from degradation by HuR. *Sci. Signal.* **6**, ra63 (2013).
859
860 64. J. W. Graff, A. M. Dickson, G. Clay, A. P. McCaffrey, M. E. Wilson, Identifying
861 functional microRNAs in macrophages with polarized phenotypes. *J. Biol. Chem.*
862 **287**, 21816-21825 (2012).
863
864 65. S. W. Kim, K. Ramasamy, H. Bouamar, A. P. Lin, D. Jiang, R. C. Aguiar,
865 MicroRNAs miR-125a and miR-125b constitutively activate the NF-κB pathway
866 by targeting the tumor necrosis factor alpha-induced protein 3 (TNFAIP3, A20).
867 *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7865-7870 (2012).
868

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

869 66. A. Weiss, L. Attisano, The TGFbeta superfamily signaling pathway. *Wiley*
870 *Interdiscip. Rev. Dev. Biol.* **2**, 47-63 (2013).
871
872 67. M. A. Travis, D. Sheppard, TGF-β activation and function in immunity. *Annu.*
873 *Rev. Immunol.* **32**, 51-82 (2014).
874
875
876 68. J. Massagué, TGFβ signalling in context. *Nat. Rev. Mol. Cell Biol.* **13**, 616-630
877 (2012).
878
879 69. R. T. Marquez, S. Bandyopadhyay, E. B. Wendlandt, K. Keck, B. A. Hoffer, M. S.
880 Icardi, R. N. Christensen, W. N. Schmidt, A. P. McCaffrey, Correlation between
881 microRNA expression levels and clinical parameters associated with chronic
882 hepatitis C viral infection in humans. *Lab. Invest.* **90**, 1727-1736 (2010).
883
884 70. G. Liu, A. Friggeri, Y. Yang, J. Milosevic, Q. Ding, V. J. Thannickal, N. Kaminski, E.
885 Abraham, miR-21 mediates fibrogenic activation of pulmonary fibroblasts and
886 lung fibrosis. *J. Exp. Med.* **207**, 1589-1597 (2010).
887
888 71. M. Yamada, H. Kubo, C. Ota, T. Takahashi, Y. Tando, T. Suzuki, N. Fujino, T.
889 Makiguchi, K. Takagi, T. Suzuki, M. Ichinose, The increase of microRNA-21 during
890 lung fibrosis and its contribution to epithelial-mesenchymal transition in
891 pulmonary epithelial cells. *Respir. Res.* **14**, 95 (2013).
892

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

893 72. J. Y. Wang, Y. B. Gao, N. Zhang, D. W. Zou, P. Wang, Z. Y. Zhu, J. Y. Li, S. N. Zhou,
894 S. C. Wang, Y. Y. Wang, J. K. Yang, miR-21 overexpression enhances TGF-β1-
895 induced epithelial-to-mesenchymal transition by target smad7 and aggravates
896 renal damage in diabetic nephropathy. *Mol. Cell Endocrinol*. **392**,163-172 (2014).
897
898 73. X. Zhong, A. C. Chung, H. Y. Chen, Y. Dong, X. M. Meng, R. Li, W. Yang, F. F. Hou,
899 H. Y. Lan, miR-21 is a key therapeutic target for renal injury in a mouse model of
900 type 2 diabetes. *Diabetologia* **56**, 663-674 (2013).
901
902 74. H. Zhu, Y. Li, S. Qu, H. Luo, Y. Zhou, Y. Wang, H. Zhao, Y. You, X. Xiao, X. Zuo,
903 MicroRNA expression abnormalities in limited cutaneous scleroderma and
904 diffuse cutaneous scleroderma. *J. Clin. Immunol.* **32**, 514-522 (2012).
905
906 75. H. Zhu, H. Luo, Y. Li, Y. Zhou, Y. Jiang, J. Chai, X. Xiao, Y. You, X. Zuo, MicroRNA-
907 21 in scleroderma fibrosis and its function in TGF-β-regulated fibrosis-related
908 genes expression. *J. Clin. Immunol*. **33**, 1100-1109 (2013).
909
910 76. L. Chen, Y. Li, Y. Fu, J. Peng, M. H. Mo, M. Stamatakos, C. B. Teal, R. F. Brem, A.
911 Stojadinovic, M. Grinkemeyer, T. A. McCaffrey, Y. G. Man, S. W. Fu, Role of
912 deregulated microRNAs in breast cancer progression using FFPE tissue. *PLoS*
913 *One* **8**, e54213 (2013).
914

