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Therapeutic microRNAs targeting the NF-kappa B Signaling Circuits of Cancers

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Abstract

MicroRNAs (miRNAs) not only directly regulate NF-κB expression, but also up- or down-regulate NF-κB activity via upstream and downstream signaling pathways of NF-κB. In many cancer cells, miRNA expressions are altered accompanied with an elevation of NF-κB, which often plays a role in promoting cancer development and progression as well as hindering the effectiveness of chemo and radiation therapies. Thus NF-κB-targeting miRNAs have been identified and characterized as potential therapeutics for cancer treatment and sensitizers of chemo and radiotherapies. However, due to cross-targeting and instability of miRNAs, some limitations of using miRNA as cancer therapeutics still exist. In this review, the mechanisms for miRNA-mediated alteration of NF-κB expression and activation in different types of cancers will be discussed. The results of therapeutic use of NF-κB-targeting miRNA for cancer treatment will be examined. Some limitations, challenges and potential strategies in future development of miRNA as cancer therapeutics are also assessed.

Keywords

miRNA; NF-κB; cancer; therapeutics

1. Introduction

1.1 MicroRNA

MicroRNA (miRNA) is a large family of single-strand non-protein coding RNAs about 22 nucleotides long, which function as negative gene regulators in both plant and animal cells [1, 2]. MiRNAs are initially transcribed by RNA polymerase II from the non-coding regions of the genome to form pri-miRNA. Pri-miRNAs are then processed by Drosha-DGCR8 complex to become pre-miRNAs, which are then transported from nucleus to cytoplasm by

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exportin-5 and cut by Dicer to form single-strand miRNAs. The majority of miRNAs bind to 3′UTR of the target gene with an imperfect match and inhibit the translation of the target gene. In the meantime, some miRNAs bind to the 3′URT with a perfect match, which leads to the mRNA cleavage of the target gene [3-5]. In either case, miRNAs inhibit protein expression of their target genes. Since the discovery of the first miRNA, lin4, hundreds of miRNAs have been found to date, and are believed to be associated with various physiological processes, such as development, apoptosis, and cancer [6-9].

In cancers, miRNAs can function as both tumor suppressors and oncogenes, depending on the target genes which they regulate [1]. However, in spite of the correlation between the expression of miRNAs and cancers, it is still not clear if it is the change of miRNA expression profile causes cancer, or development of cancer leads to a change in miRNA expression. Nevertheless, the role of miRNAs in cancer cells are critical and understanding the targets and function of miRNA as well as their downstream pathways is important and may have potential therapeutic applications. A detailed list of cancer types and the involved miRNA are listed in Table 1.

1.2 NF-κB

NF-κB is a transcript factor firstly identified in mature B cells in 1986. It was so named as it was found in the nucleus bound to the intronic enhancer region of the κ light chain gene in the B cells [10, 11]. NF-κB is a transcription factor which regulates the expression of many genes mostly related to immune and inflammatory responses, along with the genes determining developmental processes, cell growth and apoptosis [12-15]. The mammalian NF-κB family is composed of five members: p65 (RelA), RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100) [16]. All five family members have a Rel homology domain (RHD) which is responsible for DNA binding and dimerization between different or identical family members, leading to homomeric or heteromeric binding of the subunits. RelA, RelB, c-Rel share a transcriptional activation domain (TAD), while p50 and p52 do not [17, 18]. Thus, of all the possible NF-κB dimers, some function as transcriptional activators (e.g. RelA/p50 heterodimer), but others (e.g. p50/p50 homodimer) do not unless they recruit specific co-activator proteins, and some dimers are not known to bind DNA at all [19].

As a transcription factor typically involved in many signaling pathways, the activity of NF-κB is precisely regulated by both canonical and atypical pathways. For the canonical pathway, the inhibitor of NF-κB (IκB) kinase (IKK) is activated and phosphorylates IκB (inhibitor of NF-κB) at N-terminal serines (Ser32 and Ser36) [20, 21]. The phosphorylated lκB is then ubiquitin-targeted and rapidly degraded through the polyubiquitin-dependent proteasomal pathway, and the freed NF-κB translocates into the nucleus with its exposed nuclear localization signal peptide and activates its target genes [22-24]. Therefore, ubiquitination and proteasomal degradation pathways are often critical in regulating NF-κB activity. On the other hand, some stimuli such as ultraviolet radiation, regulate NF-κB through a more complicated pathway by regulating IκB translational levels via the phosphorylation of eIF2α which regulates global protein synthesis [25-28]. Other atypical NF-κB activation pathways include the IκB-independent activation of p50 and p52. Briefly,

upon stimulation, both p105 and p100 are proteasomal targeted and processed to p50 and p52 by partial proteolysis, which then translocate to nucleus and be activated [29].

The regulation of NF-κB expression and activation is tightly controlled in normal cells. One of the well-known regulatory mechanisms of NF-κB is that it can bind to the promoter region and up-regulate its inhibitor, IκB. By doing this, the activated NF-κB can increase IκB level and in turn shut down the activation of NF-κB itself. In this way, the NF-κB activation in normal cells is transient and naturally regulated. In many cancer cells, NF-κB has a constitutively high level of activity [14, 30]. This high level of NF-κB activity may be correlated with cancer development and progression [31-33]. In most cases, the activation of NF-κB leads to anti-apoptosis signaling pathways, stimulating proliferation, invasion, metastasis, and angiogenesis in cancer cells. Interestingly, the activation of NF-κB is not usually due to mutagenesis of NF-κB or IκB themselves, but due to deregulation of various signaling pathways, including but not limited to pathways that respond to virus infection, receptor activation, and constitutive activation of kinases [31, 34]. Therefore, targeting NF-κB as well as its related signaling pathways has always been considered as a potential therapeutic target for cancer treatments. As both NF-κB and miRNA are important regulators in cancer development and progression, we will discuss their relationship in various cancers in this review.

2. MicroRNA In Regulation Of NF-κB Signaling Circuits In Cancer Cells

2.1 Breast Cancer

NF-κB activation in breast cancer cells has raised much attention in the past years. In ER-negative/ErbB2-positive breast cancer tissue samples, 86% (6 out 7) showed NF-κB activation [35]. Inhibition of IKK by (NEMO)-binding domain (NBD) peptide blocked proliferation of human breast cancer SκBr3 cells induced by an NF-κB activator heregulin, suggesting that NF-κB is a potential target for breast cancer therapy. There have been many reports of reciprocal regulation between NF-κB and miRNAs in breast cancer (Fig. 1). MiR-31 targeted protein kinase C ε (PKCε) and reduced NF-κB activity indirectly [36]. PKC-mediated NF-κB activation has been suggested as an anti-tumor target as a PKC inhibitor Go6976 reduced the tumor growth *in vivo* due to suppression of the anti-apoptotic effect of NF-κB [37]. In MDA-MB-231 breast cancer cells, which express an undetectable level of miR-31, PKCε protein level was down-regulated after miR-31 overexpression, leading to reduced phosphorylated p65 level and NF-κB translocation, despite PKCε mRNA level was still intact. MiR-31 overexpression in the cells also increased the cell apoptosis by 1-fold and increased sensitivity of the cells to ionizing radiation and staurosporine. The suppressed BCL-2 partially mediated this sensitization, which was inversely related to miR-31 level in 99 patients. However, it should be noted that miR-31 overexpression also promoted cell death and radiosensitivity of normal breast epithelial MCF-10A cells, which express a higher level of miR-31 than MDA-MB-231 cancer cells. These results indicated that it would be essential to optimize the dosage of miR-31 when developing its potential for cancer treatment.

MiR-146a/b was identified as a suppressor for both TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1) in LPS stimulated THP-1 cells

[38]. TRAF6 and IRAK1 recruit transforming-growth-factor-β-activated kinase (TAK1), which phosphorylates IKK complex to activate NF-κB eventually [39]. Of interest, though miR-146a inhibits NF-κB, the expression of miR-146a induced by LPS is dependent on NF-κB binding to its promoter. Therefore, miR-146a was proposed as a key regulator in inhibition of NF-κB activity in this regulation loop. Its ability to inhibit NF-κB was also observed in breast cancer cells. Ectopic expression of miR-146a/b in MDA-MB-231 cells showed approximately 75% and 60% decrease of IRAK1 and TRAF6 protein levels, respectively [40], accompanied by 3 to 4-fold decrease of NF-κB DNA binding activity. Invasiveness was reduced by 70-80% in miR-146a/b overexpressing MDA-MB-231 cells, although migration stayed at a higher level (∼50-60% decrease). In addition, overexpressing miR-146a/b in MDA-MB-231 cells reduced their IL-8 secretion in conditioned medium to 25% [40], and IL-8 elevation in serum was reported to be correlated with breast cancer progression and post-relapse [41].

