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Review

MicroRNAs in NF-κB signaling

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Nuclear factor κB (NF-κB) is a transcriptional factor that regulates a battery of genes that are critical to innate and adaptive immunity, cell proliferation, inflammation, and tumor development. MicroRNAs (miRNAs) are short RNA molecules of 20–25 nucleotides in length that negatively regulate gene expression in animals and plants primarily by targeting 3′ untranslated regions of mRNAs. In this work, we review the convergence of miRNAs and NF-κB signaling and dysregulation of miRNAs and NF-κB activation in human diseases, particularly in cancer. The function of miR-146, miR-155, miR-181b, miR-21, and miR-301a in NF-κB activation and their impact on tumorigenesis are discussed. Given that over 1000 human miRNAs have been identified, rendering miRNAs one of the most abundant classes of regulatory molecules, deciphering their biological function and pathological contribution in NF-κB dysregulation is essential to appreciate the complexity of immune systems and to develop therapeutics against cancer.

Keywords: microRNA, NF-κB, cancer

Introduction

Nuclear factor κB (NF-κB) is a transcriptional regulator consisting of reticuloendotheliosis (Rel) protein dimers that bind a DNA sequence motif known as κB site. The Rel protein family is classified into two groups: one that requires proteolytic processing and the other that does not. The first includes NF-κB1 (also known as p105) and NF-κB2 (p100), which are processed to produce the mature p50 and p52 proteins, respectively. The second includes RelA (also known as p65), RelB, and c-Rel. All Rel proteins can form homodimers or heterodimers, except for RelB, which can only form heterodimers (Ryseck et al., 1995), while p50-RelA heterodimer is the most abundant form of NF-κB in most, if not all, unstimulated cells. We refer to the p50-RelA dimer as NF-κB in this work unless indicated otherwise. Transient NF-κB activation is regulated by two major pathways (Karin et al., 2002; Karin, 2006). The first canonical NF-κB pathway applies to dimers that are composed of RelA, c-Rel, or p50, which are retained in the cytoplasm by inhibitors of κB proteins (IκBα, IκBβ, IκBγ, IκBε, IκBζ, IκBNS, and Bcl-3). This classical pathway is normally triggered in response to microbial and viral infections and exposure to proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, all of which activate the β-subunit of IκB kinase (IKKβ) complex through the toll-like receptor (TLR). IKK phosphorylates NF-κB-bound IκBs, leading to ubiquitin-dependent degradation of IκBs and translocation of NF-κB dimers to the nucleus. The second pathway is triggered by certain members of the TNF cytokine family (such as LTβ) that selectively activate the α-subunit of IKK (IKKα) through the TNF receptor, along with another protein kinase called NIK, causing phosphorylation of p100. This phosphorylation leads to polyubiquitination-dependent degradation of the C-terminal half of p100 to generate p52, allowing the formation of p52-RelB heterodimers, which then translocate to the nucleus and activate target genes.

During the past decade, much accumulated evidence has supported the role of NF-κB in linking inflammation and tumorigenesis (Karin, 2006; Inoue et al., 2007). Several pro-inflammatory cytokines and chemokines, such as TNF-α, IL-1, IL-6, and IL-8, produced upon NF-κB activation, are associated with tumor development and progression (Karin and Greten, 2005). Furthermore, NF-κB can also be activated by many oncogenes and chemopreventive chemicals to play a crucial role in tumorigenesis and tumor progression (Bharti and Aggarwal, 2002). Recently, somatic mutation has been implicated to be causative in NF-κB activation in cancers as a large number of genetic abnormalities are found in genes involved in the canonical or alternative NF-κB pathway. The CARD11 gene encoding a cytoplasmic scaffolding protein is found to be mutated in activated B-cell (ABC)-like diffuse large B-cell lymphoma (DLBCL) and such mutations result in constitutive NF-κB activation and enhanced NF-κB activity upon antigen/receptor stimulation in lymphoma cell lines (Lenz et al., 2008). Over a dozen NF-κB-relevant genes in multiple myeloma are mutated, amplified, truncated, or deleted with a few genetic alterations confirmed to activate NF-κB (Annunziata et al., 2007; Keats et al., 2007). NF-κB activation mechanisms in solid tumors, however, have not been well understood. A recent comprehensive exome analysis of 24 pancreatic tumors, a cancer with constitutively activated NF-κB (Sarkar et al., 2007), revealed that there are plenty of mutations in genes in 12 cellular signaling

pathways and processes, but few in genes within the NF-κB network (Jones et al., 2008; Yachida et al., 2010).

