Review

miR-181a: Regulatory Roles, Cancer-associated Signaling Pathway Disruptions, and Therapeutic Potential

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Review

miR-181a: Regulatory Roles, Cancer-Associated Signaling Pathway Disruptions, and Therapeutic Potential

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Abstract: Introduction: microRNA-181a (miR-181a) is a crucial post-transcriptional regulator of many mRNA transcripts and ncRNAs, influencing cell proliferation, cancer cell stemness, apoptosis, and immune responses. Its abnormal expression has been characterized in numerous cancers, making it a significant genomic vulnerability and biomarker in cancer research. **Areas Covered:** Here, we summarize miR-181a's correlation with poor patient outcomes across numerous cancers, and the mechanisms governing miR-181a's activity and processing. We comprehensively describe miR-181a's involvement in multiple regulatory cancer signaling pathways, cellular processes, and the tumor microenvironment. We also discuss current therapeutic approaches to targeting miR-181a, highlighting their limitations and future potential. **Expert Opinion:** miR-181a is a clinically relevant pan-cancer biomarker with potential as a therapeutic target in cancer. Its regulatory control of tumorigenic signaling pathways and immune responses positions it as a promising candidate for more personalized treatments. The success of miR-181a as a target relies on the development of specific therapeutics platforms. Future research on miR-181a's role in the tumor microenvironment and the RNA binding proteins that regulate its stability will help uncover new techniques to targeting miR-181a. Further research into miR-181a serum levels in patients undergoing therapy will help to better stratify patients and enhance therapeutic success.

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Article Highlights

- **Regulatory Role:** *microRNA-181a* can function as an oncogene or a tumor suppressor by post-transcriptionally regulating many mRNA transcripts and non-coding RNAs involved in cancer.
- **Aberrant Expression:** *microRNA-181a* is aberrantly expressed in a large majority of cancers contributing to tumor progression, increased proliferation, immune suppression, and apoptosis.
- **Clinical Relevance:** *miR-181a* is a pan-cancer biomarker with altered expression profiles that can be detected in the serum of patients.
- **Therapeutic Potential:** Pre-clinical studies suggest that targeting *miR-181a* in vivo can inhibit cancer progression and that knockout of *miR-181a* is not toxic and presents a potentially favorable safety profile. Thus, *miR-181a* serves as an ideal therapeutic target.
- **Immune Microenvironment:** *miR-181a* plays a major role in immune cell development, particularly NK and T-cells, and can influence the tumor microenvironment.

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- **Next Steps:** Development of a therapeutic platform for targeting *miR-181a* via nanoparticles, natural products, small molecules, or repurposed drugs is presents a polyvalent therapeutic approach to inhibiting cancer progression.

1. Introduction

Oncogenesis is a result of dysfunctions in cellular machinery. Tumor suppressive redundancies, feedback loops, extrinsic immune surveillance, programmed cell death, and other mechanisms exist to guard against transformation. When these mechanisms fail, neoplasia and carcinogenesis result as competitive advantages that overcome these defenses, permitting cell survival, proliferation, and metastasis. Countless happenstances can result in transformation; hence, due to their wide breadth of regulatory reach, microRNAs (miRNA) have emerged as potent, corruptible mediators of oncogenesis.

miRNAs are evolutionarily conserved non-coding RNAs of about 21-25 nucleotides in length that primarily mediate post-transcriptional repression of messenger RNA (mRNA). This targeted repression of mRNA networks is conducted via homology between the miRNA's seed sequence and the 3' untranslated region (3'UTR) of the mRNA. miRNA families share a common mRNA recognition seed sequence. These family members are often derived from a single phylogenic ancestor and can be found in clusters in the genome. miRNAs are localized intragenically or, more frequently, intergenically. Intragenic miRNAs, or miRtrons, exist within the intronic regions of protein-coding genes [1,2]. Intragenic miRNAs can sometimes have their own promoters within the transcriptional region of a protein-coding gene, in addition to being regulated by the host gene's promoter [3,4]. In contrast, intergenic miRNAs have their own promoters. They are found as either independent transcription units or clusters. Clusters include two or more miRNAs transcribed from adjacent miRNA genes within ten kilobases (kb) of each other. These clusters are transcribed in the same orientation and are not separated by a transcriptional unit or miRNA in the opposite direction [5]. Thus, intergenic miRNAs can be transcribed as individual or polycistronic miRNAs [6–8].

In canonical miRNA biogenesis **(Figure 1)**, miRNA coding regions are detected by RNA polymerase II (PolII) and transcribed into large primary microRNAs (pri-miRNA) in the nucleus. There, the pri-miRNA is processed by DROSHA and DGC8 into a pre-miRNA transcript (~60-70 nt) before being shunted to the cytoplasm by Exportin 5 and Ran-GTP. Once in the cytoplasm, DICER and TRBP cleave the pre-miRNA generating a double-stranded duplex containing the mature miRNA and the complementary passenger strand. The RNA duplex is then loaded onto Argonaut (Ago), where the mature strand interacts with Ago, and the passenger strand is expelled. The Ago-miRNA unit is here-on named the RNA-induced silencing complex (RISC). RISC interacts with the target mRNA's 3'UTR via nucleotide complementarity, facilitating miRNA-mediated mRNA silencing [6,9]. In the context of cancer, these miRNAs can target oncogenic and tumor-suppressive mRNA transcripts. Thus, dysregulation of miRNAs can lead to oncogenic progression. This review will highlight the dysregulation and cancer promoting role of a particular miRNA, *miR-181a.* Abnormal expression of *miR-181a* can lead to disruption in multiple signaling pathways, providing cancer cells with the functional characteristics and hallmarks of cancer central to carcinogenesis [10].

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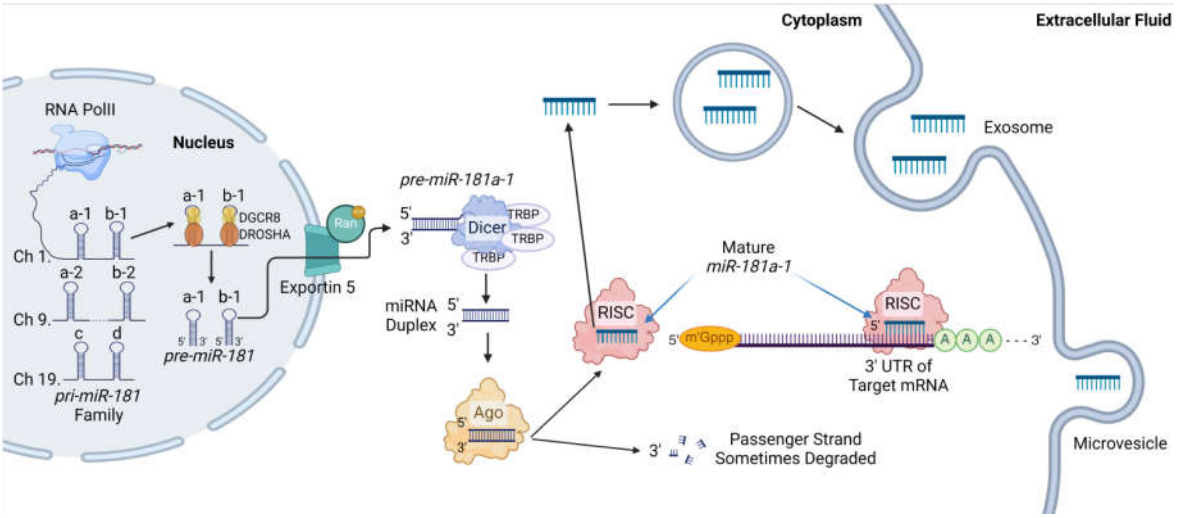

Figure 1. The *miR-181a* biogenesis pathway following canonical miRNA biogenesis. *miR-181* is transcribed by RNA Polll, generating one of three primary transcripts. *miR-181a* and *miR-181b* can be transcribed from chromosome 1 or 9. *miR-181c* and *miR-181d* are transcribed from chromosome 19. Following *miR-181a-1* synthesis, its primary *miR-181a/b-1* transcript is cleaved by the Drosha-DGCR8 microprocessor complex into individual *pre-miR181a/b* transcripts and exported into the cytoplasm via Exportin-5-Ran-GTP. In the cytoplasm, DICER-TRBP cleaves the hairpin structure to the mature *miR-181a* length. The mature strand is then loaded onto argonaut (Ago), and the passenger strand is expelled and sometimes degraded. To facilitate mRNA regulation, the Ago-mature miRNA unit, now termed RNA-induced silencing complex (RISC), is guided to the 3'UTR on target mRNA sequences and facilitates translational repression. Alternatively, mature *miR-181a* can be exported out of the cell in exosomes and microvesicles. Figure generated using BioRender.

2. miR-181a Overview

2.1. miR-181 Family

First discovered in 2003 by three independent groups, the *microRNA-181* (*miR-181*) family consists of four mature miRNA members (*miR-181a/b/c/d*) from six different loci in the genome and remains well conserved among vertebrates **(Figure 1) (Table 1)** [11–13]. *miR-181a-1* and *miR-181a-2* are located on chromosomes 1 and 9, respectively, and ultimately produce the same mature *miR-181a*. *miR-181b-1* and *miR-181b-2* are located on chromosomes 1 and 9, respectively, and ultimately produce the same *miR-181b*. On chromosome 1, *miR-181a-1* and *miR-181b-1* are 61 nucleotides (nt) apart and are encoded by the *MIR181A1B1* gene. Both *miR-181a-1* and *miR-181b-1* are co-transcribed through their own promoter [14]. The minimal regulatory region for *miR-181a-1* and *miR-181b-1* on chromosome 1 has been mapped to the 615 nt upstream of *miR-181a-1* on Chr 1q31.3. In contrast, *miR-181a*-2 and *miR-181b-2* are encoded by *MIR181A2B2* on chromosome 9 and are spaced 1158 nt apart. Distinctly, *miR-181a*-2 and *miR-181b-2* are miRtrons of the *NR6A1* gene, which is transcribed antisense to *MIR181A2B2* [14]. Since *miR-181a* and *miR-181b* are polycistronic miRNAs, they are co-transcribed within the same pri-miRNA transcript. Although their mature 5p transcripts are identical across chromosomes 1 and 9, their mature 3p sequences are not. Furthermore, their pri-miRNAs and pre-miRNAs encoded by *MIR181A1B1* and *MIR181A2B2* are unique [14,15]. The other family members, *miR-181c* and *miR-181d* are located on chromosome 19 in an uncharacterized sequence and are spaced 66 nt apart.