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

915 77. Q. Li, D. Zhang, Y. Wang, P. Sun, X. Hou, J. Larner, W. Xiong, J. Mi, MiR-
916 21/Smad 7 signaling determines TGF-β1-induced CAF formation. *Sci. Rep.* **3**,
917 2038 (2013).
918
919 78. T. D. Bhagat, L. Zhou, L. Sokol, R. Kessel, G. Caceres, K. Gundabolu, R. Tamari, S.
920 Gordon, I. Mantzaris, T. Jodlowski, Y. Yu, X. Jing, R. Polineni, K. Bhatia, A.
921 Pellagatti, J. Boultwood, S. Kambhampati, U. Steidl, C. Stein, W. Ju, G. Liu, P. Kenny,
922 A. List, M. Bitzer, A. Verma, miR-21 mediates hematopoietic suppression in MDS
923 by activating TGF-β signaling. *Blood* **121**, 2875-2881 (2013).
924
925 79. H. Xia, L. L. Ooi, K. M. Hui, MicroRNA-216a/217-induced epithelial-
926 mesenchymal transition targets PTEN and SMAD7 to promote drug resistance
927 and recurrence of liver cancer. *Hepatology* **58**, 629-641 (2013).
928
929 80. Y. Chang, C. Liu, J. Yang, G. Liu, F. Feng, J. Tang, L. Hu, L. Li, F. Jiang, C. Chen, R.
930 Wang, Y. Yang, X. Jiang, M. Wu, L. Chen, H. Wang, MiR-20a triggers metastasis of
931 gallbladder carcinoma. *J. Hepatol.* **59**, 518-527 (2013).
932
933 81. A. Parikh, C. Lee, J. Peronne, S. Marchini, A. Baccarini, V. Kolev, C. Romualdi, R.
934 Fruscio, H. Shah, F. Wang, G. Mullokandov, D. Fishman, M. D'Incalci, J. Rahaman, T.
935 Kalir, R. W. Redline, B. D. Brown, G. Narla, A. DiFeo, microRNA-181a has a critical
936 role in ovarian cancer progression through the regulation of the epithelial-
937 mesenchymal transition. *Nat. Commun.* **5**, 2977 (2014).
938

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

939 82. A. L. Smith, R. Iwanaga, D. J. Drasin, D. S. Micalizzi, R. L. Vartuli, A. C. Tan, H. L.
940 Ford, The miR-106b-25 cluster targets Smad7, activates TGF-β signaling, and
941 induces EMT and tumor initiating cell characteristics downstream of Six1 in
942 human breast cancer. *Oncogene* **31**, 5162-5171 (2012).
943
944 83. Q. Li, C. Zou, C. Zou, Z. Han, H. Xiao, H. Wei, W. Wang, L. Zhang, X. Zhang, Q.
945 Tang, C. Zhang, J. Tao, X. Wang, X. Gao, MicroRNA-25 functions as a potential
946 tumor suppressor in colon cancer by targeting Smad7. *Cancer Lett.* **335**, 168-174
947 (2013).
948
949 84. Y. Li, H. Wang, J. Li, W. Yue, MiR-181c modulates the proliferation, migration,
950 and invasion of neuroblastoma cells by targeting Smad7. *Acta. Biochim. Biophys.*
951 *Sin. (Shanghai)* **46**, 48-55 (2014).
952
953 85. M. E. Ezzie, M. Crawford, J. H. Cho, R. Orellana, S. Zhang, R. Gelinas, K. Batte, L.
954 Yu, G. Nuovo, D. Galas, P. Diaz, K. Wang, S. P. Nana-Sinkam, Gene expression
955 networks in COPD: microRNA and mRNA regulation. *Thorax* **67**, 122-131 (2012).
956
957 86. D. David, S. A. Nair, M. R. Pillai, Smurf E3 ubiquitin ligases at the cross roads
958 of oncogenesis and tumor suppression. *Biochim. Biophys. Acta.* **1835**, 119-128
959 (2013).
960
961 87. S. Cao, L. Xiao, J. N. Rao, T. Zou, L. Liu, D. Zhang, D. J. Turner, M. Gorospe, J. Y.
962 Wang, Inhibition of Smurf2 translation by miR-322/503 modulates TGF-