MiR146a/b can be up-regulated by BRCA1 in HCC1937 cells and showed competency of binding to 3′UTR of TRAF2, a regulator of NF-κB [42]. Introduction of miR146 inhibited NF-κB as well as ERK and JNK in BRCA1-deficient HCC1937 cells. MiR146a but not miR-146b was also induced by 5-fold in MDA-MB-231 breast cancer cells after the cells were transduced with metastasis suppressor-1 (BRMS1) [43], which curtails metastasis in wide range of tumors [44]. In an *in vivo* metastasis model, miR146a and miR146b overexpressing MDA-MB-231 cells reduced the cells' ability to form lung metastatic nodes by 69% and 84% in mice, respectively. Considering that BRMS1 negatively regulates NF-κB activity by reducing phosphorylated IκBα and suppressing NF-κB transcription [45, 46], miR-146a could be another contributor. Similarly, miR-146a was shown to be a downstream gene of p53-binding protein-1 (53BP1), which suppressed NF-κB and invasion of MDA-MB-231 cells [47].

MiR-520/373 family (miR-302/367, miR-371/372/373 and miR-520) was found to negatively regulate NF-κB activity using a genome-wide microRNA screen of HEK293FT cells [48]. Transfection with miR-520c and miR-373 mimics suppressed inducibility of p65 protein in response to TNFα, but not p50 expression. Further evidence showed that transforming growth factor-β (TGFβ) repression by miR-520/373 family members rather than p65 down regulation contributed to inhibit invasiveness of MDA-MB-231 cells directly. NF-κB downstream cytokines IL-6 and IL-8, which promote cancer metastasis, were reduced dramatically in both transcription level and protein secretion after introduction of miR-373 and miR-520c mimics into MDA-MB-231 and MCF-10A cells. CXCL1 and ICAM mRNA levels were also found to be decreased by more than 50%. Besides reducing IL-8 by directly targeting its 3′UTR, miR-520b is also involved in inhibiting migration of breast cancer cells through repressing hepatitis B X-interacting protein (HBXIP) [49]. Possessing the ability to activate NF-κB by promoting IκB phosphorylation, HBXIP was shown to have higher protein level not only in MDA-MB-231 and LM-MCF-7 cells but also in breast cancer samples and metastatic lymph nodes. 100 nM miR-520b caused more than a 50% decrease in migration. Considering that HBXIP increases IL-8 via NF-κB activation, suppressing HBXIP could further contribute to IL-8 inhibition. However, not all family members have antitumor effects. By targeting protein phosphatase 2A catalytic subunit

(PP2A/C), which suppresses phosphorylation of IκB [50], miR-520h promotes metastasis by enhancing NF-κB activity and the resultant up-regulation of Twist (a basic helix-loop-helix transcription factor). Suppression of miR-520h by the adenovirus type 5 E1A (E1A) was reported to mediate inhibition of invasion through the NF-κB pathway [51]. As the most down regulated miRNA in MDA-MB-231 cells, stably expressing E1A, miR-520h re-expression abrogated E1A caused significant inhibition of migration and invasion *in vitro* and lung colonization *in vivo*. Anti-miR520h led to approximately 80% decrease in both migration and invasion of breast cancer cells.

MiR-448 has been shown to be involved in epithelial-mesenchymal transition (EMT) of breast cancer cells. A feedback loop of miR-448 and NF-κB was found to regulate EMT induced by chemotherapy [52]. In adriamycin-treated MCF-7 cells, miR-448 was reduced by ∼95% accompanied with a decrease of E-cadherin and an increase of vimentin. Transfection of anti-miR-448 also endowed mesenchymal phenotype of MCF-7. MiR-448 targeted special AT-rich sequence-binding protein-1 (SATB1) mRNA inhibits NF-κB through the AR/EGFR/PI3K pathway. MiR-448 inhibition directly restored NF-κB activity in which Akt activation is indispensible. However, NF-κB was also found to inhibit miR-448 due to its competency of binding the promoter of HRT2C where miR-448 is hosted, which was required in adriamycin-induced miR-448 suppression. This bidirectional regulation further determined the expression of Twist-1, downstream of the SATB1/AR/EGFR signaling pathway, which is a key switch of EMT [53].

MiR-200 family members are involved in regulation of EMT by interfering with NF-κB downstream signaling pathways. MiR-200c was found to restore the sensitivity to anoikis (∼1 fold increase) in triple negative breast cancer cells by targeting neurotrophin 3 (NTF3), the ligand of neurotrophic tyrosine kinase receptor type 2 (NTRK2) [54]. Decreased NTF3 in the medium abrogated NF-κB-induced anoikis resistance. In addition, miR-200a/b/c mediated phosphoglucose isomerase/autocrine motility factor (PGI/AMF), an NF-κB activator, up-regulated EMT of MDA-MB-231 cells [55]. Silencing PGI/AMF decreased NF-κB activity by ∼50% in both MDA-MB-231 and BT549 cells, with a corresponding down regulation of downstream gene uPAR and MMP-9. PGI/AMF also up-regulates ZEB1/ZEB2 to promote EMT. MiR-200 members are required to inhibit ZEB1/ZEB2 as anti-miR-200s inhibited mesenchymal-epithelial transition (MET) induced by PGI/AMF knockdown.

MiR-200c has also been associated with NF-κB activation in inflammatory signaling induced by breast epithelial cell transformation, which is indispensable in sustaining malignant phenotypes [56]. Monocyte chemotactic protein-1 (MCP-1) first initiates transformation of the MCF-10a cell by transiently activating MEK/ERK and NF-κB, leading to an increase of IL-6, where a positive feedback loop maintains aberrant inflammatory status. IL-6 activates p65 and JNK2 by suppressing miR-200c, while JNK2 induced HSF1 activation stimulates IL-6 expression in turn by demethylation of its promoter. Of interest, despite the initial NF-κB activation by MCP-1 which was shown to be partially mediated by IKK, suppression of p65 by miR-200c was attributed to decreased phosphorylated IκBα by PTPRZ1 (protein tyrosine phosphatase Z1), which was IKK independent. Due to the ability to interfere with both NF-κB activation and the subsequent signaling transduction,

miR-200c shows its pivotal role in regulating breast malignancy through interaction with NF-κB.

MiR-181b-1 and miR-21 work as a switch to regulate transformation of MCF-10A expressing fused ER-Src (binding domain of estrogen receptor was linked with v-Src) by forming a positive feedback loop to activate NF-κB [57]. In this model, Src was activated by tamoxifen treatment and transformation could be achieved after 36 h, accompanied by STAT3 (signal transducer and activator of transcription 3) and Myc mRNA expression. MiR-181b-1 and miR-21 up-regulated by STAT3 were required for maintaining the transformed state, which activated NF-κB by suppressing PTEN (phosphatase and tensin homolog) and CYLD (cylindromatosis), respectively. Introduction of either miRNA allowed for cells to grow colonies on soft agar. Given that STAT3 is activated by inflammatory cytokine IL-6, which could be induced after NF-κB activation via Lin28B and Let-7 [58], a positive feedback loop was established linking inflammation to tumorigenesis. Besides in breast cells, miR-181b-1 and miR-21 promoted clonogenic ability in diverse types of cancer cells, including HCT-116, HT29, PC3, A549 and Hep3B cells. In fact, miR-21 is the most deregulated miRNA in breast cancer [59]. The genotoxic drugs, doxorubincin and camptothecin, induced DNA damage to enhance NF-κB activity in 3 triple negative breast cancer cell lines, followed by up-regulation of miR-21 [60]. An increased IL-6 level was also attributed to NF-κB activation. Of note, despite IL-6-induced STAT3 activation which positively regulates miR-21 transcription, genotoxicity activated NF-κB which was also bound to promoter miR-21 itself in a STAT3-independent manner. In addition, miR-21 was also regulated by MSK1/2-phosphoylated H3S10 and H3S28. By this process, miR-21 driven by NF-κB and p38 signaling suppresses PTEN and PDCD4 to promote breast cancer progression.

2.2 Colorectal Cancer

Constitutive NF-κB activation is also observed in various colorectal cancer cell lines and some tissue samples [61]. One of the potential risks for colon cancer development is the existence of neurotensin (NT) in the gastrointestinal tract. NT is known to regulate mitogen-activated protein kinase (MPK), and NF-κB through binding to NT receptor 1. By up-regulating NF-κB, NT is able to stimulate colonic cell proliferation and cancer transformation [62], promoting colon cancer [63, 64]. NT treatment also alters the miRNA expression profile through NF-κB activation in colon epithelial cells. MiR-21 and miR-155 are among these miRNAs that are up-regulated by NF-κB after NT treatment. The promoter regions of miR-21 and miR-155 contain functional NF-κB binding sites.