All four mature 5p members of the *miR-181* family share the same seed sequence from the second to the eighth nucleotide: "ACAUUCA." microRNA recognition sequences (MRE) are complementary binding sequences in target mRNAs that use nucleotide complementarity to interact with the seed sequence of miRNAs and facilitate mRNA repression and silencing. These MREs are commonly located in the 3'UTR; however, it is noteworthy that a large portion of *miR-181a* MREs exist in coding regions and in 5'UTRs [16]. Although only one *miR-181a* MRE is required to confer repression, increased frequencies of *miR-181a* MREs in mRNA transcripts lead to increased target repression [16].

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2.2. Genomic Variations of miR-181a

Investigation into the potential consequences of miRNA's genetic variation is still developing. Although more than 30 single-nucleotide variations (SNVs) of *miR-181a/b* within chromosome 1 are known to exist, there is limited literature describing their roles in *miR-181a's* activity.

One single-nucleotide polymorphism (SNP), rs322931 (C>T), has recently garnered attention. The minor allele of rs322931 is associated with lower *miR-181a* expression in the brain and the blood [17]. Variations in rs322931 are also associated with the *miR-181* family's role in regulating inflammation across various cell types, particularly the colon. Patients carrying the SNP rs322931 have exhibited aggravated inflammatory responses. For instance, in peripheral blood samples of Chron's disease patients, those carrying rs322931 had increased inflammatory factors, including TNF-α, IL-1β, IL-6, CRP, SSA, AAT, AAG, and HPT [18]. Additionally, rs322931 is associated with a heightened predisposition to systemic lupus erythematosus, a disease characterized by chronic inflammation and immunologic abnormalities [19]. Moreover, higher expression levels of the rs322931 SNP correlates with increased happiness and significant risk for ischemic stroke [17,20–22].

In CRC patients, a SNP in *MIR181A1* with a minor allele frequency >5%, rs12039395 G>T, was evaluated. In these patients, the G-allele was significantly more prevalent than the T-allele; however, this group was the first to identify that *miR-181a-5p* is overexpressed in both cancerous and normal tissue samples carrying the G-allele over the T-allele. Given that rs12039395 is found 1.31 Kb upstream of *pri-miR-181a-1,* primary structures containing the G-allele have a significantly different predicted loop formation with higher minimum free energy requirements than the T-allele. This suggests a potentially less complex structure with greater accessibility for protein modulators that contribute to its elevated mature expression [23]. Another, SNP rs7550394, residing in *LINC01221* genomically adjacent to *miR-181a/b-1*, has also been associated with changes in *miR-181a* expression levels and pathogenicity [17]. Further exploration of the incidence of these SNPs could elucidate the molecular underpinnings contributing to changes in *miR-181a* processing, while also identifying increased associations between normal and pathological states and their response to therapeutic options in cancer and other diseases.

Aberrations in the seed sequence of a miRNA can cause striking differences in the function, number, and types of target sequences it regulates post-transcriptionally. Mutations in the first two nucleotides of *miR-181*'s seed sequence (AC), or the fifth and sixth nucleotides (UC), can abolish the miRNA's activity, while mutations in the third and fourth nucleotides (AU), do not impair its function [24]. Chira et al. conducted a bioinformatic analysis examining the base composition in the seed region of mature *miR-181* genes and concluded that an A>G substitution in the 4th nt would result in the most significant change in the number of predicted targets, suggesting that *miR-181*'s target activity is heavily influenced by binding at this specific purine [25].

The impact of changes in the nucleotide sequences outside of the seed region on the structural conformation of *pri-* and *pre-miR-181a* is crucial for investigating the interactions of its processing and binding partners. icSHAPE (in vivo click selective 2-hydroxyl acylation and profiling experiment) is a technique used to predict the secondary structure of RNA in cells by measuring nucleotide flexibility at each base [26]. icSHAPE-MaP combined with RNA-immunoprecipitation studies (RIP) suggest that the secondary structure of *pre-miR-181a-1* matches the predicted structure found in miRBase, apart from the last few nucleotides on the 3′ end [27]. Although the wild type structure of *pre-miR-181a* is known, identification of mutant *pri-* and *pre-miR-181a* structures could be beneficial in elucidating structural components key to their function. Through ectopic expression of *miR-181a-1* mutants, Liu et al. note that mutations in the nt 8-9 (AA), 10-11 (CG), 14-15 (GU), 20-21 (GA), or 22-23 (GU) can reduce its activity, while mutations in nt 16-17 (CG) can increase its activity. Moreover, mutations in the *pre-miR-181a* loop, particularly nt 26-27 (GG), 30-31 (UU), and 32-33 (CA), resulted in reduced downstream *miR-181a* activity [24]. Chira et al. also examined how base substitutions can impact the secondary structure of *miR-181* family members. For instance, in the case of an A>G substitution in the 4th nt in *miR-181a*, this new secondary structure conformation resulted in a more extended 5′ arm by one residue and a free U residue at the 3′ end, creating a clamp-like structure [25]. Interestingly, this substitution leads to a reduced class of target genes, each associated with pro-

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oncogenic roles in human cancers, including Tripartite Motif Containing 5 (*TRIM5*) and *CDK2AP1*. This novel study highlights the potential for miRNAs to undergo "reprogramming" for a niche group of targeted interactions [25].

2.3. Canonical and Noncanonical miR-181a Processing

Canonical biogenesis of miRNAs includes the processing of pri-miRNAs by DROSHA/DGCR8, trafficking of pre-miRNAs into the cytoplasm by Exportin 5, and pre-miRNA to mature miRNA processing by DICER **(Figure 1)**. However, some miRNAs are produced in noncanonical DICER-independent pathways. Exploration of miRNA processing specific to the *miR-181* family remains in its infancy but suggests *miR-181a* could be processed via canonical and noncanonical mechanisms. In DICER-deficient murine sarcoma models, expression of all *miR-181* family members was reduced but not eliminated, demonstrating the existence of a DICER-independent processing pathway used by the *miR-181* family [28]. These findings were validated in human HCT116 *DICER*$^{-/-}$ cells where *miR-181a-5p* was expressed, although at reduced levels compared to cells with wt DICER [29]. These findings are mostly supported by the Huntsman group observing that *miR-181a-5p* was still expressed in hemizygous DICER$^{+/fl\text{-}D1693N}$ induced murine oviductal mutants and their corresponding tumors. However, in this model *miR-181a-1-3p* and *miR-181a-2-3p* were lowly expressed, suggesting that *miR-181a-5p* processing could be controlled independent of DICER while *miR-181a-3p* processing largely requires DICER [30].

Outside of DICER, other miRNA mediators have been shown to process *miR-181a* through canonical and noncanonical mechanisms. In HCT116 *DROSHA*$^{-/-}$ cells, *miR-181a-5p* levels were greatly reduced, suggesting that this miRNA is heavily processed by DROSHA [83]. These findings were further affirmed by Wang et al. demonstrating a direct interaction of *pri-miR-181a* and DROSHA in a breast cancer context [29]. In *XPO5*$^{-/-}$ HCT116 cells, *miR-181a-5p* levels were not changed, suggesting that *pre-miR-181a* may not be exported into the cytoplasm alone by Exportin 5, and there is another key transporter of *miR-181a* that remains to be identified [29]. Further investigation of *miR-181a*'s processing would elucidate critical machinery involved in its expression and could point to targetable interactions in *miR-181a* dysregulated diseases.

Upon mature *miR-181a* synthesis, little is known about this microRNA's targeting mechanisms. More than 1,300 conserved target sites within transcripts have been predicted for *miR-181a-5p* using the TargetScan [31] software and 635 targets in miRTarBase [32]. However, further analysis of the *miR-181a/b* targetome has captured 2,995 target transcripts using an Ago2-*miR-181a*-mRNA chimeric enhanced UV crosslinking and chimeric eCLIP immunoprecipitation procedure [16]. This study identified new targets not previously predicted by target identification software. The authors propose that these targets contain a noncanonical MRE that the *miR-181a* seed sequence binds to. Canonically, the seed sequence, ACAUUC, binds to a complementary MRE found in mRNA, UGUAAG. However, an alternative seed match sequence, UGUUAG, was identified in nearly half of the targets [16]. This A-to-U seed match variant demonstrates that absolute matching of seed sequences is not required for *miR-181a* targeting and suggests that the targetome of *miR-181a* could be much larger than anticipated.

Targeting of *miR-181a* to these MREs is dependent on *miR-181a*-RISC interaction [16]. Once mature *miR-181a* interacts with the 3'UTR of its targets, mRNA-mediated gene regulation occurs via RNA destabilization rather than translational inhibition. To our knowledge, this is the first time *miR-181a's* gene silencing mechanism has been characterized [16]. Further elucidation *miR-181a's* mechanism of posttranscriptional gene expression regulation could elucidate *miR-181a*-mRNA networks that are dysregulated and contribute to disease progression.

2.4. RNA Binding Protein Interactions with miR-181a

RNA binding proteins (RBP) are pivotal in governing RNA processing through splicing factors, post-transcriptional regulators, and stabilizers. However, the specific RNA binding partners associated with *miR-181a* remain relatively unidentified. One study postulated that an RBP belonging to the heterogenous nuclear ribonucleoprotein class, Synaptotagmin-binding Cytoplasmic RNA-

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Interacting Protein (SYNCRIP), plays a role in *miR-181a* processing. Knockdown of SYNCRIP reduced precursor and mature *miR-181a* levels and restored mesenchymal to epithelial transitioning (MET) via the control of TGF-β, a known target pathway of *miR-181a* [33]. While this study suggests SYNCRIP as a potential RBP of *miR-181a*, their direct interaction remains unclear.

Lin-28 is a famous RNA binding protein that regulates developmental processes and directly regulates *let-7*. It has been shown in pan-cancer models that *miR-181a* directly targets the 3'UTR of *Lin28*, an inhibitor of let-7 maturation [34]. Given that *let-7* dysregulation has been shown to play a crucial role in carcinogenesis, *miR-181a* mediated reduction in mature *let-7* expression could have greater implications on tumor formation, which remains to be explored.