MicroRNAs (miRNAs) are a class of short (20–23 nucleotides in length), endogenous, single-stranded RNAs that regulate gene expression. miRNAs are initially transcribed by either RNA polymerase II or RNA polymerase III, as a long primary miRNA transcript (pri-miRNA). It is then cleaved in the nucleus by the microprocessor complex, Drosha-DGCR8, resulting in a precursor hairpin (pre-miRNA) ranging in length from 60 to 110 nucleotides. The pre-miRNA is exported from the nucleus to the cytoplasm by exportin-5-Ran-GTP. In the cytoplasm, Dicer, a member of the RNase III family, in complex with TRBP, cleaves the pre-miRNA hairpin to a ~22 bp miRNA duplex. The mature miRNA is incorporated with argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC), where miRNA guides the complex to partial complementary binding sites located in the 3′ untranslated region (UTR) of target mRNAs to suppress gene expression. A recent report suggests that miRNA-binding sites also occur within the 5′ UTR and in the coding region (Hafner et al., 2010). It was long believed that the partial complementarity favors inhibition of translation initiation; however, recent studies suggest that miRNAs predominantly act to decrease target mRNA levels, at least in mammals (Baek et al., 2008; Guo et al., 2010). Numerous investigations have supported the role of miRNAs in the initiation and progression of human cancer, as well as in physiological function such as immune responses, cell proliferation, cell death, and inflammation, which are also known to be regulated by NF-κB (Baud and Karin, 2009). Naturally, this has led many researchers to look into the convergence of miRNAs and their target genes with NF-κB signaling cascades that are critical to tumor development and malignant progression. Here we discuss some key miRNAs intertwined with NF-κB signaling (Figure 1) and their roles in cancer.

miR-146

miR-146 was first identified as an immune system regulator in a systematic effort to find miRNAs that influence the mammalian response to microbial infection. Exposure of human monocytic THP-1 cells to lipopolysaccharides (LPS) results in rapid induction of the expression of both miR-146a and miR-146b (Taganov et al., 2006). Further characterization of miR-146a/b revealed that it is induced through TLR and the induction is NF-κB dependent. Importantly, two key adapter molecules in the TLR/NF-κB pathway, TRAF6 and IRAK1, are identified as direct targets of miR-146. This suggests a negative regulatory loop, in which NF-κB activation upregulates miR-146 gene that, upon processing and maturation, down-regulates IRAK1 and TRAF6 to reduce the activity of NF-κB. Subsequent studies have shown the pathological relevance of NF-κB/miR-146 in human breast cancer, pancreatic cancer, anaplastic thyroid carcinomas, brain tumors, and mesenchymal stem cells (Bhaumik et al., 2008; Lukiw et al., 2008; Hurst et al., 2009; Li et al., 2010b; Pacifico et al., 2010; Suzuki et al., 2010). In an experiment that directly demonstrates miR-146 as an NF-κB negative regulator, the phosphorylation of IκBα on serine 32, which is essential for its degradation, is reduced to ~40% or 20% of control levels in cells expressing miR-146a or miR-146b (Bhaumik et al., 2008). Moreover, miR-146a and miR-146b have also shown to inhibit migration and invasion in breast cancer cells (Bhaumik et al., 2008; Hurst et al., 2009).

In human lung alveolar epithelial tumor A549 cells, IL-1β induces miR-146a and negatively regulates the release of IL-8 and RANTES (regulated upon activation, normal T-cell expressed, and secreted; also known as CCL5) (Perry et al., 2008). IL-8 and RANTES are regulated by NF-κB activation, providing additional support for the negative feedback regulation of inflammation following activation of the innate immune response. However, this is only observed at high IL-1β concentrations, indicating that it may be an important feedback mechanism during severe inflammation. miR-146 is also reported to be a contributor in pancreatic β-cell function (Lovis et al., 2008). Increased levels of miR-146 are observed in pancreatic β-cells incubated with free fatty acids (FFAs) and are also found in pancreatic islets from diabetic db/db obese mice. Moreover, induction of miR-146 promotes β-cell apoptosis, while miR-146 inhibition reduces β-cell death elicited by FFAs. Activation of NF-κB is proposed to be a key event in the progressive loss of β-cells in diabetes, as inhibition of this process protects β-cells against cytokine-induced apoptosis (Roggli et al., 2010). Treating cells with oligonucleotides blocking miR-146 partially protects them against palmitate-induced apoptosis, suggesting that miR-146 contributes to the detrimental effects of palmitate on β-cells (Roggli et al., 2010). Overall, miR-146 is an NF-κB transactivational target and negatively regulates IRAK1 and TRAF6, constituting a negative feedback loop. Upregulation of miR-146a is reported in papillary thyroid carcinoma, cervical cancer, breast cancer, and pancreatic cancer, whereas reduced miR-146a expression is associated with prostate cancer (Williams et al., 2008). Thus, it is unknown whether miR-146a dysregulation is causal to cancer.