MiR-21 was shown to be able to inhibit PTEN, while miR-155 was shown to inhibit PPP2CA. Both PTEN and PPP2CA are negative regulators of Akt, by inhibition of PTEN and PPP2CA, Akt can be activated and further activate NF-κB. Through this positive feedback loop, NF-κB can be further activated and play a role in colon cell proliferation and cancer formation [65]. MiR-155 has also been demonstrated to be up-regulated by adrenaline-induced NF-κB in colon cancer cells. Adrenaline, also known as epinephrine, belongs to the family of catecholamines, which is involved in regulation of genesis and progression of multiple cancers, such as breast, lung, pancreatic, *etc.* [66, 67]. In colon

cancer cells, adrenaline has been shown to be able to induce multidrug resistance by up-regulation of ABCB1 gene expression [68], but the detailed mechanism still remains unknown. Further research confirmed that adrenaline increased cell proliferation and resistance to cisplatin in colon cancer HT29 cells [69]. As for the signaling pathways activated by adrenaline, evidence showed that adrenaline up-regulated NF-κB activity [69-71], which can further increase the expression of miR-155 in a NF-κB dependent manner [69, 72]. MiR-155 was further confirmed to contribute to increased cell proliferation, migration and inhibition of cell apoptosis in colon cancer cells, as well as an increase in the chemoresistance under adrenaline treatment [69, 73].

MiR-1290 is another miRNA which is up-regulated by NF-κB in colon cancer tissues compared to adjacent normal tissue. By comparing SW620 colon cancer cell line stably expressing miR-1290 and wild type SW620 cell, miR-1290 has been shown to be able to target kinesin family member 13B (KIF13B) directly. By silencing KIF13B, miR-1290 could suppress cytokinesis, resulting in cells with large volume and a large increase in the number of tetraploid cells. On the other hand, miR-1290 also targets NF-κB repressing factor (NKRF), which in turn activates the Akt and NF-κB pathways in order to maintain cell proliferation. Moreover, overexpression of NF-κB induces the miR-1290 expression by about two fold compared to the control, which suggests that NF-κB also promotes miR-1290 transcription. By functioning as a positive feedback loop, miR-1290 affects the NF-κB and wnt pathways, thus modulates cell reprogramming [74].

MiR-124 is one of the down-regulated miRNAs in colon rectal cancer (CRC) tissues and cell lines compared to normal ones. Based on prediction and *in vitro* 3′UTR luciferase reporter activity, miR-124 can directly regulate inhibition of apoptosis-stimulating protein p53 (iASPP). Further analysis showed that knockdown of iASPP or overexpression of miR-124 had similar effects in increasing NF-κB expression, yet how it affects NF-κB activity remains unknown. Physiologically, overexpression of miR-124 or iASPP knockdown attenuated CRC cell viability, proliferation and colony formation, which could possibly be due to the manipulation of NF-κB signaling pathways [75].

MiR-143 expression is also reduced in colon tumors compared to normal tissues, and the overexpression of miR-143 reduces colon cancer cell viability. MiR-143 appears to be a down-regulator of NF-κB and be able to sensitize the cancer cells to drug treatments, such as 5-fluorouracil (5-FU). MiR-143 overexpressing HCT116 colon cancer cells are more sensitive to 5-FU-induced cytotoxicity than the wild type HCT116 cells. Moreover, the treatment of 5-FU and the increasing of miR-143 down-regulate the protein expression of NF-κB, ERK5, and Bcl-2, though the detailed mechanism of NF-κB-regulation is unclear. It's also unclear how the change of the NF-κB protein level will affect its downstream signaling pathways [76]. In addition to miR-143, overexpression of prostate apoptosis response protein 4 (PAR-4) has also been shown to sensitize human colon cancer cells to 5-FU. Par-4 is known to be a NF-κB inhibitor, therefore, it is not surprising that overexpression of Par-4 in CRC cells inhibits NF-κB activity, and as a consequence restrains cell viability as well. On the other hand, as shown by miRNA array and qPCR analysis, overexpression of PAR-4 altered the miRNA expression profile: miR-7, 18a, 193b, 221, 222 were all down-regulated, while miR-30d, 34a, and 195 were up-regulated. Among

these miRNA, miR-34a has been showed to possibly regulate the BCL2 protein, thus may also play a role in regulating cell apoptotic pathways. Therefore, both NF-κB and microRNAs may mediate the function of PAR-4 in sensitizing 5-FU treatment in human colon cancer [77]. A schematic illustration of the miRNA involved signaling pathways in colorectal cancer is summarized in Fig 2.

2.3 Gastric Cancer

Gastric cancer is the second leading cause of cancer mortality worldwide. Previous research suggested that cigarette smoking is one of the major risk factors associated with gastric cancer. Nicotine, the active component in cigarettes, can induce a variety of pathways involve in cell proliferation, apoptosis, and cancer transformation [78, 79]. MiR-16 and miR-21 are associated with nicotine-promoted cancer cell growth. In gastric adenocarcinoma cells, nicotine treatment has been demonstrated to be able to increase both miR-16 and miR-21. On the other hand, NF-κB has been shown to directly bind to the promoter region of miR-16 and miR-21 and increase the expression level of both miRNAs. Moreover, the inhibition of NF-κB activity by BAY11-7085 or NF-κB knockdown by siRNA can inhibit the induction of nicotine-induced miR-16 and miR-21. One possible signaling pathway is that nicotine targets on prostaglandin E receptors mediates the activation of NF-κB. The activated NF-κB further increases the expression of miR-16 and miR-21, which leads to increased cell proliferation [80].

MiR-9 expression level is significantly decreased in human gastric adenocarcinoma compared to adjacent normal tissues. By overexpression of miR-9, the gastric adenocarcinoma cell proliferation rate will be decreased, and xenograft tumor growth will also be reduced in *in vivo* assays. NF-κB1 (the p105 subunit of NF-κB) is one of the direct targets of miR-9. Overexpression of pri-miR-9, the precursor of miR-9 will cause a decrease in NF-κB1 mRNA level, as well as both p105 and p50 protein level. Moreover, overexpression of NF-κB1 together with miR-9 can recover all the physiological effects caused by overexpression of miR-9 alone [81].

MiR-146a is known to be up-regulated by NF-κB when innate immune response is triggered [38]. Innate immune response often leads to NF-κB activation by releasing multiple cytokines, which is associated with various cancers, including gastric carcinogenesis [82, 83]. In gastric cancer, it has been revealed that miR-146a is up-regulated in gastric cancer mouse models, as well as 73% of investigated human gastric adenocarcinomas cells. MiR-146a directly targets caspase recruitment domain-containing protein 10 (CARD10) and COP9 constitutive photomorphogenic homolog subunit 8 (COPS8). Both CARD10 and COPS8 are members of G protein-coupled receptor (GPCR) pathways, which up-regulate NF-κB activity. Therefore, mediation by CARD10 and COPS8, and overexpression of miR-146a could inhibit NF-κB activity, thus inhibiting LPA-induced expression of cytokines and growth factors. Although induced by NF-κB, miR-146a further inhibits NF-κB activity through CARD10, and COPS8 forms a negative regulatory loop containing the two. Yet, the reason why this inflammatory and tumor suppressor miRNA is up-regulated in gastric cancers remains largely unknown [84].

2.4 Glioma

The association between NF-κB and brain tumor activity has recently been noticed. A recent report has shown that inhibition of NF-κB interfered in fibronectin processing and therefore reduced invasiveness in glioblastoma multiforme (GBM) cells [85]. Another recent study also pointed out that resistance to ionizing radiation was partially attributed to NF-κB activation in GBM cells, which hinders one of the major therapy for tumors in the brain [86]. Therapeutic targets are still quite limited for brain tumors, exploring the NF-κB-related miRNAs might provide some clues about new therapeutic strategies. MiR-30e* was shown to be highly expressed in World Health Organization (WHO) Grade II, III and IV gliomas (n=115) when compared with grade I tumors (n=12) and normal brain tissue (n=5) [87]. This aberrant expression of miR-30e* was inversely correlated with favorable prognosis. MiR-30e* targeted 3′UTR of IκBα to reduce its protein level despite that up-regulation of IκBα mRNA and miR-30e* could be present at the same time. NF-κB translocation to nucleus was required for miR-30e* induced invasiveness in U87MG, LN444 and SNB19 cells through MMP-9. A negative correlation of IκBα and a positive correlation of MMP-9 with MiR-30e* were also established in 127 clinical glioma samples. It is of note that even though they originated from the same precursor, miR-30e alone did not have an impact on NF-κB activity and invasiveness, which might be due to its different targets [88]. But the possibility of miR-30e/miR-30e* cooperation could not be excluded in glioma carcinogenesis since both of them were up-regulated in the mice brain when fed with carcinogen RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine) [89].

MiR-182 was elevated in a subset of glioma which showed low expression of miR-30e* MiR-182 activates NF-κB by targeting CYLD (cylindromatosis), a K63-specific deubiquitinase which functions as an NF-κB inhibitor by decreasing ubiquitination of NEMO, TRAF2 and TRAF6 to interfere with IKK activation [90, 91]. Although comparable CYLD mRNA levels were present in both tumor and normal tissue, direct binding of miR-182 to 3′UTR of CYLD activated NF-κB in tumor cells. Blocking IκBα abolished miR-182 induced anchorage-independent growth and angiogenesis. In the same study, miR-182 was shown to be induced by TGF-β, which is an NF-κB activator and a frequently up-regulated cytokine that promotes glioma progression [92, 93]. The activation of the TGF-β signaling cascade was further confirmed in clinical glioma specimens (n=161), showing a positive correlation between phosphorylated Smad2 and miR-182 as well as an inverse correlation between miR-182 and patient survival time.