2.5. miR-181a's Classical Role

miR-181's regulatory network encompasses key mediators of stemness and cell fate to control development in various tissue types [35,36]. Properly orchestrated vasculogenesis and angiogenesis are critical from early embryonic development through adulthood [37,38]. The nature of these processes requires tight spatial and temporal regulation and coherence of various biological processes [38]. The *miR-181* family can execute the multi-pathway regulation required to coordinate endothelial differentiation and angiogenesis through direct targeting of key mediators such as *PROX1, ERK, MMP-2, MMP-9, PDGFRA*, and more [37,39]. Furthermore, *miR-181a/b* are indispensable for controlling differentiation. For example, in retinal development, proper axonal guidance and cell-fate specification by facilitating a process that allows the TGF-β pathway to tune MAPK signaling [40]. Here, TGF-β increases mature *miR-181a* expression via SMAD2/3 mediated processing which in turn targets *ERK2* mRNA for degradation. Knockdown of *miR-181a* in vitro and in vivo ablates this crosstalk, resulting in developmental defects of the visual system. Paradoxically, *miR-181a* promotes differentiation in adipocytes and osteoblasts through negative regulation of TGF-β signaling, indicating tissue-specific regulatory activity [41–43]. In these cell types, *miR-181a* can promote differentiation by blunting the TGF-β pathway by directly targeting *TGF-βR1* [44]. Furthermore, in osteogenesis, *miR-181a*/b promote differentiation by targeting *PTEN*, thus enhancing PI3K signaling [41,45].

miR-181a has also emerged as a critical regulator of T-cell development [46]. Appropriate T-cell maturation is predicated on clonal deletion of self-recognizing thymocytes. This inextricable feature for avoiding autoimmunity is carefully orchestrated within the thymus, wherein even weakly affinitive self-peptides can induce negative selection. High expression of *miR-181a* has been shown to govern T-cell receptor (TCR) signaling making early thymocytes more sensitive to cognate antigens. This results in a more rigorous negative selection during development only to be downregulated in differentiated T-cells, thus increasing the activation threshold as a protective measure against autoimmunity [46,47]. In this way, *miR-181a* primes TCR signaling intermediates by governing the expression of multiple negative regulators. Li et al. demonstrated that *miR-181a* acts as a rheostat through repression of key phosphatases that negatively regulate the TCR signaling cascade [47]. *miR-181a* primes TCR signaling intermediate *LCK* uniquely through multi-target repression of phosphatases meant to restrain the pathway such as PTPN22, *SHP-1*, and *DUSP5/6* (via ERK dephosphorylation) [47]. *miR-181a* has also been implicated as a key mediator of γδ T-cell maturation. High intrathymic *miR-181a* expression in immature γδ T-cells restricts differentiation by targeting *MAP3K2* and *Notch2* [48]. Importantly, *miR-181a* expression declines in T-cells over time, suggesting that *miR-181a* plays a key role in age-related adaptive immunity [46]. This decline in expression is thought to be controlled by transcription factor YY1, binds to the enhancer region of *MIR181A1B1.* As the expression of *YY1* decreases with age, so does *pri-miR-181a* [46].

Beyond lymphocytic cells, *miR-181a's* role in myeloid cells has also been recently described. For example, CD34+ hematopoietic progenitor cells require *miR-181a* to differentiate into mature CD56+ NK cells. At each stage of NK cell development, *miR-181a* expression steadily increases, emphasizing its role in NK cell development. Further, *miR-181a* is essential for CD56+ NK cells to produce IFN-γ but does not affect natural cytotoxicity receptors on the surface of NK cells [49,50]. Interestingly, *miR-181a-5p* expression levels decrease in CD27+ NK cells as animals age [50,51]. In M1, M2, and M3 acute myeloid

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leukemia (AML) patients, *miR-181a* is elevated suggesting that it plays a prominent role in leukemogenesis. The authors go on to show that *miR-181a* decreases during granulocytic and macrophage differentiation [52].

Taken together, these studies highlight *miR-181a's* role in regulating cellular plasticity and differentiation in numerous cellular contexts. It is precisely these characteristics of *miR-181a*'s regulome that make its dysregulation a potent and pervasive mediator of oncogenesis. In this review, we describe the current literature surrounding *miR-181a* as a highly relevant miRNA in human cancer, discussing both the critical signaling pathways it regulates and is modulated by.

3. *miR-181a* in a Cancer Context

miR-181a plays a pivotal role in cancer, either as a tumor suppressor or an oncomiR. This duality is due to context-specific expression levels or genomic modifications, underscoring its use as a biomarker in clinical settings. The expression level of *miR-181a* in cells concerning its ability to enhance, stabilize, or inhibit target mRNA functions is critical in defining its role in cancer.

miR-181a can function as a tumor suppressor and upon dysregulation is downregulated in a subset of cancers, including, leukemias, gliomas, melanomas, oral squamous cell carcinomas (OSCC), non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), cervical cancer, and retinoblastomas (RB) [53–65]. In RBs, *miR-181a* targets *NRAS* through 3'UTR binding, thereby regulating RAS-induced oncogenic signals [61]. In colorectal cancer (CRC), *miR-181a* represses PLAG1/IGF2 signaling, subduing proliferative signals and sensitizing CRC cells to 5-fluoruracil chemotherapy treatment [66]. *miR-181a*-2-3p also targets *HIF-2α*, suppressing cancer stem cell (CSC) phenotypes [67]. In the ABC subgroup of diffuse large B cell lymphoma (DLBCL), *miR-181a* suppresses constitutive activity of NF-Kβ signals, controlling cellular proliferation and mediating cell death [68]. Collectively, it is evident that increased *miR-181a* expression levels in these cases are crucial in defining the anti-oncogenic role of *miR-181a* compared to normal cells.

Despite evidence supporting *miR-181a*'s tumor suppressive roles, significantly more studies suggest its oncogenic functions in tumor initiation and progression. Higher expression levels of *miR-181a* are associated with poor prognosis and increased the risk of disease progression in patients diagnosed with breast cancer (BC) [69–75], high-grade serous cancer (HGSC) [76–79], endometrial cancer [80], CRC [81–84], gastric cancer (GC) [60,85–87], NSCLC [88], brain stem gliomas [89], AML [52,90,91], acute lymphoblastic leukemia (ALL) [55,92,93], chronic lymphocytic leukemia (CLL) [94], pancreatic cancer (PaCa) [95], hepatocellular carcinoma (HCC) [96], or thyroid cancer [97]. In HGSC, the most aggressive subtype of ovarian cancer (OC), *miR-181a* has been shown to induce stem-like properties and drive tumor progression and recurrence [76,79,98–101]. *miR-181a* is also enriched in the serum and circulating tumor cells of BC, GC, and OC patients, further emphasizing its role as a putative biomarker [73,75,79,102,103]. In BC, *miR-181a* expression positively correlates with tumor aggressiveness and with primary grade 3 tumors, showing higher *miR-181a* expression levels than grade 1 or 2 [104]. The expression levels of *miR-181a* also increase during the progression from non-neoplasia to dysplasia in inflammatory bowel disease-associated CRC [105]. Moreover, *miR-181a* promotes transformation, tumor growth, and suppresses immune stimulation in several cancers leading to a pro-tumorigenic environment [71,81,98,106]. These hallmark capabilities acquired upon high *miR-181a* expression underscoring this miRNA's role as an oncogenic driver

Mechanistically, both overexpression and under-expression of *miR-181a* are associated with aberrant activation of several cellular pathways involved in tumorigenesis, including TGF-β, STAT, WNT, PTEN/AKT, and MAPK. Regulation of *miR-181a* is critical for maintaining homeostasis, and its dysregulation is concomitant with cancer pathogenesis. Here, we will provide a concise overview of numerous crucial signaling pathways that contribute to cancer and involve *miR-181a*. Understanding these dynamics is essential in discovering novel therapeutic modalities of targeting *miR-181a* in these oncomiR-driven diseases.

4. Genomic and Epigenetic Changes at the *miR-181a* Loci in Cancer

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The diversity seen in *miR-181a* expression levels across cancer lineages can be tied to changes in ploidy number due to genomic instability and epigenetic alterations at the *miR-181a* loci on chromosome 1 & 9. In head and neck cancer and ovarian cancer, where *miR-181a* overexpression is established as a known driver of oncogenesis; there are notable increased copy number gains at this locus [107–109]. In contrast, *miR-181a* copy number losses are notable in lymphoid cancer lineages [110]. In this cancers, *miR-181a* can function as a tumor suppressor and is known to be downregulated [55]. Ultimately, *miR-181a* copy number alterations could confer cancer progression, tumor heterogeneity, and drive resistance.

4.1. Acetylation Marks

Increased chromatin accessibility, marked by elevated H3K27ac marks, is a recognized driver of disease pathogenesis, particularly in cancer. For instance, in HGSC where *miR-181a* is oncogenic, H3K27ac modifications at the *miR-181a* promoter are elevated and are further amplified as cells become platinum-resistant [100]. Our group further showed that treatment with bromodomain and extra-terminal motif (BET) inhibitors, which target acetyl-lysine groups on open chromatin and suppress transcription, strongly reduce *miR-181a* expression. However, one study challenged this concept showing that, treatment with TSA, a histone deacetylase inhibitor (HDACi) which improves chromatic accessibility, has been shown to further elevate *miR-181a* expression in ALL where *miR-181a* is overexpressed [111]. Conversely, in contexts where *miR-181a* is tumor suppressive, including some CRC cases, butyrate supplementation, another HDACi, has been demonstrated to synergize with *miR-181a*, enhancing anti-proliferative effects [112].

4.2. Methylation Marks

The Polycomb group of proteins Repressive Complex 1 and 2 (PRC1 and PRC2) are epigenetic regulators that establish gene silencing. PRC2 mediates histone methyltransferase activity on histone H3 lysine 27. Trimethylation of this residue (H3K27me3) induces chromatin compaction and has been shown to recruit PRC1 to aid in the repression of target gene sites [113,114]. Dysregulation of these epigenetic writers is well characterized in cancer.

Interestingly, in BC and prostate cancer (PrCa) where *miR-181a* can be tumor suppressive, PRC2 H3K27me3 deposition at the *miR-181a* and *miR-181b* loci represses their expression enabling cancer progression. The histone methyltransferase subunit of PRC2, EZH2, was shown to play a significant role in this transferase activity, where inhibition of EZH2 increased *miR-181a* expression and vise versa. Furthermore, in a normal cell context, *miR-181a* would be able to directly target *BMI1* and *RING2*, E3 ubiquitin-protein ligase subunits of PRC1 that modulate monoubiquitylation of histone H2A lysine 119, thereby preventing the transcriptional repression of target genes. However, inhibition of *miR-181a* expression by aberrantly expressed EZH2 prevents *BMI1* and *RING2* inhibition, leading to cancer progression [115]. The role of EZH2 in regulating *miR-181a* has also been implicated in astrocytic and GBM tumors [116].