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

963 β/Smad2 signaling and intestinal epithelial homeostasis. *Mol. Biol. Cell* **25**, 1234-
964 1243 (2014).
965
966 88. X. Liu, X. Gu, L. Sun, A. B. Flowers, A. W. Rademaker, Y. Zhou, H. Kiyokawa,
967 Downregulation of Smurf2, a tumor-suppressive ubiquitin ligase, in triple-
968 negative breast cancers: involvement of the RB-microRNA axis. *BMC Cancer* **14**,
969 57 (2014).
970
971 89. S. Vimalraj, N. C. Partridge, N. Selvamurugan, A Positive Role of MicroRNA-
972 15b on Regulation of Osteoblast Differentiation. *J. Cell. Physiol.* **229**, 1236-1244
973 (2014).
974
975 90. Y. Liu, W. Liu, C. Hu, Z. Xue, G. Wang, B. Ding, H. Luo, L. Tang, X. Kong, X. Chen,
976 N. Liu, Y. Ding, Y. Jin, MiR-17 modulates osteogenic differentiation through a
977 coherent feed-forward loop in mesenchymal stem cells isolated from periodontal
978 ligaments of patients with periodontitis. *Stem Cells* **29**, 1804-1816 (2011).
979
980 91. W. Wang, F. Ren, Q. Wu, D. Jiang, H. Li, Z. Peng, J. Wang, H. Shi, MicroRNA-497
981 inhibition of ovarian cancer cell migration and invasion through targeting of
982 SMAD specific E3 ubiquitin protein ligase 1. *Biochem. Biophys. Res. Commun.* **449**,
983 432-437 (2014).
984
985 92. J. P. Edwards, H. Fujii, A. X. Zhou, J. Creemers, D. Unutmaz, E. M. Shevach,
986 Regulation of the expression of GARP/latent TGF-β1 complexes on mouse T cells

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

987 and their role in regulatory T cell and Th17 differentiation. *J. Immunol.* **190**,
988 5506-5515 (2013).
989
990 93. Q. Zhou, S. Haupt, I. Prots, K. Thümmler, E. Kremmer, P. E. Lipsky, H. Schulze-
991 Koops, A. Skapenko, miR-142-3p is involved in CD25+ CD4 T cell proliferation by
992 targeting the expression of glycoprotein A repetitions predominant. *J. Immunol.*
993 **190**, 6579-6588 (2013).
994
995 94. J.-H. Luo. Oncogenic activity of MCM7 transforming cluster. *World J. Clin Oncol.*
996 **2,** 120–124 (2011).
997
998 95. S. Baskerville, D. P. Bartel, Microarray profiling of microRNAs reveals
999 frequent coexpression with neighboring miRNAs and host genes. *RNA* **11,** 241-
1000 247 (2005).
1001
1002 96. F. Petrocca, A. Vecchione, Emerging Role of miR-106b-25/miR-17-92 Clusters
1003 in the Control of Transforming Growth Factor β Signaling. *Cancer Res* **68,** 8191-
1004 8194 (2008).
1005
1006 97. I. Matsumura, H. Tanaka, Y. Kanakura, E2F1 and c-Myc in cell growth and
1007 death. *Cell Cycle* **2,** 333-338 (2003).
1008
1009 98. Z. Zhang, Y.-W. Qin, G. Brewer, Q. Jing, MicroRNA degradation and turnover:
1010 regulating the regulators. *Wiley Interdiscip. Rev. RNA* **3,** 593–600 (2012).

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com

1011

1012 99. E. Berezikov, Evolution of microRNA diversity and regulation in animals

1013 *Nature Rev. Genetics* **12,** 846-860 (2011).

1014

1015 100. C. Backes, E. Meese, H.-P. Lenhof, A. Keller, A dictionary on microRNAs and

1016 their putative target pathways. *Nucl. Acids Res.* **38,** 4476-4486 (2010).

1017

1018 101. M. Lindow, S. Kauppinen, Discovering the first microRNA-targeted drug. *J.*

1019 *Cell Biol.* **199,** 407-412 (2012).