miR-155

miR-155 is processed from a non-protein-coding primary transcript, called 'BIC'. BIC/miR-155 has been shown to be highly expressed in a variety of human B cell lymphomas, including Hodgkin lymphoma, primary mediastinal B-cell lymphoma, and DLBCL, suggesting that this miRNA may contribute to the etiology of lymphomas (Kluiver et al., 2005). In monocytes, macrophages, and myeloid dendritic cells, miR-155 is induced substantially after exposure to a variety of inflammatory cytokines such as IFN-β and IFN-γ (O'Connell et al., 2007). Yet there are some discrepancies regarding whether the induction of BIC and miR-155 is NF-κB dependent. Overexpression of an IκBα dominant negative protein does not block antigen-B cell receptor (BCR)-mediated induction of BIC in Ramos cells, a Burkitt lymphoma cell line (van den Berg et al., 2003). However, inhibition of IKK leads to a reduction in BIC and other NF-κB-regulated transcripts in ABC-like DLBCL cells with constitutively active NF-κB (Lam et al., 2008). A third report supports BCR signaling regulation of pri-miR-155 expression via activation of the PKC-NF-κB pathway in Ramos cells because blocking either PKC signaling or NF-κB activation abrogates the induction of BIC expression (Kluiver et al., 2007). Induced or ectopic expression of BIC in Ramos, HEK293, normal tonsillar B cells, and other Burkitt lymphoma cell lines results in miR-155 overexpression in all cell

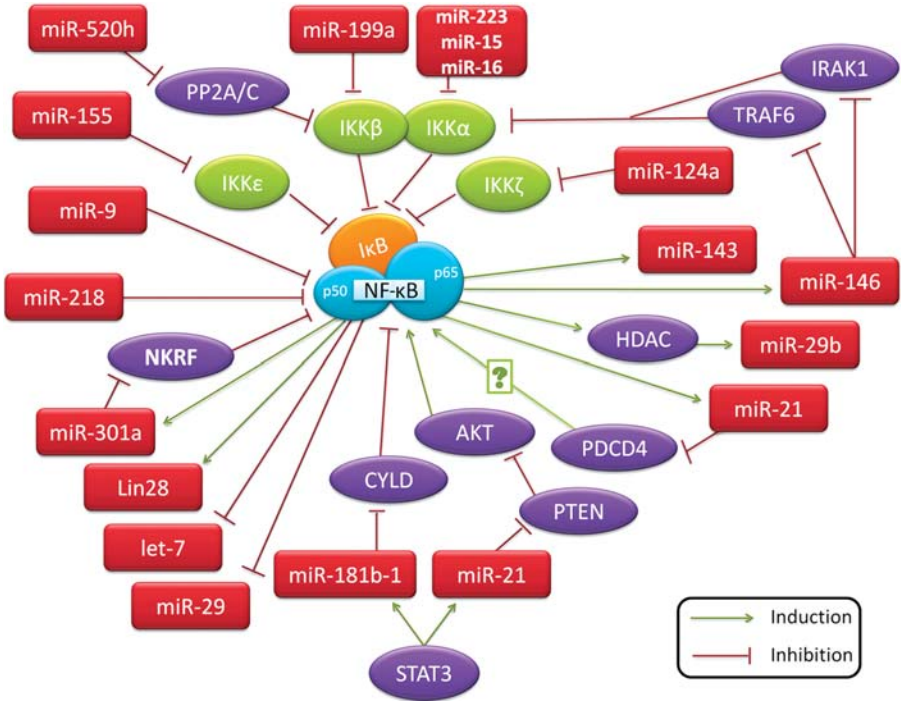

Figure 1 Panoramic view of miRNAs converged with the NF-κB network.

lines except Ramos cells (Kluiver et al., 2007), suggesting an unknown mechanism of differential miR-155 and BIC regulation in Ramos cells.