The EpimiR database has shown that H3K27me3 reduces *miR-181a/b* expression [117]. Interestingly, *miR-181a* expression is upregulated upon DNA methyltransferase inhibitor (DNMTi) treatment in melanoma and CRC cell lines [118–120]. Further, hypermethylation of CpG islands in CRC reduced *miR-181a* expression and this effect was reversed upon DNMTi [66]. The role epigenetic regulation plays on *miR-181a* accessibility, expression, and activity remains relatively unexplored, and further investigation could point to new therapeutic avenues for controlling this oncomiR.

5. Post-Transcriptional Modifications on *miR-181a* in Cancer

Post-transcriptional modifications, such as RNA methylation via S-adenosylmethionine, can control the temporal binding of microRNAs to their target mRNAs. Recent findings indicate that the *miR-181a* transcript is frequently methylated in PaCa [121]. Further exploration of *miR-181a* methylation levels in serum samples could provide a unique diagnostic strategy for PaCa patients.

6. Competing Endogenous RNAs with *miR-181a* in Cancer

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Just like miRNAs, long non-coding RNAs (lncRNAs) and circular RNAs (cirRNAs) also harbor distinct MREs that allow them to regulate the post-transcriptional activity of miRNAs. This ability to function as competitive inhibitors of miRNA activity characterizes this group of non-coding RNAs as competing endogenous RNAs (ceRNAs). In some cancer contexts including, CRC, NSCLC, BC, PrCa, endometrial, multiple myelomas, and colon adenocarcinoma (COAD), *miR-181a* functions as a tumor suppressor. In these cancers, ceRNAs like *ANRIL, SNHG7, LUCAT1, CCAT1, LINC01232*, and lncRNA-*MALAT1* complementarily bind to and sequester *miR-181a* through a process known as miRNA sponging, which ultimately promotes oncogenesis [122–129]. Some of these ceRNAs against *miR-181a*, such as *lncRNA-MALAT1*, have even been implicated in secondary complications—like cirrhosis—associated with their primary cancers, such as in chatliver cancer [130]. In other cases, lncRNAs such as muscleblind-like 1 antisense RNA 1 (lncRNA-*MBNL1-AS1*) impede PrCa progression by sequestering *miR-181a-5p*, thereby abrogating *miR-181a*'s repression of *PTEN* and activation of the PI3K pathway [131]. Similarly, in glioma models, *lncRNA-CASC2* directly binds to *miR-181a* in the RISC complex, thereby preventing *miR-181a's* ability to function as an oncomiR—this sponging of *miR-181a* reduces tumor growth by upregulating PTEN and increasing sensitivity to temozolomide (TMZ) chemotherapy. However, overexpression of *miR-181a* can abrogate these effects by inhibiting *lncRNA-CASC2* [132]. Alternatively, *miR-181a* can be upregulated by lncRNAs like *HOTAIR* and *RELA* in papillary thyroid cancer (PTC) [133].

7. Signaling Pathways Modulating *miR-181a* Expression

miR-181a has been shown to regulate numerous signaling pathways that will be discussed later. Conversely, many of these same pathways contain negative and positive feedback loops that control expression of *miR-181a*. Here, we briefly summarize the direct regulators of *miR-181a* expression and activity in both tumor suppressing and tumor promoting roles.

7.1. TGF-β Signaling

Canonical transforming growth factor-β (TGF-β) signaling through the SMAD pathway is responsible for regulating the expression of hundreds of genes, particularly in developmental signaling pathways. Stimulation of this pathway suppresses early-stage tumor growth but promotes transformation, epithelial to mesenchymal transition (EMT), and metastasis in later stages of disease. *miR-181a* has been shown to both be regulated by TGF-β and modulate downstream TGF-β signaling – the latter of which will be discussed later in this review (section 8.1). Notably, TGF-β upregulates *miR-181a* expression [134,135]. The Lutz group mapped the transcriptional start sites of *miR-181a* and identified that the *MIR181A2B2* promoter was strongly transactivated by SMAD3 and SMAD4 transcription factors, suggesting that TGF-β signaling increases *miR-181a* expression [14].

TGF-β upregulates *miR-181a* more strongly in metastatic BC than nonmetastatic BC, promoting metastasis, micrometastatic outgrowth, and increased BC lethality [136,137]. This TGF-β dependent increase in mature *miR-181a* expression occurs in a time and dose-dependent manner. However, it is noteworthy that TGF-β supplementation also dose dependently decreases both *pri-miR-181a* and *pre-miR-181a* transcripts. Mechanistically, the authors suggest that TGF-β induces the binding of SMAD2/3 and DROSHA to the *pri-miR-181a-1* transcript, leading to increased processing of the *pri-* transcripts into mature transcripts [137]. Likewise, *miR-181a* gene expression was found to be dependent on both TGF-β and activin in multiple cancer cell lines. Specifically in BC, the upregulation of *miR-181a* through activin and TGF-β is necessary for cell migration [138]. These findings contrast with Zhang et al.'s conclusion that *miR-181a* is suppressed by activin in mouse granulosa cells [139] **(Figure 2)**.

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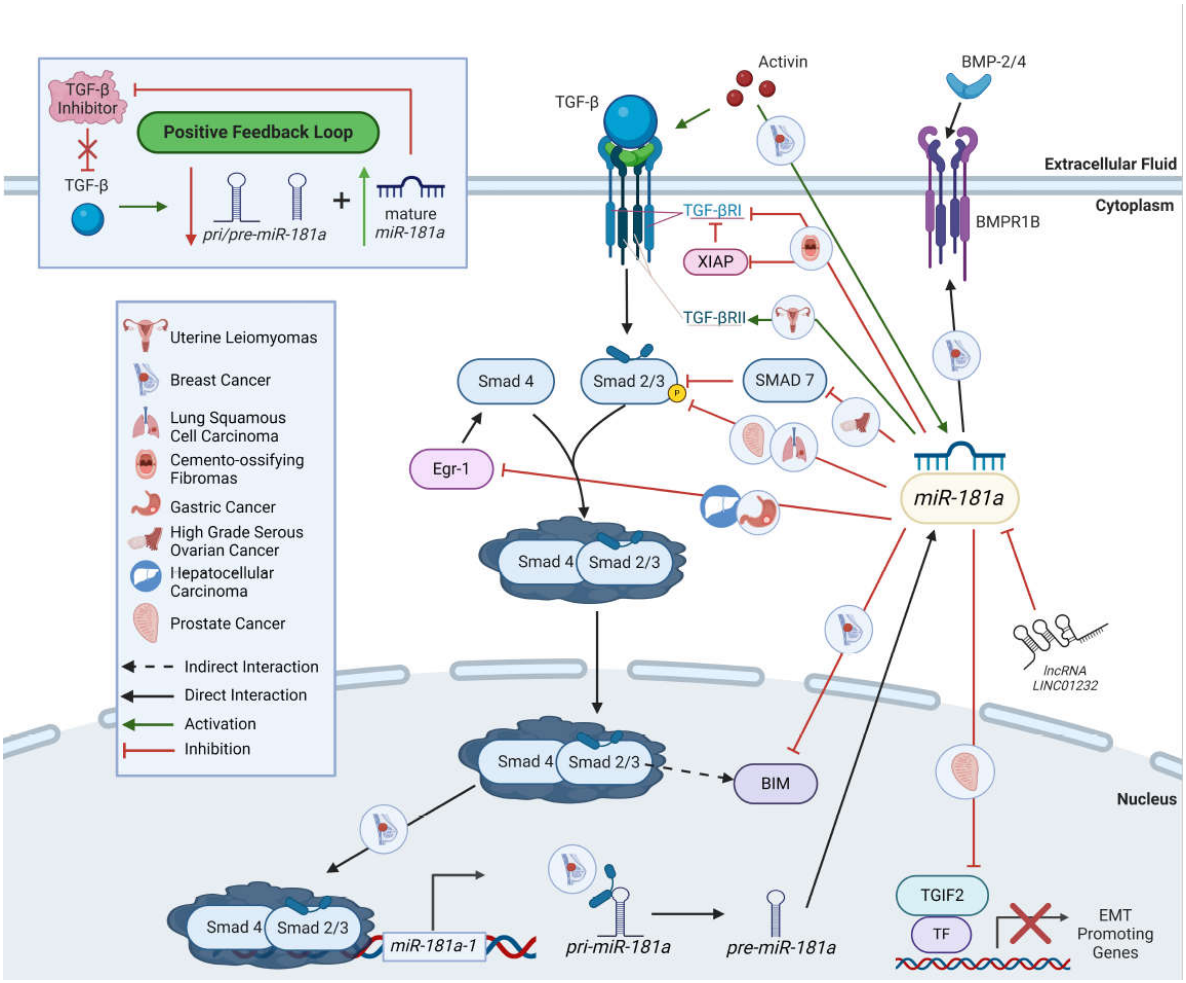

Figure 2. The role of *miR-181a* in the regulation of TGF-β signaling across cancer types. *miR-181a* can promote and TGF-β signaling by upregulating TGF-βRI, inhibiting *XIAP*, an inhibitor of TGF-βRII, and directly inhibiting *SMAD7*, an inhibitor of TGF-β signaling. Increased TGF-β signaling also leads to SMAD2/3/4 transactivation of *miR-181a* transcription, SMAD2/3 complexing with *pri-miR-181a*, and increased processing of *pri-/pre-miR181a*, elevating mature *miR-181a* levels. Activin treatment also promotes TGF-β signaling and *miR-181a.* Alternatively, *miR-181a* can inhibit TGF-β signaling by targeting *SMAD2, Egr-1, TGF-βRI, BIM* and *TGIF2* directly. Alternatively, *SMAD2* expression is also affected by *LINC01232*, which can sponge miR-181a, facilitating the increase of *SMAD2*. *miR-181a* is predicted to interact with *BMPR1B*, a crucial regulator of SMAD signaling. Figure generated using BioRender.