MiR-221 and miR-222 expressions are highly expressed in glioblastoma and their expressions are regulated by NF-κB [94, 95]. Suppression of highly activated NF-κB in U87 cells decreased both miR-221 and miR-222 by more than 50%. Two p65 binding sites in the upstream region of the miR-221 and miR-222 promoters were identified and an interaction between p65 and c-Jun in binding the same site was required for enhancing miRNAs transcription [96]. Because p27, a cell cycle inhibitor, was the target of these two miRNAs, NF-κB impaired this tumor suppressor gene indirectly [97]. Similar regulation was found in the PC3 prostate cancer cell line.

2.5 Hematological Cancer

Hematological cancers are tumors often involved in immune system affecting blood, lymph nodes, and bone marrow. Adult T-cell leukemia/lymphoma (ATL) is a cancer of T cell in the immune system. Human T cell leukemia virus type I (HTLV-1) is believed to be the major cause of the disease [98]. According to microarray analysis comparing clinical ATL samples and control $CD4^+$ T cells from healthy donors, about 60 miRNAs showed significantly altered levels of expression. MiR-31 is the most profoundly repressed miRNA in adult T cell leukemia (ATL) individuals, with about 250-fold decrease compared to normal groups. MiR-31 has been further demonstrated to directly bind to the 3′-UTR region of NF-κB-inducing kinase (NIK) and suppress its expression. By inhibiting NIK, the phosphorylation level of IKKα and IκB are also decreased accordingly. Mediated by NIK, overexpression of miR-31 could inhibit the activity of NF-κB, thus inhibiting ATL cell growth and promoting apoptosis [99]. In leukemia U936 cells, it has also been found that miR-183 can regulate NF-κB through β-transducin repeat-containing protein (β-TrCP). MiR-183 was demonstrated to be able to bind to β-TrCP, which is known to be a NF-κB inhibitor, and inhibit its expression directly. Through the inhibition of β-TrCP, miR-183 can up-regulate the activity of NF-κB. Compounds such as piceatannol, an inhibitor of TNFα-mediated signaling pathways, have shown the potential to inhibit the expression of miR-183, thus down-regulating NF-κB activity, and leading to inhibition of tumor growth [100].

MiR-29b is found to be down-regulated in acute myeloid leukemia (AML), especially one subset of AML driven by overexpression of wild type or mutant Kit (also known as CD117) protein [101, 102]. *Kit* encodes a tyrosine kinase receptor which regulates its downstream pathways leading to cell proliferation and survival [103, 104]. At the promoter region of *Kit*, there are binding sites for both SP1 and NF-κB. Examined by overexpression and knockout assays of SP1 and NF-κB, it has been shown that binding with SP1 and NF-κB up-regulates Kit expression. Overexpression or mutant Kit would inhibit miR-29b expression through up-regulation of Myc. Although miR-29b was originally also up-regulated by Sp1/NF-κB HDAC1 complex, it would be inhibited by Kit and lose its regulation of Sp1 [105]. Thus, SP1 forms an autoregulatory loop by suppression of miR-29b expression, and in turn increase the expression of itself, which can further promote the expression of Kit. A couple of compounds, e.g. proteasome, NF-κB or Sp1 inhibitors, have shown potential to decrease Kit expression, and thus have the possibility to become therapeutic approaches [102].

MiR-155 and MiR-125a/b, two NF-κB activators, are highly expressed in diffuse large B-cell lymphoma (DLBCL). MiR-155 and its precursor B-cell integration cluster (BIC) have been shown to be overexpressed in lymphoma, as well as other cancers, such as breast, lung and cancer. [106, 107]. In DLBCL, the high miR-155 expression level is usually correlated with high NF-κB activity [108]. In B lymphoma cell lines, when infected with Epstein-Barr virus (EBV), a herpesvirus associated with lymphoma as well as other cancer development, or when overexpression of the chief transforming protein LMP1 from EBV occurs, both the level of BIC and miR-155 will be increased [109, 110]. Moreover, by adding an NF-κB inhibitor, the EBV-induced miR-155 induction can be inhibited, which indicates that the EBV-induced miR-155 induction is possibly regulated by NF-κB [111]. On the other hand, miR-155 has also been shown to be able to be induced by B-cell receptor triggering, and this

pathway is mediated through NF-κB since transfected the cells with non-degradable IκB could inhibit the miR-155 induction. However, the induction of BIC cannot be simply equal to the induction of miR-155 in some cases. As shown in Burkitt lymphoma cells, there is no miR-155 expression even the cells are transfected with BIC, which indicates that there may be other unknown factors regulating the processing mechanism besides the regulation of NF-κB at the transcriptional level [112]. In addition to miR-155, miR-125a/b is also related to DLBCL. MiR-125 directed targets TNFAIP3, a negative NF-κB regulator, as it is an ubiquitin-editing enzyme interfering with the interaction between E3 ligases and E2 ubiquitin conjugation. By targeting TNFAIP3, miR-125a/b directly inhibits the expression level of TNFAIP3, and induces NF-κB activity, which leads to increased aggressiveness in both cell line assays and in primary tumors [113].

MiR-146a is one of the miRNAs induced by the LPS-induced activation of NF-κB in the human monocytic cell line. The induction of miR-146a has shown to be NF-κB-dependent, and can down regulate NF-κB through inhibition of two NF-κB activators, TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1) [38, 114]. A study on NK/T cell lymphoma (NKTL) has also presented that miR-146a down-regulated NF-κB via TRAF6, and its overexpression leads to decreasing in proliferation, survival rate, and enhancement in chemosensitivity of NKTL cancer cells. In addition, NKTL patients' prognosis and chemotherapeutic response are consistent with their miR-146a level, with high miR-146a levels correlates to high frequency of response to chemotherapy [114]. Further *in vivo* study has shown that mice with miR-146a knockout develop myeloid and lymphoid tumors with progressively enlarged spleens, as well as bone marrow myeloproliferative diseases, starting from month five to six. Detailed examination on spleen and bone marrow of miR-146-deficient mice showed increased activation of NF-κB, while reduction of NF-κB level could successfully protect from the myeloproliferation in spleen and bone marrow [115].

2.6 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer and is the third most common cause of the cancer-related death in the world. Hepatitis, caused by hepatitis virus, is a major cause of acute and chronic liver diseases, and induces liver inflammation, which favors the development of liver cancer. Among all types of hepatitis virus, HCV and HBV are the most common ones, which count for a substantial portion of HCC development. By comparing miRNA expression profiles of liver cancer cell HepG2 and liver normal cell L02 using microarray analysis, about 143 miRNA expression levels were noticed to be changed, among which 66 were up-regulated and 77 were down-regulated [116]. The functions of most of these miRNA remain unknown, yet researchers have provided some clues on some of the miRNA functions. MiR-155 was significantly increased in HCV-infected cells in both *in vitro* and *in vivo* assays. Inhibition of miR-155 could lead to the regression of cancer cell proliferation and promotion of cell apoptosis through wnt/β-catenin signaling pathways. HCV infection is also known to activate NF-κB activation. Upon the overexpression of NF-κB, the miR-155 level can also be increased; while after treating with NF-κB inhibitors the miR-155 level will be decreased. This result indicates that the HCV-induced miR-155 may be mediated through NF-κB pathways [117]. In addition to HCV-induced HCC, NF-κB

regulated miR-155 can also be observed in Choline-deficient and amino acid-defined diet (CDAA diet)-induced tumors in C57BL/6 mice. Based on microarray assay, miR-155 is one of the 30 miRNAs changed in the early weeks of CDAA diet-treated mice. MiR-155 is also related to inflammatory responses. While prominent level of inflammation developed in CDAA-diet mice, a higher miR-155 expression level has also been correspondingly observed in CDAA-diet mice compared to control groups. Further analysis has proved that CDAA-diet induces NF-κB activation, which in turn binds directly on the miR-155 promoter region and induces miR-155 expression. The elevation of miR-155 causes the down-regulation of tumor suppressor C/EBPβ, which mediates the effect of miR-155 on the growth of HCC cells [118].

MiR-143 is shown to be up-regulated in HBV infected-cells by HBV-coated HBV X protein (HBx). HBx is also known to be a positive regulator of NF-κB.[119-121]. It has been further shown that HBx-induced NF-κB can up-regulate miR-143 in both cell based assays and p21-HBx transgenic mouse. In both p21-HBx transgenic mouse with the development of HBV-HCC, and in HepG2 cells transiently transfected with HBx gene, miR-143 are highly expressed. Although not affect apoptotic pathways, miR-143 does affect metastasis pathways by inhibiting fibronectin protein FNDC3B. Based on the data, a novel pathway for HBV/NFκB/miR-143/metastasis in HCC models has been established [122].