Most recent publications on miR-155 support a positive correlation between miR-155 upregulation and NF-κB activation. First, when the expression pattern of miR-155 in twenty DLBCL cell lines is examined, it is found that this miRNA is expressed at a higher level in ABC-like DLBCL cells (Rai et al., 2008). Second, both miR-155 expression and NF-κB activation are significantly elevated at early stages of choline-deficient and amino-acid-defined (CDAA) diet-induced hepatocarcinogenesis mouse model (Wang et al., 2009). Third, miR-155 is induced during *Helicobacter pylori* infection, which stimulates NF-κB (Xiao et al., 2009). Fourth, in human mesangial cells, IFN-γ and TNF-α induce miR-155 expression and regulate inflammatory and immune responses, which are dependent on transforming growth factor-β-activated kinase-1 (TAK1)-binding protein 2 (TAB2) and NF-κB (Imaizumi et al., 2010). Finally, in a mouse model of alcoholic liver disease, chronic alcohol consumption increases miR-155 in macrophages via NF-κB, and the increased miR-155 levels contribute to alcohol-induced elevation in TNF-α production (Bala et al., 2011).

A few target genes (FADD, IKKε, Ripk1, and PU.1) of miR-155 have been identified (Tili et al., 2007; Vigorito et al., 2007; Thompson et al., 2011). In addition, the splenocytes of Eμ-miR-155 transgenic mice, which specifically overexpress miR-155 in B cells, displayed lower levels of IKKβ transcripts than their wild type counterparts (Costinean et al., 2006). Thus, miR-155 may control the expression of both IKKβ and IKKε, which leads to repression of, or at least limitation of NF-κB activation, constituting a negative feedback loop. Taken together, these results indicate that miR-155 is an NF-κB transactivational target and is involved in a negative feedback loop through down-regulation of IKKs and other genes. miR-155 is upregulated in B-cell lymphomas and chronic lymphocytic leukemia (Eis et al., 2005), as well as in solid tumors of lung (Yanaihara et al., 2006), breast (Iorio et al., 2005), colon, pancreas (Gironella et al., 2007; Greither et al., 2010), and thyroid (Nikiforova et al., 2008), indicating its oncogenic role.

miR-181b

miR-181b-1 has recently been identified as a key player in a positive feedback loop linking inflammation to an epigenetic switch that controls cellular transformation in human mammary epithelial MCF-10A cells (Iliopoulos et al., 2010). Inhibition of miR-181b-1 in colon, prostate, and hepatocellular cancer cell lines reduced colony formation. Signal transducer and activator of transcription 3 (STAT3), a transcription factor upregulated during transformation, and miR-181b-1 expression levels are positively correlated in colon adenocarcinomas as well as in MCF-10A cells during transformation. Furthermore, miR-181b-1 and CYLD are inversely correlated in these tumors and in MCF-10A cells. CYLD is a tumor suppressor and deubiquitinating enzyme known to negatively regulate NF-κB (Trompouki et al., 2003). miR-181b-1 is found to be transactivated by STAT3, resulting in a positive feedback loop: STAT3 binds promoter regions in the miR-181b-1 gene to increase its transcription, which then inhibits CYLD production, which in turn causes increased NF-κB activation. NF-κB works to complete this feedback loop by increasing IL-6 production, leading to STAT3 phosphorylation and activation. However, miR-181b-1 is not simply a downstream effector of this signaling cascade. Transient transfection of MCF-10A cells with miR-181b-1 caused stable transformation of these cells, allowing them to be passaged for at least 30 days while retaining the

ability to form colonies in soft agar, suggesting the involvement of an epigenetic switch. Therefore, miR-181b is indirectly regulated by NF-κB in a positive feedback loop (NF-κB → IL-6 → STAT3 → miR-181b → CYLD → NF-κB) and participates in an exclusive epigenetic circuit to promote cell transformation. Overexpression of miR-181b is associated with the progression of leukoplakia to oral carcinoma (Cervigne et al., 2009), as well as poor prognosis and therapeutic outcome in colon cancer (Schetter et al., 2008). Yet down-regulation of miR-181b-1 is observed in human glioma cells (Shi et al., 2008) and astrocytic tumors (Conti et al., 2009), suggesting that miR-181b may have a tumor-type-specific role.