7.2. Wnt Signaling

Canonical Wnt/β-catenin signaling is crucial in the regulation of cell fate. Evidence suggests that Wnt signaling can transcriptionally activate *miR-181a* in HCC [140]. Functionally, seven putative β-catenin/TCF4 binding sites have been identified in the promoter region of *miR-181a-2* [140]. Further studies have demonstrated a direct interaction of the TCF4/β-catenin complex and the TCF/LEF complex in the promoter region of *miR-181a-2/miR-181b-2* [140]. Importantly, these findings suggest the existence of a positive feedback loop between *miR-181a* and Wnt signaling. Further supporting this conclusion, *miR-181a* regulates the expression of *NLK* (Nemo-like kinase) and *SFRP4*, both inhibitors of Wnt/β-catenin signaling, ultimately stimulating further downstream activity [101,141,142] **(Figure 3)**.

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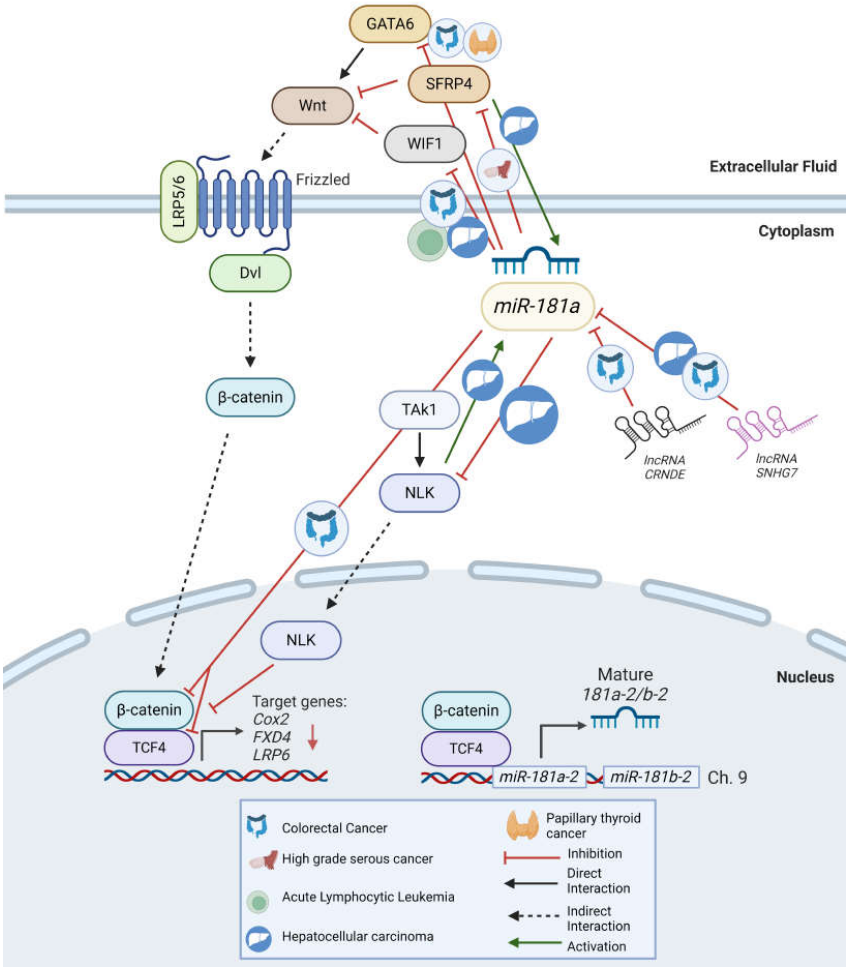

Figure 3. The role of *miR-181a* in the regulation of Wnt signaling across cancer types. In HGSC, *miR-181a* suppresses the Wnt antagonists *SFRP4* and *NLK*. In ALL, HCC and CRC, *miR-181a* directly inhibits *WIF1*, altering cell growth and proliferation. In CRC alone, *miR-181a* overexpression reduced the Wnt target gene expression of *COX2*, *FXD4*, and *LRP6*. *miR-181a* can also function as a tumor suppressor in the Wnt/ β-catenin pathway. In CRC, *miR-181a* suppresses the Wnt signaling activators *β-catenin* and *TCF4*. Alternatively, β-catenin and TCF4 bind to the promoter region of *MIR181A2B2* leading to increased *miR-181a* expression. *miR-181a* is also sponged by *lncRNA-SNHG7*, leading to *GATA6* upregulation in HCC and PTC. Additionally, in HCC *miR-181a* is directly regulated by Wnt signaling through NLK and SFRP4. Figure generated using BioRender.

7.3. STAT Signaling

The STAT (signal transducer and activators of transcription) family of proteins are well characterized mediators of apoptosis, cell signaling, cell proliferation, and differentiation. STAT1, a transcription factor activated by cytokines and growth factors, stimulates the transcription of genes crucial for cell viability. Activation of STAT1 can occur through Type-1 interferon (IFN) signaling, where IFN-α/β activates TYK2 and JAK1, leading to phosphorylation of STAT2. STAT2 then forms a heterodimer with STAT1, translocates to the nucleus, and activates IFN response elements for transcription. Alternatively, Type-2 interferon signaling activates STAT1 through IFN-γ receptor-mediated phosphorylation of JAK1 and JAK2, followed by STAT1 homodimer formation and nuclear translocation to initiate transcription of genes containing Gamma-Activated-Sequences (GAS) – particularly c-GAS, which activates STING-mediated signaling [143].

In tumorigenesis, STAT1 acts as a tumor suppressor by increasing major histocompatibility complex (MHC) expression and exhibiting anti-angiogenic properties [143]. In CRC, where high *miR-181a* expression correlates to poor patient prognosis, STAT1 inhibits CRC cell growth by down-regulating *miR-181a*. STAT1 directly inhibits *miR-181a* transcription by binding to the -890 base pair

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region of the *miR-181a* promoter. In this model, increased STAT1 promotes PTEN-mediated tumor suppression and reduces Akt activation. Taken together, STAT1 attenuates the increased cell viability and proliferative phenotypes concomitant with high *miR-181a* expression in CRC [144]. Notably, Zhu et al. subsequently demonstrated that *miR-181a* directly binds to the 3'UTR of *STAT1*, inhibiting its mRNA expression and suggesting a competitive dynamic between *miR-181a* and STAT1 [145]. In summary, the intricate interplay between STAT1 and *miR-181a* highlights their reciprocal regulation and significant impact on cancer cell maintenance and potential to evade immune activation.

7.4. SOX2

The sex determining region Y (SRY-type high mobility gene box) (SOX) gene family encodes transcription factors that are strongly involved in embryogenesis, gonad development, and differentiation. Aberrantly high *SOX2* expression has been involved in tumorigenesis, and cancer stem cell maintenance [146,147]. In BC, SOX2 regulates *miR-181a-5p* expression given that knockdown reduced *miR-181a-5p* expression [148]. Interestingly, *miR-181a* directly targets the 3' UTR of *TUSC3*, a tumor suppressor protein downstream of SOX2 that is dysregulated in ovarian and breast cancer [148–151]. Thus, cancer progression could be modulated through the SOX3-*miR-181a-5p-TUSC3* axis [148].

7.5. HBx

In HCC, Hepatitis B Virus X protein (HBx) has been correlated with cancer development. Interestingly, HBx increases *miR-181a* promoter activity, engendering *miR-181a* induced proliferation and anti-apoptosis in hepatoma cells [152]. Limited research exists exploring this novel mechanism of HCC tumorigenesis; however, further in vivo studies elucidating this process may provide an effective therapeutic approach to targeting Hepatitis B Virus-induced HCC.

8. Signaling Pathways miR-181a Regulates

8.1. TGF-β

Dysregulation of the TGF-β signaling pathway has become synonymous with tumor progression across tumor lineages. The TGF-β signaling pathway has paradoxical roles in cell proliferation. In a pre-malignant state, it functions as a tumor suppressor by exerting cytostatic, pro-apoptotic, and tumor-suppressive effects. Upon loss of TGF-β's tumor suppressive phenotypes, TGF-β signaling promotes oncogenic activity via cytoskeletal rearrangement, EMT, mobilization of cancer associated fibroblasts in the tumor microenvironment (TME), and stimulation of pro-metastatic cytokines [153].

Activation of this this pathway occurs upon TGF-β binding to and activates TGF-β receptors I and II. This receptor complex then phosphorylates and activates SMAD2/3, which subsequently engages with SMAD4 to translocate to the nucleus and interact with DNA-binding transcription factors to regulate transcription. SMAD7 can compete with SMAD2/3 to inhibit their activation outside the nucleus and DNA binding activity inside the nucleus. Numerous studies have implicated *miR-181a's* interaction with these pathway mediators in both tumor suppressive and oncogenic contexts [154].

miR-181a activity in BC is highly controversial, with some groups suggesting *miR-181a-5p* is upregulated and acts as an oncomiR, while others suggest it is downregulated [74,122,155–157]. A bioinformatic analysis of three BC patient datasets revealed *BMPR1B*, a prominent modulator of TGF-β could be regulated by *miR-181a* [158]. Liu et al. showed that TGF-β-targeted DNA repair genes *ATM* and *BRCA1* are direct targets of *miR-181a* and that this targeting can modulate tumor response to PARP inhibitors [159].

TGF-β-induced EMT is well documented in multiple other tumor types, causing enhanced invasion and metastasis [160]. Functionally, studies by Parikh et al. revealed *miR-181a's* involvement in downregulating a TGF-β inhibitor, *SMAD7*, to promote EMT signatures in HGSC. Re-expression of *SMAD7* lacking a 3'UTR prevented *miR-181a* binding and, thus, rescued EMT phenotypes [76]. Similar lung squamous cell carcinoma (LUSC) studies show that *miR-181a* directly targets *SMAD2*.

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However, in LUSC, lncRNA, *LINC01232*, can sponge *miR-181a,* facilitating increased *SMAD2* expression, TGF-β pathway stimulation, and enhanced proliferation, migration, and invasion [129].