MiR-224 is one of the most substantially up-regulated miRNAs in HepG2 cells. In the putative miR-224 promoter region, there are three conserved NF-κB binding sites according to the sequence analysis and binding-site scanning. LPS, TNFα and LTα treatments were adopted to activate NF-κB activity, and the results showed that the level of miR-224 increased accordingly. On the other hand, when treated with BMS, an IKK inhibitor, which inhibited NF-κB activity, the level of miR-224 decreased. Based on these results, it is suggested that miR-224 transcription is activated by the induction of the NF-κB pathway [123]. Mediated by NF-κB, miR-224 has been also shown to positively regulate HCC cell migration, invasion, and proliferation, but has little effect on cell cycle regulation [116, 123].

MiRNA-301a is also one of the highly expressed miRNAs in human HCC tissues and hepatoma cell lines compared to normal liver tissues [116, 124]. By both TargetScan prediction of the binding site for miR-301a in the 3′-UTR region and *in vitro* luciferase assay, miR-301a has been shown to target at Homeobox gene Gax (also known as Meox2). Inhibition of miR-301a or over expression of Gax protein could decrease both the mRNA and protein level of NF-κB. Based on the previous research showed that Gax could target NF-κB gene in endothelial cells [125], there is reason to believe that miR-301a inhibits Gax while Gax inhibits NF-κB, thus miR-301a indirectly up-regulates NF-κB expression through Gax. MiR-301a has been further shown to be related to liver cancer cell progression, since inhibition of miR-301a can inhibit cell growth, migration, invasion, and cellular apoptosis in HepG2 cells [124].

MiR-520e, on the other hand, is one of the most substantially decreased miRNAs in HCC. MiR-520e down regulation is possibly mediated by DNA methylation [116, 126]. Instead of targeting the 3′UTR of CD46 and sensitizing the breast cancer cells to complement attack in

breast cancer, in HCC, miR-520e directly targets the 3′UTR of NIK and thus inhibits the expression of NIK [127]. NIK is a well-accepted NF-κB positive regulator, so miR-520e has been further shown to inhibit NF-κB activity as well as its nucleus translocation in HepG2 cells, but not in its normal cell counterpart Chang cells. In both *in vitro* and *in vivo* assays, the overexpression of miR-520e suppresses the growth of hepatoma, but has no effect on normal cells [126].

miR-199/214 cluster is also significantly decreased in HCC compared to normal cells. miR-199/214 cluster has been shown to be able to inhibit XBP-1, which is a major transcriptional regulator of the URR, essential for tumor cell survival and solid tumor formation and growth. The data also demonstrated that the decreasing of miR-199/214 is regulated by UPR-induced NF-κB activity, since the suppression of miR-199/214 could be compromised by NF-κB inhibitor PDTC. Overexpressing miR-214 was also shown to inhibit cell proliferation and induce apoptosis in HepG2 and SMMC-7721 cells, possibly by regulating XBP-1 [128].

MiR-140 is also one of the down-regulated miRNAs in HCC. miR-140$^{-/-}$ mice are more prone to hepatocarcinogenesis compared to normal mice, which makes miR-140 a potential liver tumor suppressor. MiR-140 is regulated by DDX20, a miRNA-containing ribonucleoprotein component, which is involved in miRNA profile change in HCC. miR-140 can directly target and silence DNA methyltransferase 1 (Dnmt1). Dnmt1 can hypermethylate the promoters of metallothionein genes, resulting in decreased metallothionein expression leading to enhanced NF-κB activity. Therefore, miR-140 can inhibit NF-κB in canonical-independent regulatory signaling pathways [129]. As DDX20 is decreased in most HCC cells and tissue samples, miR-140 is decreased accordingly, yet NF-κB activity increases and leads to HCC development and progression [130]. Moreover, other miRNAs such as miR-152 also affects Dnmt1 in HCC cells, although it is not clear if miR-152 and miR-140 share the same pathways in regulating NF-κB activity and downstream signaling pathways [131].

MiR-129-5p is also believed to be a tumor suppressor in HCC. By prediction using the Targetscan database, miR-129-5p is one of the candidates which can bind to the 3′UTR region of the Valosin containing protein (VCP) [132]. VCP belongs to the AAA family (ATPase with multiple cellular activities), and is associated physically with ubiquitinated IκB in targeting IκB to the proteasome for degradation [133, 134]. Because of its role in regulating NF-κB, it is intensively studied in various cancers, including HCC. In human HCC tissues, the mRNA expression of VCP is significantly higher than in normal tissues, while the knockdown of VCP using siRNA can significantly reduce the tumor size. The direct regulation of miR-129-5p on VCP was confirmed by site-directed mutagenesis on the predicted binding region at the 3′UTR. In both HepG2 and SK-HEP1 liver cancer cells, knockdown of VCP or overexpression of miR-129-5p can cause the induction of IκB protein level, and consequently inhibit NF-κB activity. MiR-129-5p has been further revealed to be able to induce apoptosis and reduce migration of HCC cells in a VCP down-regulation-dependent manner [132].

MiR-221, unlike miR-140 and miR-129-5p, is considered to be a tumor promoter miRNA. MiR-221 is shown to be up-regulated by staphylococcal nuclease domain-containing 1 (SND1)-induced NF-κB. SND1 is considered to be an oncogenic gene, which is ovexpressed in various cancers, including HCC. The SND1-induced NF-κB can further activate miR-221. The augment of miR-221 causes the increasing expression of CXCL16, as well as angiogenin; both proteins are positively related with angiogenesis and promote HCC progression [135]. Moreover, the *in vivo* assay also demonstrated that inhibition of NF-κB activity by overexpression of mutant IκB, could significantly decrease tumor development and progression mediated by the inhibition of vessel formation [135, 136].

MiR-9 is another oncogenic miRNA found in HCC. Up-regulated by NF-κB, miR-9 suppresses the protein level of CD166 [137]. CD166 is a transmembrane glycoprotein of the immunoglobin superfamily; its expression is related to cellular dynamic growth and migration, thus it is intensively studied in HCC, as well as other cancer types [138]. In HCC HepG2 and GQY-7701 cell lines, serum depletion (SD) can cause approximately 4-fold increase of CD166 mRNA after 24 h, and even greater increase after 48 h, yet its protein increased at 24 h but decreased at 48 h. Simultaneously, SD can also activate and facilitate the translocation of the p50/p65 NF-κB dimer. The recruitment of the p50/p65 dimer to the promoter region of CD166 can up-regulate its expression. The activation of NF-κB also activates the pri-miR-9-1 gene, which increases the expression of miR-9, in a delayed manner. The delayed activated miR-9 suppressed the protein level of CD166, which may explain the suppression of CD166 protein level at 48 h, but not at 24 h post SD. In addition, mediation of miR-9 activation by the inhibition of CD166 can also promote HCC cell migration [137]. A schematic illustration of the miRNA involved signaling pathways in HCC is summarized in Fig 3.

2.7 Lung Cancer

Radiation resistance is one of the major obstacles that occur in traditional cancer treatments. The IR-induced NF-κB1 (p105) activation is one of the mechanisms that cause radiation resistance since NF-κB1 is involved in DNA damage repair and cell survival pathways [139]. To induce sensitivity to IR, inhibition of IR-induced NF-κB1 activity becomes one of the feasible treatment methods in lung cancer. MiR-9 is one of the NF-κB1 regulators which can bind directly to the 3′-UTR of NF-κB1, thus inhibiting both mRNA and protein levels of NF-κB1. Moreover, the expression level of miR-9 was decreased after IR in a time-dependent manner. It has been further shown that with the overexpression of miR-9, the sensitivity of cancer cells is increased in response to IR. Interestingly, another miRNA let-7g was also decreased upon IR, and its overexpression could also down-regulate NF-κB1, yet with no predictable binding site on the 3′-UTR of NF-κB1 [140]. Based on these results, there is reason to believe that overexpression of certain miRNAs, such as miR-9 and let-7g, in combination with IR treatment, have the potential to lead to better therapeutic results.

In addition to normal miRNA regulatory mechanisms, some miRNAs function in a different way. MiR-886 was found to be suppressed in lung cancer cell H1299 compared to lung epithelial cell CRL2741 using microarray hybridization, along with miR-205 and miR-200 family members (miR-200a, 200b, 200c, and 141). Unlike other precursor microRNAs, pre-

miR-886 was neither a genuine nor a vault RNA. It physically associated with PKR, induced eIF2α phosphorylation, and led to global protein synthesis shutdown. IκB protein level was also decreased along with eIF2 α phosphorylation, which in turn led to NF-κB activation, and as a result caused impaired cell proliferation. This characterization of pre-miR-886 may exist in other cancer cell lines and in clinical specimens [141].