miR-21

Unlike miR-181b-1, the function of miR-21 has been elucidated to a greater extent, its pervasive overexpression patterns in cancer have been fleshed out, and many of its predicted targets have been confirmed (Liu et al., 2010a). Instead of providing the scientific community with more answers, however, this plethora of information only serves to raise more questions. One of which is the mechanism behind miR-21's complex relationship with NF-κB (Young et al., 2010). In MCF-10A cells, miR-21 is characterized as part of the positive feedback loop linking inflammation to cellular transformation involved in STAT3 (Iliopoulos et al., 2010). Inhibition of STAT3 resulted in lower expression levels of miR-21. PTEN, a target of miR-21, is a known inhibitor of AKT phosphorylation that promotes NF-κB activation and enhances tumorigenesis. Thus, miR-21 works within the inflammation-transformation positive feedback loop by down-regulating PTEN expression to increase NF-κB activity.

Opposing this feedback loop in which miR-21 serves to enhance NF-κB activation is the recent study reporting that miR-21 is induced by LPS to attenuate pro-inflammatory effects of TLR4 signaling by negatively regulating NF-κB activity (Sheedy et al., 2010). Mice deficient in PDCD4, a confirmed miR-21 target (Lu et al., 2008), exhibit lower LPS-induced mortality rates, lower IL-6 production, and increased IL-10 protein levels. As depletion of the NF-κB subunit p65 abolished LPS-induced miR-21 expression, the authors then show that miR-21 is an NF-κB transactivational gene, which is supported in another report (Shin et al., 2011). Inhibition of miR-21 blocks PDCD4 down-regulation induced by LPS and increases NF-κB activation as well as the pro-inflammatory cytokine IL-6, yet decreases the levels of the anti-inflammatory cytokine IL-10. Thus, miR-21 acts as an anti-inflammatory agent within a negative regulatory loop: NF-κB activity is necessary for miR-21 induction, but by targeting PDCD4, miR-21 works to inhibit NF-κB and its pro-inflammatory transcriptional targets. Cell-type specificity may cause the irreconcilable difference of miR-21 in NF-κB activity: in epithelial cells, miR-21 acts to down-regulate PTEN, activate AKT, and increase NF-κB activation; in LPS-stimulated macrophages, miR-21 negatively regulates PDCD4, which activates NF-κB through an unknown mechanism. It is notable that during earlier stages of liver regeneration, miR-21 is upregulated, leading to down-regulation of pellino (Marquez et al., 2010), an activator of NF-κB, supporting that miR-21 is an NF-κB inhibitor. Work from animal models provides no insight in this regard: the expression of miR-21 target genes is not widely upregulated in tissues of miR-21 knockout mice as one would expect (Hatley et al., 2010; Patrick et al., 2010). There is an omnipresent overexpression of miR-21 in all types of human carcinomas (Liu et al., 2010a), as well as chronic lymphocytic leukemia (Fulci et al., 2007), diffuse large-B-cell lymphoma (Lawrie et al., 2007), acute myeloid leukemia (Jongen-Lavrencic et al., 2008), and Hodgkin lymphoma (Navarro et al., 2008). Interestingly, NF-κB activation has been reported in all of these cancers (Baud and Karin, 2009), underscoring the interplay of miR-21 overexpression and NF-κB activation in cancer. Further evidence is needed to dissect the role of miR-21 in NF-κB signaling and inflammation.

miR-301a

miR-301a is a newly identified miRNA that activates NF-κB. Using an NF-κB-dependent reporter screening, miR-301a stands out as the most potent NF-κB activator from hundreds of human miRNA minigenes (Lu et al., 2011). It was then demonstrated that miR-301a down-regulates NF-κB repressing factor (NKRF) and elevates NF-κB activation. When NKRF was first discovered, it was deemed to interact with specific negative regulatory elements (NREs) to mediate NF-κB's transcriptional activity, which regulates the expression of three NF-κB-responsive genes including IL-8, IFN-β, and NOS2A (Nourbakhsh and Hauser, 1999; Nourbakhsh et al., 2001; Feng et al., 2002). Yet much evidence is provided to suggest that NKRF, like IκBα, broadly inhibits the expression of NF-κB transactivational targets such as MMP2 and COX2 without NREs. As the promoter to miR-301a also contains a *bona fide* κB site (Lu et al., 2011), these results support a positive feedback loop as a mechanism for persistent NF-κB activation in which miR-301a represses NKRF to elevate NF-κB activity, which in turn, promotes miR-301a transcription.