In contrast, other studies in PrCa have identified a possible mechanism in which *miR-181a* inhibits SMAD function through transcriptional repressor *TGIF2* favoring EMT transitions and inducing drug resistance mechanisms [161,162]. Additionally, Bi et al. demonstrated that *miR-181a-5p* is a negative regulator of early growth response factor 1 (*Egr1*), downregulating in TGF-β/Smad signaling [163]. *miR-181a* and TGF-β1 have also been shown to interact to promote peritoneal dissemination of GC [164]. In T-cell prolymphocytic leukemia (T-PLL), Erkeland et al. showed that *miR-181a/b* is highly expressed, correlates with poor survival, and significantly downregulates predicted target *TGF-β3* [165].

miR-181a also targets various classes of TGF-β receptors across cancer types. High *miR-181a-5p* expression is correlated with increased TGF-βR2 and Insulin-like Growth Factor 2 mRNA Binding Protein 1 in advanced uterine leiomyomas [166]. *miR-181a-5p* is also upregulated in cemento-ossifying fibromas (COFs) and is correlated with increased *XIAP* expression, an inhibitor of cell-death caspases 3, 7, and 9 and *TGF-βR1*. Given that *miR-181a* directly targets the 3'UTR of *XIAP* and *TGF-βR1,* upregulation of *miR-181a* may lead to increased proliferative capacity [167] **(Figure 2)**.

8.2. Wnt/β-Catenin

The Wnt/β-catenin signaling stands as one of the most evolutionarily well conserved paracrine and autocrine signaling pathways responsible for orchestrating cell fate determination and organogenesis during embryonic development. Mutations and dysregulation within this pathway consistently serve as critical drivers of recalcitrant cancer subtypes. Activation of Wnt signaling can occur through either the canonical (β-catenin dependent) or non-canonical (β-catenin independent) pathways. Canonical Wnt signaling stimulates the transcription of various transcription factors, extracellular matrix components, and cell adhesion proteins. In contrast, non-canonical Wnt signaling triggers other pathways, including the Wnt-dependent calcium or planar cell polarity pathway. Stimulation of canonical Wnt target genes augments tumor progression, fostering increased cancer cell initiation, persistence, invasion, and metastatic phenotypes.

Within the framework of Wnt signaling, *miR-181a* serves as a significant oncomiR across diverse cancer lineages by targeting WNT and its regulators [154]. As a result, *miR-181a* has garnered attention as an attractive target in Wnt/β-catenin-directed therapies. Recent research led by Nagaraj et al. showcased *miR-181a* suppression of the Wnt antagonist *SFRP4*, thereby promoting tumor initiation and stemness in HGSC [101]. Re-expression of *SFRP4* in a *miR-181a* high cell line notably reduced β-catenin gene expression, leading to a robust decrease in tumor sphere formation ability. Furthermore, an inverse relationship between *miR-181a* and *SFRP4* in primary and recurrent ovarian tumors underscores the significance of targeting this axis in HGSC treatment. In a similar study focusing on highly invasive HCC's positive for epithelial cellular adhesion molecule (EpCAM) and alpha fetoprotein (AFP), Ji et al. demonstrated that *miR-181a*'s directly suppresses another Wnt antagonist, *NLK*, prolonging HCC stemness [142,168].

Evidence of constitutive Wnt/β-catenin activation has been reported in ALL, HCC, and CRC through the direct inhibition of *WIF1* (Wnt inhibitory factor 1) by *miR-181a-5p* [55,81]. Thus, in ALL, blocking *miR-181a* activity significantly alters cell growth and proliferation by inducing cell cycle arrest at the G1/S phase [55]. Further, potentiation of Wnt signaling by *miR-181a* in CRC was evident through a TOPFlash assay, where treatment with a *miR-181a* mimetic reduced the expression of Wnt target genes *COX2, FXD4,* and *LRP6* [112].

Other studies suggest that *miR-181a* functions as a tumor suppressor in the Wnt/β-catenin pathway. *miR-181a* can directly bind to the 3'UTR of *β-catenin* and *TCF4*, crucial activators of Wnt signaling. These studies additionally implicate *lncRNA-CRNDE* as an overexpressed sponge of *miR-181a* in CRC, promoting cell proliferation and chemoresistance [169]. Similarly in HCC, *lncRNA-SNHG7* sponges *miR-181a-5p* leading to upregulation of *GATA6,* a crucial transcription regulator of intestinal cell phenotypic reprogramming [127]. Given that GATA6 has been shown to stimulate Wnt signaling [170], it is possible that the *SNHG7/miR-181a/GATA6* axis could activate Wnt signaling in

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CRC. These results are corroborated by a new study in PTC where *miR-181a* promotes tumorigenesis by directly binding to *GATA6* [142]. Combined, these studies suggest that *miR-181a* may exert repressive effects on Wnt signaling in some cancer subtypes **(Figure 3)**.

8.3. Notch

The *Notch* gene is a transmembrane receptor initially discovered for its notched wing phenotype in *Drosophila melanogaster.* Years later, the Notch pathway became recognized as critically important in several fundamental cellular and developmental mechanisms, including regulation of cellular proliferation, stem cell differentiation, maintenance during embryogenesis, and apoptosis [171]. This signaling pathway is also highly oncogenic and is hyperactive in several cancers such as T-cell acute lymphoblastic leukemia (T-ALL), BC, and NSCLC [154]. Despite years of research, a Notch inhibitor has only recently been approved for the clinical treatment of desmoid tumors, highlighting the necessity to identify new targetable vulnerabilities in this pathway. Recent studies have implicated *miR-181a* modulation of the Notch pathway.

The Notch receptor, Notch2, is overexpressed in many cancers, including GBM. In this context, *miR-181a* is an oncosuppressor. Xiu et al. report that *miR-181a* downregulates *Notch2*, inhibiting the proliferation and differentiation of glioma cells [172]. Huang et al. support these findings, showing that increased *miR-181a* expression in GBM inhibits the stem-like properties of tumor-initiating glioblastoma cells via targeting the 3'UTR of *Notch2* [173]. Furthermore, low *miR-181a* expression correlates with high *Notch2* expression and poor patient prognosis, emphasizing the prognostic significance of *miR-181a* and its role as a tumor suppressor in Notch-driven GBMs.

Recognizing *miR-181a*'s crucial role in lymphocyte development and differentiation, it is not surprising that dysregulation of *miR-181a* causes perturbations in hematopoietic differentiation and stimulates oncogenesis through the Notch pathway. *miR-181a* and *miR-181b* are necessary for natural killer (NK) cell development and IFN-γ production. *miR-181a* downregulates *NLK*, a negative regulator of NK cell development, by directly targeting its 3'UTR, thus potentiating Notch proliferative signaling [49,142]. Additionally, *miR-181a* functionally targets the 3'UTR of *Narp*, a critical regulator of thymocyte development in the Notch pathway. In T-ALL, *miR-181a* acts as an oncogene, and loss of *miR-181a*/b promotes survival via potentiation of the intracellular domain of Notch1 (ICN1). Through loss of function analyses, Fragoso et al. demonstrated that deleting *miR-181a-1/b-1* inhibits the development of notch oncogenes and tumor transformation [174]. These studies emphasize the significant onco-regulatory role of *miR-181a* on the Notch pathway and the treatment potential for targeting *miR-181a* in Notch-dependent cancers.

8.4. PTEN-PI3K-AKT-mTOR Network

The PI3K/AKT/mTOR (PAM) signaling pathway is a signal transduction network highly conserved across the Eukarya domain. This pathway is frequently activated in cancer and involves cell growth, survival, progression, and chemotherapy resistance [175]. The PAM signaling pathway has been recently linked to *miR-181a* expression in several cancer types, largely due to *miR-181a's* inhibitory effect on the phosphatase and tensin homolog protein (PTEN). PTEN is a tumor suppressor in numerous cancers and a negative regulator of PI3K/AKT activity in normal cells [131].

In 2014, *PTEN* was identified as a direct target of *miR-181a* in CRC, and this interaction has since been confirmed in BC, NB, and HCC [21,152,176]. *miR-181a* knockdown effectively restores *PTEN* and E-cadherin expression and reduces the expression of EMT markers, resulting in inhibited cellular migration and invasion [96,152]. Reinforcing these findings in NB cells, PTEN overexpression reversed the effects of *miR-181a* upregulation, inducing cell death and increased ROS production [21]. Furthermore, in endometrial cancer, a study found that non-obese patients showed a correlation between increased *miR-181a* expression and decreased *PTEN* expression [80]. In CRC, *miR-181a* overexpression repressed PTEN and increased AKT activation, the direct downstream target of PTEN inactivation [144,176]. This increase in p-AKT induced a metabolic shift, increasing lactate secretion, glucose uptake, and glycolytic flux [176]. Together, these studies emphasize the regulatory interactions of PTEN and *miR-181a* to drive PAM-mediated cancer progression.

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miR-181a has also been shown to regulate and interact with other modulators of PTEN activity. NDRG2, an N-myc downregulated gene, was identified as a PTEN binding protein, regulating the phosphatase activity of PTEN. In BC, *miR-181a* directly targets the 3' UTR of *NDRG2,* demonstrating an inverse correlation between *miR-181a* and NDRG2 expression. *miR-181a-5p* suppresses NDRG2, resulting in PTEN dephosphorylation and increased phosphorylation of Akt, thus facilitating tumor progression through NDRG2-induced activation of PTEN/AKT signaling pathway [74]. Further, *miR-181a* and *lncRNA-XIST* (X-inactivated specific transcript) directly regulate each other; knockdown of *miR-181a* increases *XIST* expression and vice versa. Interestingly, overexpression of *XIST* abolished *miR-181a*'s inhibitory effects on *PTEN* [96]. In PrCa, lncRNA-*MBNL1-AS1* inhibits tumor progression by sponging *miR-181a-5p*, thereby abrogating *miR-181a*'s repression of PTEN and downstream activation of the PAM pathway [131].