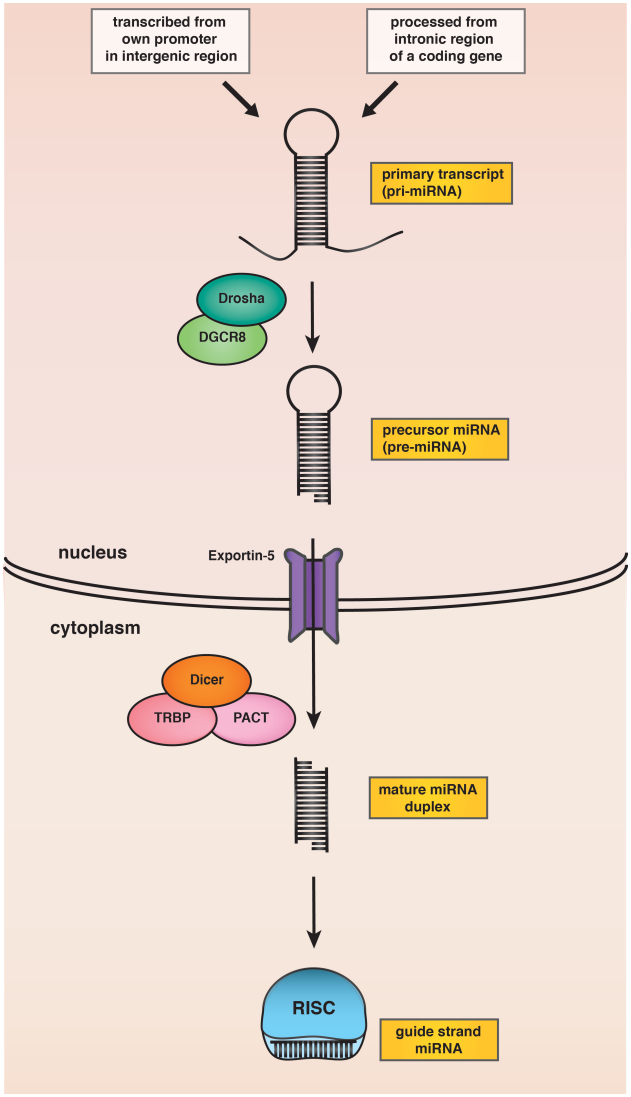
1020

1021 102. A. Bouchie, First microRNA mimic enters clinic. *Nature Biotechnology* **31,**

1022 577 (2013).

1023

Published in final edited form as:
Cell Mol Life Sci. 2015 Sep; 72(18):3531-42. doi: 10.1007/s00018-015-1940-0.
The final publication is available at link.springer.com



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1025 Fig.1 Biogenesis of microRNA. In the nucleus, miRNA is either transcribed from
1026 its own promoter in intergenic region or processed from the intronic region of a
1027 coding gene as a primary transcript (pri-miRNA). It is processed by an RNase III-
1028 type endonuclease family protein Drosha, with an accessory dsRNA-binding
1029 protein DGCR8, into a precursor molecule (pre-miRNA) with stem-loop structure
1030 of around 60-70nt in length and a 2nt overhang at the 3' end. It is then exported
1031 to the cytoplasm by a transport protein Exportin-5 . In the cytoplasm, pre-miRNA
1032 is further processed by another RNase III-type endonuclease family protein
1033 Dicer, in some cases also with the help of accessory dsRNA-binding proteins

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PACT and TRBP, into a dsRNA duplex of 19-21nt base pair region and a 2nt overhang at each 3' end. One of the two strand (guide strand) from this miRNA duplex is loaded onto RISC complex to effect its gene silencing function.

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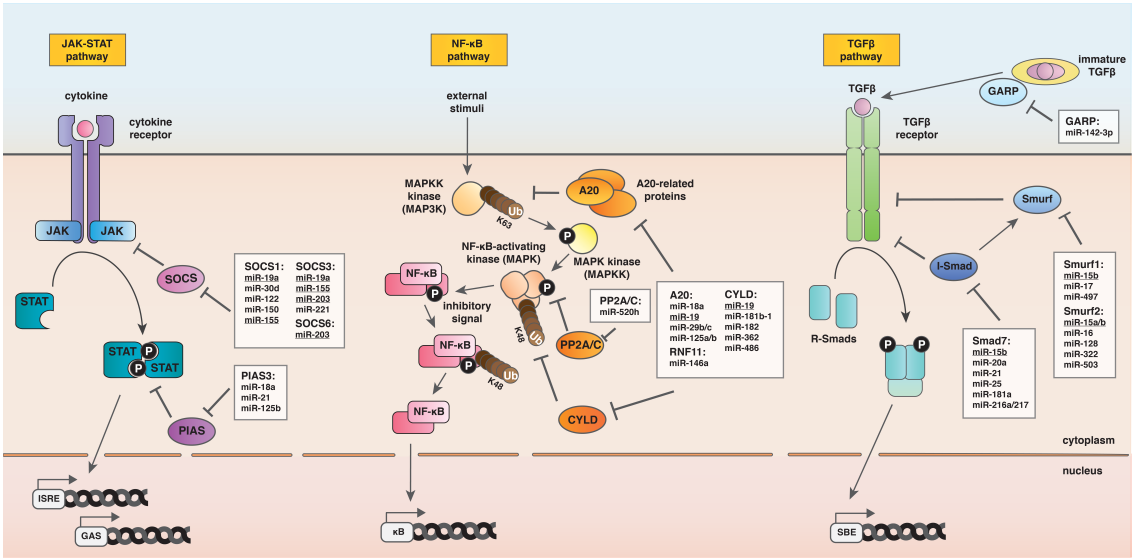