Interestingly, miR-301a was first reported to be only specifically overexpressed (34.2-fold with a *P*-value of 1.11E−05) in pancreatic adenocarcinoma tumors and tumor cell lines compared with normal pancreas and pancreatitis, but not in other types of cancers (Lee et al., 2007). Yet, later, it was found to be upregulated (2.19-fold with a *P*-value of 0.0213) in hepatocellular carcinoma at a much lower level and less significantly (Jiang et al., 2008). Examination of NKRF expression reveals that it is down-regulated in human pancreatic adenocarcinoma tissues. Moreover, miR-301a inhibition or NKRF upregulation in pancreatic cancer cells led to reduced NF-κB target gene expression and attenuated xenograft tumor growth (Lu et al., 2011), indicating that miR-301a overexpression contributes to NF-κB activation. Revealing this novel mechanism of NF-κB activation by a miRNA offers new avenues for therapeutic interventions against pancreatic cancer. There are still some unanswered questions about miR-301a and NF-κB. First, what drives miR-301a overexpression in pancreatic cancer? Second, is miR-301a overexpression a cause or a consequence of pancreatic cancer pathogenesis? Third, why do mice without the NKRF allele have no obvious phenotype (Froese et al., 2006)? Answers to these questions will allow us to gain a comprehensive view of miR-301a in NF-κB signaling and pancreatic tumor development.

Other miRNAs in the NF-κB network

There are other miRNAs that suppress genes coding for NF-κB, IκB, and IKK proteins. miR-9 is another miRNA induced by LPS via MyD88 and NF-κB (Bazzoni et al., 2009). Induction of miR-9 is also mediated by the proinflamatory cytokines IL-1β and TNF-α, but not by IFN-γ. As NF-κB1 encoding p105 (processed into p50) is experimentally confirmed as a miR-9 target, a new model is proposed with the induction of miR-9 acting as a fine-tuning mechanism to prevent negative regulation by p50 homodimers in monocytes in anti-inflammatory response. Moreover, miR-9 inhibits ovarian and gastric cancer cell growth through modulation of the NF-κB pathway (Guo et al., 2009; Wan et al., 2010; Wang et al., 2010). miR-199a negatively regulates IKKβ expression to reduce NF-κB activity in ovarian cancer cells (Chen et al., 2008). MyD88-positive epithelial ovarian cancer cells have high levels of IKKβ due to lower miR-199a expression, and *vice versa*. Twist1 is found to regulate the expression of both miR-199a and miR-214 within the same primary transcript to modulate IKKβ/NF-κB and PTEN/AKT pathways and subsequently impact the differentiation of epithelial ovarian cancer stem cells (Yin et al., 2010). miR-124a is the first reported miRNA targeting a member of the IκB family, IκBζ, though the biological importance of this regulation remains elusive (Lindenblatt et al., 2009). miR-143 is also an NF-κB transactivational target, and it promotes live tumor cell invasion and metastasis with FNDC3B as a direct and functional target (Zhang et al., 2009). Overexpression of miR-143 decreases cell viability and increases cell death of colon cancer cells upon exposure to 5-fluorouracil with an impact on NF-κB p65 expression (Borralho et al., 2009). IKKα is targeted by miR-223, miR-15 and miR-16 during monocyte–macrophage differentiation, and decreased expression of these miRNAs may prevent macrophage hyperactivation (Li et al., 2010a).