2.8 Melanoma

Melanoma is a malignant tumor of melanocyte, which is often considered incurable because of its high metastatic rate. Accumulated evidence has shown that miR-9 plays a critical role in tumor cell proliferation and tumor progression in cancers including gastric, cervical and ovarian cancers [81, 142, 143]. However, the role of the regulation of miR-9 on cell metastasis is still controversial in various cancer types. In cutaneous melanoma, miR-9 expression level is shown to decrease in metastatic melanoma compared to primary melanoma or radial growth phase melanoma. In cutaneous melanoma cells, as well as in ovarian cancer [144], miR-9 binds directly to the NF-κB1 (p105) 3′-UTR, and down-regulates the expression of NF-κB1. The activity of NF-κB1 is known to be critical in the induction of cytoskeletal reorganization, which is important in cell metastasis [145, 146]. In melanoma cells, it has been further shown that the overexpression of miR-9 and down-regulation of NF-κB1 and Snail1 pathways lead to the inhibition of the expression of cytoskeleton proteins including Rab8, p65, MMP-2, and MMP-9. By inhibiting NF-κB1-Snail 1 pathways, miR-9 decreases the cell growth as well as the cell motility of all types of melanoma [147, 148].

2.9 Ovarian Cancer

An inverse correlation between NF-κB (p50) and ovarian cancer patient's survival was observed in a clinical investigation; and inhibiting IKK-ε in ovarian cancer cells effectively reduced tumor progression [149, 150], suggesting NF-κB activation contributes to ovarian cancer development. Several miRNAs have been reported to regulate NF-κB activity via different pathways. MiR-9 and miR-214 are suppressors of NF-κB. MiR-9 directly targeted NF-κB1 mRNA and suppressed expression of both p105 and p50 subunits of NF-κB in ES-2 cells. Down-regulation of miR-9 in ovarian cancer tissues was shown to contribute to NF-κB activation [144]. Introduction of pri-miR-9 in ES-2 cells inhibited proliferation and colony formation by ∼30% and ∼40%, respectively. MiR-214 suppresses PTEN, which is an NF-κB activator. MiR-214 along with miR-199a are encoded by pri-miR-199a, which is located on chromosome 1 [151]. This gene cluster is positively controlled by Twist1. In type II/CD44- EOC cells which expressed Twist1, higher expression level of both miR-214 and miR-199a were accompanied by reduced IKKβ activity and enhanced AKT phosphorylation. Knockdown of Twist1 also increased RANTES (Regulated on activation, Normal T-cell Expressed and Secreted) in type II/CD44- EOC cells, suggesting Twist1 negatively regulates downstream cytokines of NF-κB. In addition, the differentiation from type I/CD44+ into type II/CD44- EOC cells by losing stem cell characteristics was reported [152]. By suppression of Twist1, type II/CD44- EOC cells regained the ability for tube formation in matrigel, which was a property of type I cancer stem cell.

MiR-199a-mediated NF-κB activation in ovarian cancer cells has been shown to be involved in inflammatory cytokines secretion, which potentially facilitates tumorigenesis and cancer progression [153, 154]. Epithelial ovarian cancer (EOC) cells are divided into type I (MyD88 positive) and type II (MyD88 negative) according to their differential response to TLR signaling [154, 155]. Only type I EOC cells, which have a much higher ratio of IKKβ/IKKα and enhanced NF-κB activity, produce diverse cytokines/chemokines. IL-6 and MCP-1 production decreased sharply after NF-κB inhibition [154]. MiR-199a was identified to target IKKβ and suppress NF-κB activation, which was down-regulated in type I and up-regulated in type II EOC cells.

2.10 Pancreatic Cancer

Pancreatic cancer, one of the most malignant diseases of which the 5-year survival rate is about 5%, is still lacking of effective therapeutics [156]. Constitutive NF-κB activation in pancreatic adenocarcinoma cells can be characterized by RelA activation and up-regulation of IκB [157]. Moreover, a correlation between constitutive NF-κB activation and poor outcome has been recognized in a portion of pancreatic cancer patients [158]. MiR-301a up-regulation was reported to be a signature in pancreatic adenocarcinoma for its 34.2-fold increase in tumor cells [159]. MiR-301a up-regulated NF-κB activity by 5 fold in a reporter screening assay and enhanced its DNA binding activity by ∼2.8 fold as validated in EMSA [160]. This activation was attributed to NF-κB-repressing factor (NKRF) mRNA degradation caused by miR-301a binding and the resultant suppression of NFRF protein level. Furthermore, activated NF-κB up-regulates miR-301a by promoting transcription of miR-301a so that a positive feedback loop is formed. Although transient introduction of anti-miR-301a did not reduce tumor growth *in vivo* due to the low efficiency, stable inhibition of miR-301a in PANC-1 cells by tough decoy RNA (TuD) transfection significantly reduced tumor volume by NF-κB inhibition.

MiR-146a has been found to be lower expressed in Colo357 and PANC-1 cells compared with human pancreatic duct epithelial (HPDE) cells [44]. In agreement with its role in breast cancer, miR-146a suppresses NF-κB activity through IRAK1. MiR-146a was further shown to down-regulate both EGFR and phosphorylated EGFR (Tyr992), as well as MTA-2, all of which contributed to tumor progression [161]. In addition to targeting IRAK1, NF-κB inhibition by miR-146a correlated with EGFR down-regulation. 25 μM 3, 3′-diinodolylmethane (DIM) or isoflavone treatment was shown to induce miR-146a in pancreatic cancer cells and lead to suppressed invasion [44].

MiR-200 family members were proposed to paly a role in pancreatic tumorigenesis in mice bearing both activated K-ras and deficient INK4a/Arf mutation. These mice developed pancreatic tumors from day 45 to 80 [162]. Notch signaling was reported to promote pancreatic cancer growth [163, 164] and was also observed in tumors derived from the compound transgenic mice. Enhanced Notch signaling characterized by up-regulated Notch-2 and Notch-4 was shown to partially mediate NF-κB activation. Overexpression of miR-200b, which was suppressed in tumors in mice, inhibited Notch signaling by down-regulation of Jagged-1, a Notch receptor ligand, suggesting that miR-200 might suppress tumorigenesis by inhibiting Notch and then NF-κB signaling.

3. Potential Therapeutics, Challenge and Limitation

Growing evidence has shown that NF-κB is highly activated in a variety of different cancer types [35, 61, 157]. Inverse correlations between NF-κB activation and prognosis of patients have been reported in pancreatic and ovarian cancers [149, 158]. As an important cell survival regulator, NF-κB is "utilized" by malignant tumor cells to resist and escape from apoptosis induced by chemotherapy as it is already known that chemotherapy drugs lead to NF-κB activation [165]. Upon SN38 or doxorubicin treatment, IκBα was phosphorylated and degraded in HeLa cells, followed by enhanced NF-κB activity [166]. Sensitivity to DNA damaging reagents was restored when NF-κB induction was blocked, suggesting the anti-apoptotic function of induced activation of NF-κB. It is also notable that even low-dose exposure to chemotherapy drug was able to activate NF-κB in cervical cancer cells and that the acquired drug resistance lasted for passages [167]. Besides inducible NF-κB, constitutive activation of NF-κB in cancer cells could work as an intrinsic shield against apoptosis. It was reported that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) up-regulated NF-κB in 5 different cancer cell lines. However, this induction was not directly associated with resistance to apoptosis. Introducing non-degradable IκBα only sensitized Panc-1 cells to TRAIL, which had a much higher basal NF-κB activity. It was the constitutive NF-κB activation that upregulated the expression of X-linked inhibitor of apoptosis protein (XIAP) to inhibit apoptosis. Therefore, targeting NF-κB in cancer cells might not only reduce tumor progression and invasiveness directly [85, 150], but also restore sensitivity of tumor cells to chemotherapy and ionizing radiation [86, 168].

Given that cancer cells are developing their resistance towards first-line chemotherapeutic drugs, targeting NF-κB by miRNAs could be a promising and more specific treatment strategy. NF-κB in different cancer types can be either up-regulated or down-regulated by miRNAs (Table 2). Due to the implication of constitutive NF-κB activation in promoting malignant phenotypes, it is not surprising that some positive regulators of NF-κB are highly expressed while the NF-κB inhibitors are more likely to be suppressed [59, 88, 144]. Significant differences between oncogenic and cancer-suppressing miRNAs have been discovered from multiple perspectives, and are not limited to expression level [169].

Antagonists could be designed for suppressing miRNAs which upregulate NF-κB signaling in cancer cells. Chemically modified antisense oligonucleotides, especially 2′-O-methyl RNA, has shown its potential for miRNA inhibition, showing stronger binding affinity and resistance to nucleases than DNA oligonucleotides [170, 171]. RNA antisense oligonucleotides conjugated with cholesterol, called "antagomir", effectively down-regulates targeted miRNA after direct intravenous injection in mice [172]. As reviewed in this article, many miRNAs that positively regulate NF-κB in cancer cells could be good targets for antagonist. MiR-21, one of the highest expressed miRNAs in breast carcinomas has been shown to play an important role in both chemoresistance and tumorigenesis [57, 59, 60, 173]. MiR-21 activates NF-κB by suppressing PTEN while itself can be up-regulated by chemotherapy-induced NF-κB. Hence, targeting miR-21 will break down this positive feedback loop so that NF-κB activation could be attenuated. As a "hot" oncogene, there have been several reports of chemically synthesized oligonucleotides which target miR-21 [170, 174, 175]; despite these, more *in vivo* experiments would still be required to validate

the effectiveness. In addition, combination with traditional chemotherapeutic drugs might be worthwhile since targeting NF-κB would impair chemoresistance of cancer cells.