Without PTEN activation, downstream proliferative signaling proteins, such as PI3K, AKT, and mTOR, can also be regulated by *miR-181a* [177]. In CRC, increased *miR-181a* expression significantly decreases *PIK3R3* expression, the regulatory subunit of PI3K, and a predicted target of *miR-181a* [112]. Knockdown of *miR-181a* in HCC reduces p-AKT and p-mTOR expression and subsequently markers of EMT signaling [96]. *miR-181a* and *miR-181d* also directly suppress *PHLPP2* and *INPP4B* phosphatases, augmenting Akt phosphorylation, S-phase entry, and cell proliferation in estrogen receptor-positive BC [136,178]. In GC, Hao et al. found that *miR-181a* downregulates TCL1 family Akt coactivator A's (*TCL1A*) expression and, therefore the activation of Akt family members. Interestingly, this *miR-181a* mediated inhibition of Akt activation increased c-MYC expression, further upregulating *miR-181a,* and establishing a *miR-181a-5p*-TCL1A-Akt/mTOR-c-MYC feedback loop. However, it is worth noting that *miR-181a* does not directly bind to the 3'UTR of *TCLA1* or *c-MYC*, so the molecular mechanism of this interaction remains unknown [179].

miR-181a also modulates chemoresistance via the AKT/ERK pathway. In BC, *miR-181a* directly inhibits *EPDR1* (Ependymin Related 1), a transmembrane protein that facilitates cell adhesion. EPDR1 inhibits PI3K/Akt activation, so increased expression of *miR-181a* in BC facilitates activation of PI3K/Akt signaling and reduces sensitivity to Epirubicin, a BC chemotherapeutic [180]. In NSCLC, Ping et al. show that *miR-181a* is upregulated in gefitinib-resistant NSCLC, and knockdown of *miR-181a* reduced p-AKT in gefitinib-resistant cells [181]. In T-cell leukemias and lymphomas, high *miR-181a* expression also correlates to increased p-AKT expression, cell proliferation, and acquired chemoresistance [182].

Contrary to these findings, some studies show that when *miR-181a* acts as a tumor suppressor, PAM signaling can be suppressed. For example, hormone replacement therapy, including norethisterone, correlates with an increase BC. In this cancer subtype *miR*-181a acts as a tumor suppressor. Here, Cai et al. report that exogenous *miR-181a* suppresses hormone-induced tumorigenesis through the deactivation of PAM pathway proteins including mTOR, EGRF, PTEN, and PGRMC1 [183]. In another BC and CRC example, the transmembrane protein MMP-14 (matrix metallopeptidase) phosphorylates EGFR, activates the MAPK and PI3K signaling pathways, and promotes oncogenic signaling [184]. Thus, upregulation of *MMP-14* correlates with poor patient prognosis. *miR-181a* directly targets the 3'UTR of *MMP-14,* reducing PAM signaling, in vivo tumor invasion, angiogenesis, and migration [185]. These studies point out that *miR-181a* function as a tumor suppressor by inhibiting PAM signaling.

In summary, *miR-181a* plays a crucial role in regulating the PTEN-PAM signaling network in both oncogenic and tumor-suppressive contexts **(Figure 4A)**. Further evaluation of the context-dependent mechanisms of *miR-181a's* regulatory control of this pathway may elucidate how PTEN-PAM signaling promotes cancer progression and chemoresistance.

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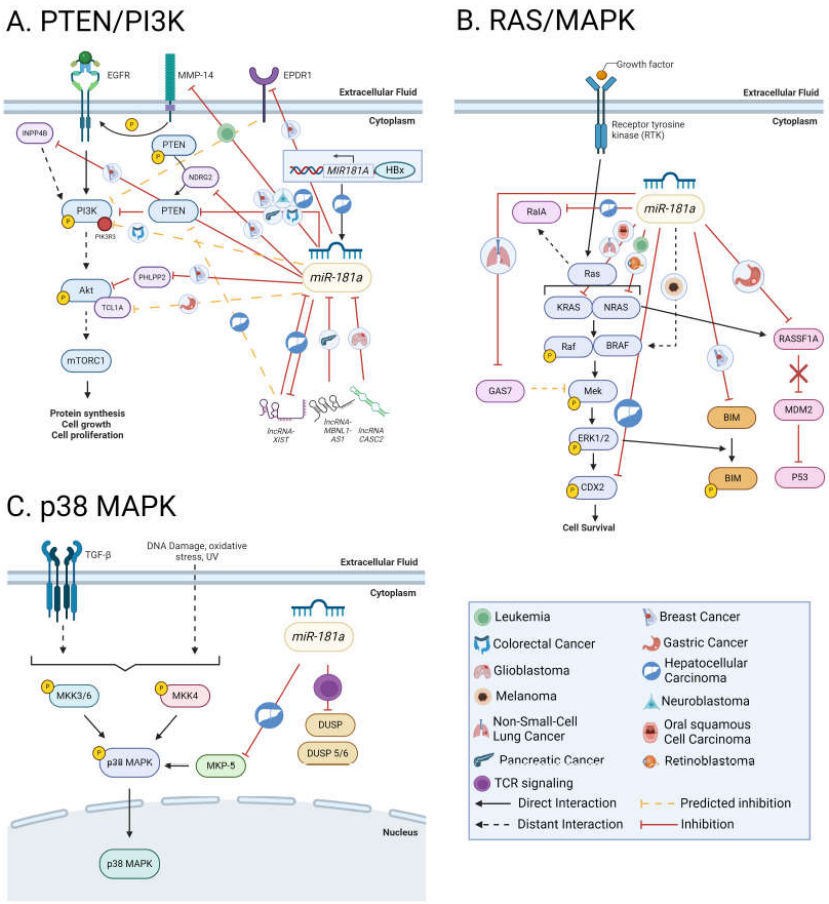

Figure 4. (A) The role of *miR-181a* in regulating PI3K/PTEN signaling across cancer types. To upregulate this pathway, *miR-181a* directly targets of critical tumor suppressor *PTEN* in CRC, BC, NB, and HCC. In BC, *miR-181a* also directly targets *NDRG2*, resulting in PTEN dephosphorylation and increased Akt phosphorylation. To downregulate this pathway, *miR-181a* also interacts with several lncRNAs, such as the *XIST, CASC2,* and *MBNL1-AS1*. *miR-181a* also reduces *PIK3R3* in CRC, *PHLPP2* and *INPP4B* phosphatases in BC, *EPDR1* in BC, and *TCL1A* expression in GC. *miR-181a* also directly targets *MMP-14* in CML thereby preventing propagation of PAM signaling. **(B)** The role of *miR-181a* in the regulation of Ras signaling across cancer types. *miR-181a* can function as a tumor suppressor by directly regulating Ras isoforms, *NRAS* and *KRAS*, in AML, OSCC, and NSCLC. Further, *miR-181a* inhibits *RalA* leading to increased cell cycle arrest and apoptosis. Further, *miR-181a* supplementation has been shown to increase chemo-sensitivity when combined with BRAF inhibitors.As an oncomiR, *miR-181a* can inhibit *RASSF1A* thereby preventing the degradation of MDM2 leading to inhibition of P53. In HCC, *miR-181a* targets *CDX2*, promoting cell differentiation and enriched stem cell properties. *miR-181a* also modulates chemoresistance by targeting proteins *GAS7* in NSCLC. In BC, *miR-181a* directly represses *Bim*, a pro-apoptotic factor that interacts with ERK, thereby promoting cancer progression. **(C)** The role of miR-181a in the regulation of P38/MAPK signaling across cancer types. *miR-181a* interacts with *DUSP5/6* to enhance TCR signaling. Additionally, *miR-181a* can regulate p38 MAPK activation by targeting *MKP-5* in HCC. Figure generated using BioRender. .

8.5. RAS/MAPK/ERK

The Ras family of proto-oncogenes is a group of small GTPases, including NRAS, HRAS, and KRAS. This family plays diverse roles in regulating cell division, differentiation, survival, metabolism, and programmed cell death. Downstream of these GTPase proteins are the canonical RAS/RAF1/MAPK and PI3K/AKT pathways, which drive gene expression, governing cell survival and cell cycle progression, respectively [186]. Dysregulation of these GTPases is well-characterized across various cancer types particularly, CRC and PaCa. A growing body of literature from the last

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decade suggests that *miR-181a* could significantly influence these pathways as either a tumor suppressor or an oncomiR.

In the Ras-MAPK-ERK pathway, *miR-181a* can function as a tumor suppressor. It directly regulates the Ras family by binding to the 3' UTRs of *NRAS, KRAS,* and *MAPK1,* but not *HRAS* [44,60,187]. Huang et al. report that *miR-181a* downregulates *NRAS, KRAS*, and *MAPK1* in AML, thereby decreasing cell proliferation [187]. In RB's, *miR-181a-5p's* 3'UTR inhibition of *NRAS* was shown to decrease proliferation, migration, and invasion while enhancing apoptosis in RB cells [61]. Similarly, *miR-181a's* regulation of *KRAS* in OSCC and NSCLC downregulates *KRAS* gene and protein expression, resulting in increased senescence and suppressed proliferation, migration, colony formation, and anchorage independent cell growth [60,188]. In CML, Gu et al. found that *miR-181a* directly targets the 3'UTR of *RalA*, a signaling molecule downstream of Ras, inducing G2 phase arrest and promoting apoptosis [189]. They later showed that *miR-181a* mimetic treatment combined with imatinib increases treatment sensitivity and reduces phosphorylation of P38 MAPK, SAPK/JNK, Akt, and Erk1/2 [106].

Downstream of Ras signaling, *miR-181a* can also enhance MAPK and ERK's oncogenic activity through other mechanisms. Treatment with *miR-181a* mimics in BC models enhances the basal phosphorylation and activation of ERK, Akt, and c-JUN [136,190]. Additionally, *miR-181a* expression modulates caudal-type homeodomain transcription factor (CDX2), an intestine-specific transcription factor downstream of MAPK and ERK. In highly invasive EpCAM+ and AFP+ HCC, CDX2 is a major protein regulator of MAPK and ERK signaling. In this model, *miR-181a* promotes HCC stemness by targeting the 3'UTR of *CDX2* and *GATA6,* promoting HCC cell differentiation and enriched stem cell properties [141,142,191]. In BC, *miR-181a* directly interacts with the 3'UTR of *Bim*, a pro-apoptotic factor that interacts with ERK, thereby repressing Bim translation and promoting tumor dissemination [136].

miR-181a has also been shown to modulate chemoresistance through aberrant RAS/MAPK/ERK signaling. Ping et al. noted that *miR-181a-5p* is upregulated in gefitinib-resistant NSCLC. Here, *miR-181a* drives chemoresistance by directly targeting *GAS7* (growth arrest specific-7), increasing AKT and ERK activation, migration, and invasion [181]. In contrast, *miR-181a* expression can promote chemo-responsiveness. BRAF inhibitors, such as dabrafenib, are used in melanoma treatment but lack efficacy due to developed resistance. Barbato et al. found that *miR-181a/b* replacement therapy in melanoma cells with downregulated *miR-181a/b* increased dabrafenib sensitivity when combined with BRAF inhibitors. Clinically, this group saw increased chemo-responsiveness in patients with higher *miR-181a* [58].