miR-15a and miR-16-1 are encoded by the miR-15/16 cluster, which resides at chromosome 13q14.3, a genomic region frequently deleted in chronic lymphocytic leukemia (CLL) and other malignancies, such as multiple myeloma, mantle cell lymphoma, and prostate carcinoma (Aqeilan et al., 2010). miR-15a and miR-16-1 are reported to inhibit cell proliferation, induce cellular apoptosis, and suppress tumorigenesis by targeting multiple oncogenes, including Bcl-2, Mcl1, CcnD1, and Wnt3A (Bonci et al., 2008). A recent report on miR-15a, miR-16, and miR-223 suggests that these miRNAs play a role in noncanonical NF-κB pathway (Li et al., 2010a). During human monocyte–macrophage differentiation, these three miRNAs were down-regulated by 75%–90%. Li et al. (2010a) further identified that IKKα, but not IKKβ or IKKγ, is a target gene of miR-15, miR-16, and miR-223. Additionally, the expression of TRAF2, NIK, and p52, which are important components in the noncanonical NF-κB pathway, were also affected with modulation of these miRNAs in macrophage cells and/or HeLa cells. An ensuing study of miR-16 in gastric cancer cells further supports the regulation of IKKα by miR-16 and provides evidence that miR-16 is an NF-κB transactivational target (Shin et al., 2011), indicating that miR-16 modulates the noncanonical NF-κB pathway through a feedback loop.

During the past two years, more miRNAs have been found to be involved in NF-κB signaling by targeting NF-κB regulators and effectors. One high-profile paper reports that transient Src activation triggers an inflammatory response mediated by NF-κB that directly activates Lin28 transcription and rapidly reduces let-7 miRNA levels. As let-7 directly inhibits IL-6 expression, reduced let-7 expression results in high levels of IL-6 to activate STAT3 and transform epithelial cells. Src-mediated inflammation activates this positive feedback loop that maintains the epigenetic transformed state for many generations in the absence of the inducing signal. This provides direct evidence linking inflammation to cellular transformation (Iliopoulos et al., 2009). miR-520h is involved in adenovirus type 5 E1A-mediated tumor suppression through the signal cascade, E1A → miR-520h → PP2A/C → IKK → NF-κB → Twist (Su et al., 2010). miR-29b is identified as a miRNA that is repressed by NF-κB. miR-29b is found in a signal cascade, KIT (a tyrosine kinase receptor) → Sp1 → NF-κB → HDAC → miR-29b, in which miR-29b represses Sp1 expression to promote KIT-driven leukemia (Liu et al., 2010b). By repressing the expression of another target YY1, miR-29b plays the role of a tumor suppressor regulated by the NF-κB/YY1 pathway, whose abnormal expression may contribute to myogenesis and rhabdomyosarcoma (Wang et al., 2008). NF-κB repression of miR-29b is also found in cholangiocytes and cholangiocarcinoma cells (Mott et al., 2010). Another NF-κB-regulated miRNA, miR-10a, is found to be a novel regulator in smooth muscle cell differentiation from embryonic stem cells (Huang et al., 2010). Other NF-κB-regulated miRNAs include miR-125b-1, miR-30b, miR-130a, the clusters miR-17-92 and miR-23b-27b-24-1, all of which may regulate epithelial antimicrobial defenses (Zhou et al., 2009, 2010).

Conclusion

miRNAs target scores of genes encoding NF-κB, IκB, IKK, regulators, and effectors in the NF-κB signaling network with the vast majority of them participating in positive or negative feedback loops. It remains to be ascertained whether dysregulation of miRNAs (rather than accompanied NF-κB activation) is causal to tumor development and progression. Nonetheless, miRNAs seem to be potent targets as many tumor cells are sensitive to up- or down-regulation of miRNAs. The seminal work from Slack and colleagues has demonstrated that miR-21 is such an oncomiR in mouse pre-B-cell lymphoma (Medina et al., 2010). Development of miRNA replacement therapy, therefore, may potentiate our current findings into future clinical applications against cancer and other diseases that have a component of constitutive NF-κB activation. The major challenges for targeting miRNAs directly using modified DNA or RNA oligonucleotides are similar to that of clinical delivery of RNA interference (RNAi), which include (but are not limited to) biological barriers, toxicities, and tissue specificity (Pecot et al., 2011). On the other hand, targeting miRNAs that are effectors of NF-κB using small chemical inhibitors of IKK/NF-κB may show disproportionate side effects with excessive and prolonged NF-κB inhibition, given the importance of NF-κB in immunity (Baud and Karin, 2009). We are cautiously optimistic that the development of new miRNA inhibition tools such as cholesterol conjugates (Krutzfeldt et al., 2005) and locked nucleic acids (LNAs) (Elmen et al., 2008) and the discovery of dysregulation and/or mutation

of specific components of NF-κB signaling in tumor initiation, progression, and maintenance will pave the way towards translating the immense popularity of miRNA-based therapeutic strategies into effective and marketable clinical solutions.

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