MiRNA mimics may increase expression level of miRNAs which serve as NF-κB inhibitors. These miRNAs are frequently found to be lowly expressed in cancer cells [36, 127, 141]. For example, restoration of miR-520b in metastatic breast cancer cells sharply reduced the cell migration by targeting an NF-κB activator, hepatitis B X-interacting protein (HBXIP) which was positively expressed in a large portion of cancer tissues [49]. Meanwhile, miR-520b also targeted IL-8, the downstream cytokine of NF-κB, to further contribute to repressing cell migration. In this case, overexpressing miR-520b has the advantage to target more than one oncogene simultaneously. There are growing reports of successful miRNAs replacement therapy *in vivo*. Delivery of let-7 as well as miR-34 to established NSCLC xenografts inhibited tumor progression, both of which employed lipid-based vehicle or reagent to enhance uptake of miRNAs [176, 177]. Metastatic prostate tumor in bone was shown to be inhibited in mice by introducing synthetic miR-16, which was mediated by atelocollagen [178]. With the improvement of miRNA delivery technology, the influence of NF-κB inhibition *in vivo* could be applied in the future.

Interestingly, besides upstream of NF-κB, miRNAs which are regulated by NF-κB might also be considered when designing therapeutic targets. For instance, miR-155 upregulation following NF-κB activation plays important roles in many cancer types [65, 108, 117]. Therefore, cutting off the signal transduction from NF-κB might attenuate malignant phenotypes. As the evidence that suppression of miR-155 *in vivo* began to accumulate [179, 180], miR-155 inhibition has shown the competence in treating lymphoma by intravenous administration of nanoparticle-based anti-miR-155 in mice [181]. Moreover, since there is a positive feedback loop involving miR-155 in colon cancer that activates NF-κB, targeting miR-155 may impair the oncogenic signaling to a greater extent [65].

When determining miRNAs for therapeutic targets, we should be aware of specificity of tumors because of the different expression levels and biological functions of miRNAs. Though miR-124 promoted growth of colorectal cancer cells and showed the competency of activating RelA [75], it worked as a powerful tumor suppressor in breast cancer cells by inhibiting CD151 [182]. This reminds us that the multiple targets of miRNAs may demonstrate diverse or even opposite physiological functions in different cancer types. While targeting NF-κB, other pathways cannot be overlooked. Although bringing the miRNA targeted drugs from benchtop to bedside still requires researchers to overcome many obstacles, the phase 2 clinical trial of miravirsen, an antagonist for miR-122, is still ongoing as a pioneer [183]. It not only provides new hope for HCV treatment by showing the effectiveness in patients who did not respond to traditional drugs, but also serves as a model for all therapeutic miRNAs targeting oncogenic signaling pathways.

As miRNA therapy attracting more and more interest these days, it still has a long way to go for substantial clinical trials. One the major concerns of miRNA therapeutics is the off-target regulation, as one miRNA usually regulates multiple genes. Most of the miRNA research started with miRNA target prediction based on sequence complementarity with software such as TargetScan, PicTar, and TargetRank, followed by *in vitro* assays to validate the

regulation [1, 184, 185]. In most of the recent miRNA-related research, only one target gene of the miRNA is studied, yet most miRNAs have more than one target. Therefore, in therapeutic approaches, in addition to the target gene, other genes will also be affected, which may cause severe side effects.

Other therapeutic methods include up-regulation or down-regulation of certain miRNAs, since alteration of miRNA levels has been observed in various cancers. As for the up-regulation method, miRNA stability and delivery are still major obstacles [186, 187]. As for miRNA stability, the ‘uncovered’ miRNA is quickly degraded by nucleases in the cells. Although modification such as using phosphorothioate to replace phosphodiester, or using fluoro, O-methyl group, or 2-methoxyethyl group on the miRNAs claims to be able to protect them from nuclease attack and keep them stable, the biological role of these modified miRNAs still remain large uncertified [188, 189]. The therapeutic miRNAs can also be ‘coated’ with particles for stability and delivery. Liposome or polymer-nanoparticle coated miRNA delivery is prevalent today, yet both of the methods still face potential dose toxicity effects, and how these materials are going to be degraded in the body is still largely unknown. For the miRNA down-regulation approaches, antagomirs, which are cholesterol-conjugated with complementary sequence to a specific miRNA, are commonly used, as they can compete with the target miRNA for its target molecule [172]. However, the antagomir design is always a concern, since miRNAs are not perfectly complimentary to their target genes. Moreover, some other critical factors such as the off-target toxicity, tissue delivery, and pharmacokinetics are also barriers for this approach.

Overall, the research on miRNA and its therapeutic role in treating cancer is still in early stage, which aims at the fundamental signaling pathways or mechanisms manipulated by these miRNAs. These studies undoubtedly provide solid and essential information on the detailed miRNA mechanisms, yet as reviewed in this manuscript, most experiments are performed on cell-based assays, while only limited amounts of *in vivo* assays are involved. Even though in some studies, the therapeutic roles of miRNAs in treating cancers have been successfully demonstrated in mouse model, the information on the side-effect or the off-target toxicity study is still inadequate, not to mention the difference between human and mice. Though miravirsen, an antagonist for miR-122 is currently under phase 2 clinical trial, the cautiousness still existing on the therapeutic translation from miRNAs to eventual a cancer medicine [183].

4. Conclusion

In conclusion, a large number of miRNAs are able to up- or down-regulate expression and activity of NF-κB, which plays a central role in control of cancer development and progression as well as the responses of cancer patients to chemo and radiation therapies. MiRNAs can directly affect NF-κB activity; but more often they alter NF-κB function indirectly via interfering with upstream or downstream signaling pathways of NF-κB. While miRNA-mediated NF-κB signaling pathways have been shown as promising targets for cancer treatment in cell and animal models, clinical use of miRNAs as cancer therapeutics still have a long way to go due to multi-targets and instability of miRNAs. However, with improved knowledge of the regulatory mechanisms of the miRNA-mediated NF-κB

signaling network and the development of new technologies in construction of miRNAs and their mimetics as well as carrying vehicles, NF-κB-targeting miRNAs will have potential being used for treating various cancers especially in combination with chemo and radiation therapies.

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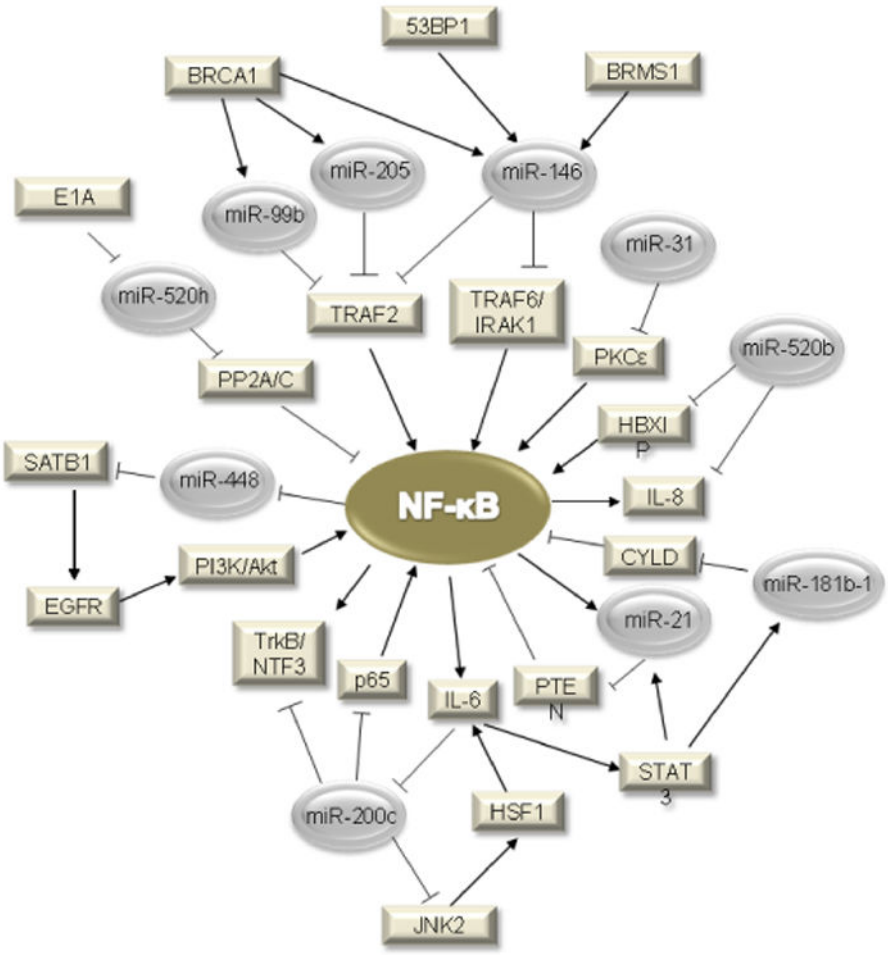

Figure 1.
Diagram illustrating the relationship between NF-κB and miRNAs in breast cancer.