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1039 Fig.2 Regulation of key regulators of cellular pathways by miRNAs. Left: JAK-
1040 STAT pathway. Binding of cytokine to its cognate receptor pair activates and
1041 phosphorylates receptor-associated JAK kinase which then phosphorylates
1042 downstream transcription factor STATs. Activated STATs dimerize and expose
1043 nuclear localization signal to enter nucleus and promote transcription from
1044 promoter carrying interferon-stimulated responsive element (ISRE) or IFN-γ
1045 activated sequence (GAS). SOCS proteins negatively regulate JAK kinase by
1046 blocking the binding with STATs and promoting the degradation of cytokine
1047 receptors; and PIAS proteins negatively regulate STAT transcription factors by
1048 blocking its binding to DNA and recruiting corepressor to inhibit transcription.
1049 Both SOCS and PIAS are under tight regulation by miRNAs. Middle: NF-κB
1050 pathway. Both canonical and non-canonical NF-κB pathway are activated
1051 through similar mechanism. Under unstimulated condition, the activity of NF-κB
1052 is suppressed by an inhibitory signal (canonical: inhibitor of κB (IκB); non-
1053 canonical: ankyrin repeats on p100). To remove this inhibitory constrain, the

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inhibitory signal needs to be labeled by K48-polyubiquitin chain and targeted to proteosomal processing. Prior to K48-polyubiquitination by E3 ligase, the target protein must be phosphorylated by an NF-κB-activating kinase, also known as mitogen-activating protein kinase (MAPK) (for example, canonical: IκB kinase (IKK); non-canonical: NF-κB-inducing kinase (NIK). In order to become activated to mediate downstream phosphorylation event, MAPK needs to be phosphorylated by an upstream kinase, also known as MAPK kinase (MAPKK) (for example TGFβ activated kinase-1 (TAK1)). This activating phosphorylation can be removed by protein phosphatase PP2A/C. Like MAPK, activation of MAPKK requires the phosphorylation of another upstream kinase, also known as MAPKK kinase (MAP3K). MAP3K can be activated by K63-polyubiquitination in response to external stimuli. In terms of tumor necrosis factor (TNF) stimulation, the MAP3K protein, receptor-interacting protein (RIP)1 kinase, can be deactivated by deubiquitinase A20, as well as other A20-related proteins, including its binding partner Ring Finger Protein 11 (RNF11) as well as another deubiquitinase CYLD, by removing its K63-polyubiquitin chain and recruiting E3 ligase to tag a K48-polyubiquitin chain to promote its degradation. All these regulators, A20, RNF11, CYLD, and PP2A/C, can be regulated by miRNAs. Right: TGFβ pathway. Binding of TGFβ to its cognate receptors phosphorylates and activates receptor Smad (R-Smad) proteins, such as Smad2 and 3. R-Smad proteins bind other coactivator and translocate into nucleus to drive transcription from promoter with Smad-binding element (SBE). Activation of R-Smad proteins can be inhibited by inhibitory Smad (I-Smad) such as Smad7 through direct blockage of receptor and recruitment of other deactivating

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enzymes such as Smad ubiquitin regulatory factor (Smurf) E3 ligase. TGFβ is normally secreted in a closed immature form into the extracellular matrix. Glycoprotein A repetitions predominant protein (GARP) expressed by T regulatory (Treg) cells can facilitate the maturation of TGFβ, thus acting as a positive regulator of the pathway. I-Smad, Smurf and GARP can be regulated by miRNAs. miRNAs that can regulate multiple cellular targets from the same signaling pathway are underlined.

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