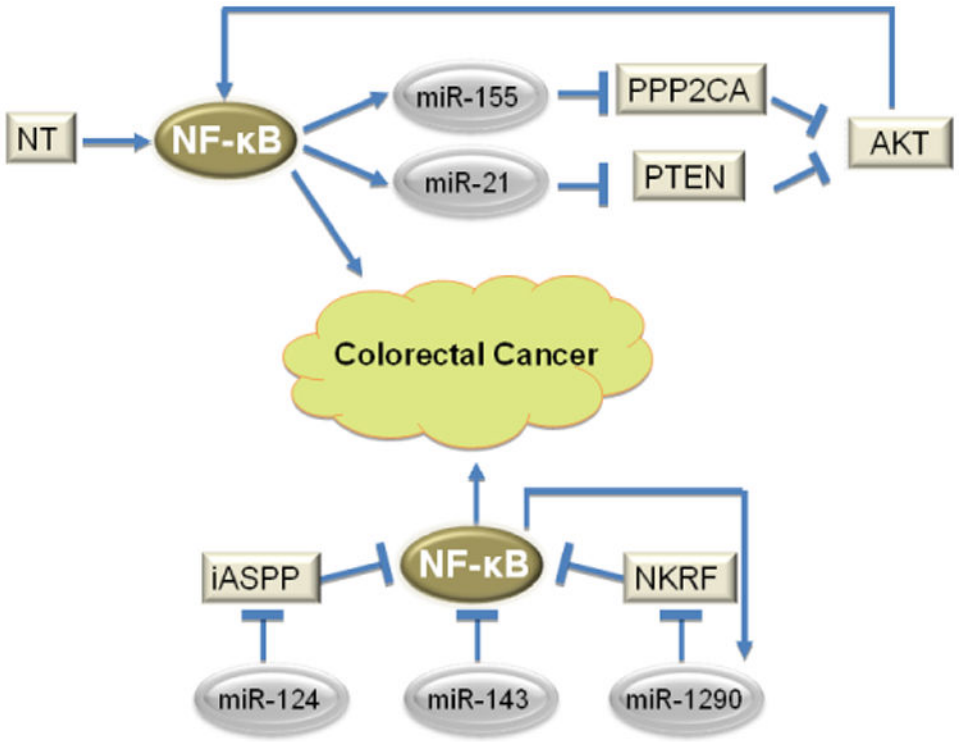

Figure 2.
Diagram illustrating the relationship between NF-κB and miRNAs in colorectal cancer.

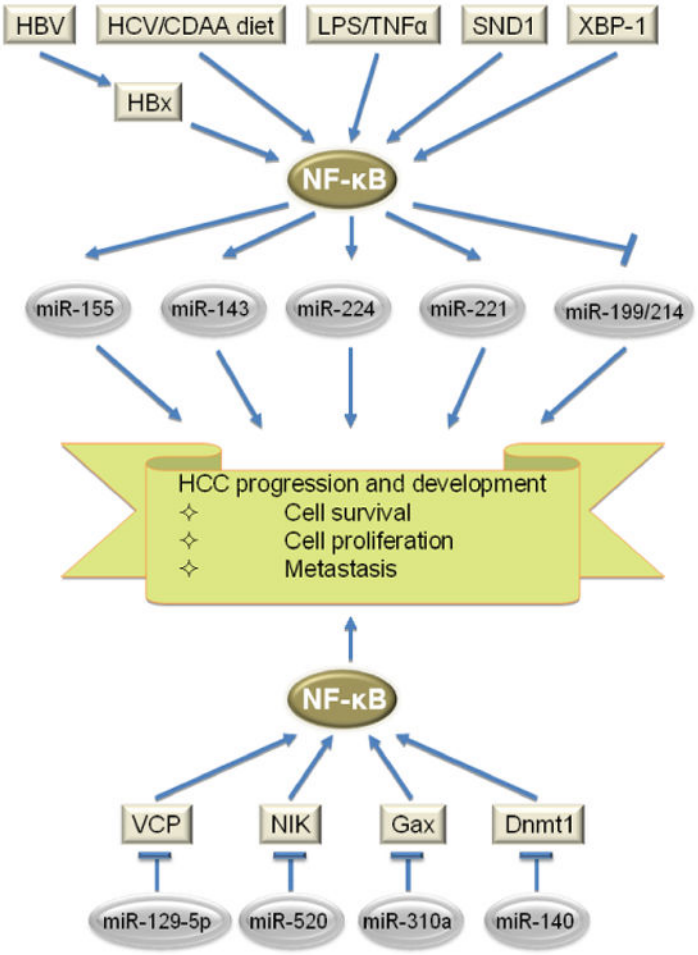

Figure 3.
Diagram illustrating the relationship between NF-κB and miRNAs in HCC.

Table 1

miRNAs with related cancer types.

Cancer Type	MiRNAs
Breast Cancer	miR-21 [57]; miR-31 [36]; miR-146 [40, 47]; miR-181b-1 [57]; miR-200c [56]; miR-373 [48]; miR-448 [52]; miR-520b [49]; miR-520c [48]; miR-520h [51]
Colorectal Cancer	miR-21 [65]; miR-124 [75]; miR-143 [76]; miR-155 [65, 69, 72, 73]; miR-1290 [74]
Gastric Cancer	miR-9 [81]; miR-16 [80]; miR-21 [80]; miR-146a [84]
Glioma	miR-30e* [87]; miR-182 [91]; miR-221 [94-96]; miR-222 [94, 96]
Hematological Cancer	miR-29b [102]; miR-31 [99]; miR-125a/b [113]; miR146a [114, 115]; miR-155 [106, 111, 112]; miR-183 [100]
Hepatocellular Carcinoma	miR-9 [137]; miR-129-5p [132]; miR-140 [129, 130]; miR-143 [122]; miR-155 [117]; miR-199 [128]; miR-214 [128]; miR-221[135]; miR-224 [116, 123]; miR-301a [124]; miR-520e [126]
Lung Cancer	miR-9 [140]; miR-886 [141]; Let-7g [140]
Melanoma	miR-9 [147, 148]
Ovarian Cancer	miR-9 [144]; miR-199a [154]
Pancreatic Cancer	miR-146 [44]; miR-200b [162]; miR-301a [159, 160]

Table 2

Reciprocal effects of miRNA and NF-κB in cancer cells.

MiRNA Upstream Of NF-κB	Effect of miRNA On NF-κB		Cancer Type	Ref
let-7g	↓		Lung	[140]
miR-9	↓		Ovarian; Lung; Gastric; Melanoma	[81, 139, 140, 144]
miR-30e*	↑		Glioma	[87]
miR-31	↓		Breast; Leukemia	[36, 99]
miR-124	↑		Colon	[75]
miR-125a/b	↑		Lymphoma	[113]
miR-129-5p	↓		HCC	[132]
miR-140	↓		HCC	[130]
miR-143	↓		Colon	[76]
miR-146	↓		Breast; Pancreatic; Gastric	[40, 44, 47, 84]
miR-182	↑		Glioma	[91]
miR-183	↑		Leukemia	[100]
miR-199a	↓		Ovarian	[154]
miR-200b	↓		Pancreatic	[162]
miR-301a	↑		HCC	[124]
miR-373	↓		Breast	[48]
miR-520b	↓		Breast	[49]
miR-520c	↓		Breast	[48]
miR-520e	↓		HCC	[126]
miR-520h	↑		Breast	[51]
miR-886	↓		Lung	[141]
MiRNA Downstream Of NF-κB	**Effect of NF-κB On miRNA**		**Cancer Type**	**Ref**
miR-9	↑		HCC	[137]
miR-16	↑		Gastric	[80]
miR-21	↑		Gastric	[80]
miR-29b	↑		Leukemia	[102]
miR-143	↑		HCC	[122]
miR-155	↑		Leukemia; HCC	[106, 111, 117]
miR-199	↓		HCC	[128]
miR-214	↓		HCC	[128]
miR-221	↑		HCC; Glioma; Prostate	[94-96, 135]
miR-222	↑		Glioma; Prostate	[94, 96]
miR-224	↑		HCC	[116]
MiRNA Reciprocally Related with NF-κB	**Effect of miRNA on NF-κB**	**Effect of NF-κB on miRNA**	**Cancer Type**	**Ref**
miR-21	↑	↑	Breast, Colon	[57, 65]

miR-146a	↓	↑	Lymphoma	[114]
miR-155	↑	↑	Colon	[65]
miR-181b-1	↑	↑	Breast, Colon	[57]
miR-200c	↓	↓	Breast	[56]
miR-301a	↑	↑	Pancreatic	[159, 160]
miR-448	↓	↓	Breast	[52]
miR-1290	↑	↑	Colon	[74]