In GC, where *miR-181a* functions as an oncogene, studies show that *miR-181a-5p* is a novel regulator of the Ras-MAPK pathway through its interaction with the Hippo pathway. *miR-181a* negatively regulates the highly conserved tumor suppressor, Ras-associated domain family member (*RASSF6*) by binding to its 3'UTR [86,192]. This prevents degradation of MDM2, thereby inhibiting P53 and ultimately inducing multiple proliferative phenotypes, including EMT, invasion, and metastasis. Inhibition of *miR-181a* reverses these phenotypes and prevents cell cycle transition. These findings highlight the various influential roles *miR-181a* has across the different dimensions of cancer-promoting signaling pathways **(Figure 4B)**.

8.5.1. p38 MAPK

The p38 subgroup of MAP kinases serves as a center for signal transduction while playing a dual role in regulating cell death and survival. Mechanistically, p38 MAPK activation is mediated by dual phosphorylation of its Thr and Tyr residues in the TXY motif, differentiating it from ERK and JNK kinases. p38 is generally activated by environmental stress, inflammatory cytokines, and TGF-β signaling [193].

miR-181a interacts with the p38 MAPK pathway via its downstream dual specificity phosphatases (DUSPs). DUSPs are a class of genes and associated proteins that dephosphorylate Ser, Thr, and Tyr residues, ultimately regulating protein activity [194]. Specifically, DUSP5 and DUSP6 are controlled by *miR-181a* [47]. Okada et al. expanded on this work, suggesting that *miR-181a's*

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regulation of DUSPs could be exploited as a promising cancer immunotherapy [195]. It is known that *miR-181a* augments the sensitivity of TCR-mediated T-cell responses and that DUSP5/6 negatively regulate TCR signaling. The authors suggest that *miR-181a* directly targets *DUSP5* and *DUSP6*, which in turn upregulate TCR signaling and reduce the T-cell activation threshold. Leveraging this *miR-181a/DUSP* interaction could serve as a potential therapeutic angle to shift immune-cold environments to immune-hot microenvironments [195].

miR-181a can also regulate p38 MAPK activation by targeting the 3'UTR of MAPK phosphatase 5 (*MKP-5*) [196]. MKP-5 blocks the enzymatic activation of p38 [197]. However, in HCC cell lines exposed to carcinogens benzo[a]anthracene and benzo[k]fluoranthene, *miR-181a* is significantly upregulated, leading to *MKP-5* suppression and continued p38 MAPK activation. Phenotypically, this drives migration [196]. Given these juxtaposing roles of *miR-181a* in driving p38 MAPK-mediated tumor suppression or, conversely, cancer migration, further characterization *of miR-181a's* activity in the p38 MAPK pathway is needed **(Figure 4C)**.

8.6. Snail

The Snail family of zinc-finger transcription factors consisting of SNAI1 (Snail), SNAI2 (Slug), and SNAIL3 (Smuc) plays a significant role in embryonic development [198]. Corruption of this crucial pathway illustrates how developmental pathways are repurposed to drive cancer progression. In the context of cancer, the Snail family promotes EMT and CSC maintenance [199]. Specifically, SNAI1 and SNAI2 stimulate tumor metastasis and immune suppression within the TME [200]. In BC, *miR-181a,* along with transmembrane bax inhibitor motif-containing 6 (TMBIM6), induces SNAI1 and SNAI2 expression by initiating upstream ERK activation [190]. In PrCa, migration and invasion inhibitory protein (MIIP) inhibits *miR-181a-5p,* resulting in reduced Snail and Twist expression and inhibited tumor growth [201]. Additionally, *miR-181a* is also implicated in the migration and invasion of salivary adenoid cystic carcinoma via regulation of the MAPK-Snai2 pathway. The Inhibition of *miR-181a* increases the expression of MAP2K1, MAPK1, and SNAI2, leading to enhanced metastatic potential [199]. In summary, *miR-181a* significantly influences the Snail family, particularly, SNAI1 and SNA2, to modulate carcinogenesis.

8.7. Hippo-YAP/TAZ

The canonical Hippo pathway is a kinase cascade that restricts the activity of two transcriptional activators, Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ). Activated YAP and TAZ induce cytoplasmic retention and ubiquitin-mediated proteasomal degradation and prevent activation of the transcription factor TEAD. TEAD stimulates the transcription of various genes responsible for cell proliferation, migration, and survival [202]. The interaction between this pathway and several noncoding RNAs has been well-researched, as discussed in a recent review from Zhang et al [203].

Recent studies implicate *miR-181a*'s involvement in the Hippo pathway. In PTC, exosomal *miR-181a* targets the 3'UTR of mixed lineage leukemia 3 (*MLL3*/*KMT2C*), a histone-lysine N-methyltransferase. These exosomes reduce the enhancer activity of disheveled binding antagonist of beta-catenin 2 (DACT2) in adjacent endothelial cells, thus activating the YAP/VEGF signaling pathway and promoting proliferation, migration, tumor formation, and capillary network formation [204].

In multiple myeloma, *miR-181a-5p* is downregulated. Re-expression of *miR-181a* in myeloma cells inhibits proliferation, adhesion, and promotes apoptosis. The authors show that this re-expression via a *miR-181a* mimic increases LATS1, p-YAP1, and reduces total YAP1 mRNA and protein expression. Another connection between the *miR-181a* and Hippo-YAP/TAZ pathway was identified in CML. Long-term exposure to the tyrosine kinase inhibitor, imatinib mesylate (IM), results in dysregulation of the Akt/Erk1/2 signaling pathway, *miR-181a* inhibition, decreased YAP levels, and increased sensitivity to IM treatment [205]. Although multiple studies demonstrate that elevated *miR-181a* levels are concomitant with activation of YAP/TAX, the molecular mechanism driving *miR-181a*'s stimulation of the Hippo pathway remains elusive [206].

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8.8. JAK/STAT

The Janus kinase/signal transducers and activator of transcription (JAK/STAT) signaling pathway mediates cellular processes crucial for proliferation, differentiation, migration, apoptosis, immune cell development, and hematopoiesis [207]. Its activation is triggered by cytokines such as IL-6 and growth factors like EGF, which lead to the phosphorylation of intracellular JAKs and downstream STAT signaling.

Several studies have linked the post-translational regulation of this pathway to miRNAs, including *miR-181a*. In BC, *miR-181a* inhibits *PIAS3* but not *SOCS3*, both of which are inhibitors of the JAK/STAT pathway [72]. Elevated levels of *miR-181a* in breast tumor exosomes can activate STAT signaling by targeting *SOCS3*, which favors the expansion of immature early myeloid-derived suppressor cells and suppresses T-cell immunity [72]. Conversely, other research in T-cell large granular lymphocytic leukemia (T-LGL) indicates that *miR-181a* targets the 3'UTR of both *SOCS3* and *DUSP6*, an ERK signaling inhibitor [208]. Knockdown of *miR-181a* restores DUSP6 and SOCS3 expression and induces apoptosis [208]. In GBM models, Schafer et al. found that aberrantly high metalloprotease-disintegrin ADAM8 expression downregulates *miR-181a* via activation of STAT3 and MAPK [209]. In liver metastasis of CRC, *miR-181a-5p* enrich extracellular vesicles appear to activate hepatic stellate cells directly by targeting *SOCS3* and activating IL6/STAT3 signaling. Overall, *miR-181a* influences tumor progression and immune stimulation in various cancers by regulating JAK/STAT signaling **(Figure 5A)**.

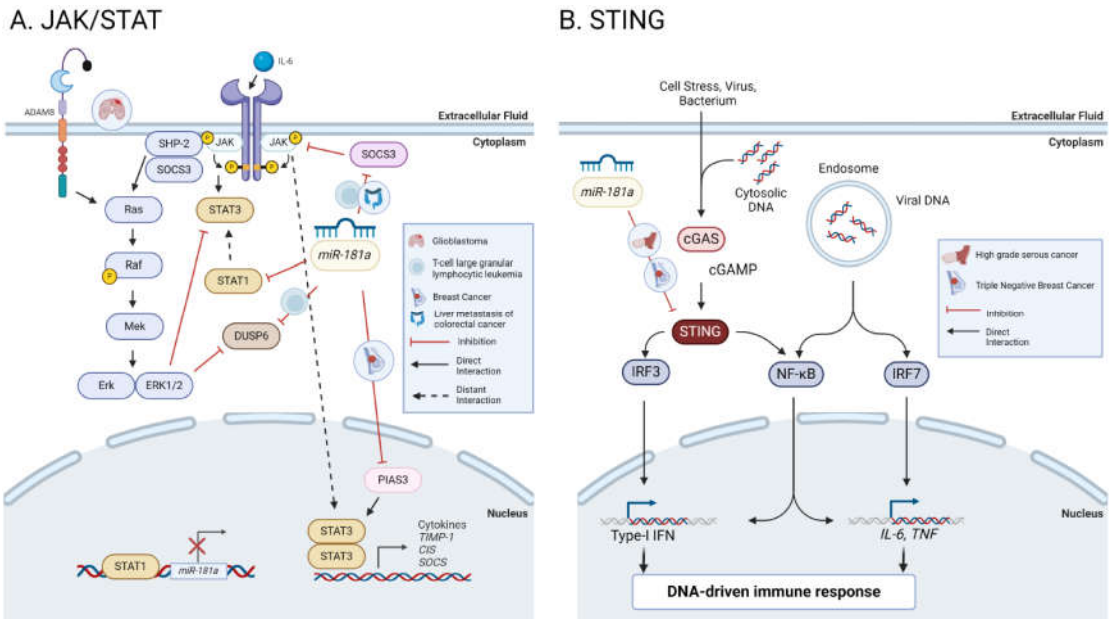

Figure 5. (A) The role of *miR-181a* in the regulation of JAK/STAT signaling across cancer types. *miR-181a* directly inhibits *PIAS3* and *SOCS3* in BC. Similarly, in T-LGL and CRLM, *miR-181a* seems to directly target *DUSP6* and *SOCS3*. A competitive regulatory loop also exists where *miR-181a* directly inhibits STAT1 and STAT1 binds to the promoter of *miR-181a* preventing its transcription. **(B)** The role of *miR-181a* in the regulation of STING activation and signaling across cancer types. As we report, *miR-181a* regulates STING activation in high-grade serous cancer and in triple-negative breast cancer thus stimulating innate immune responses. Figure generated using BioRender.

8.9. STING

The cGAS-STING (Cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING)) innate immune signaling pathway is a vital host defense network that produces Type-1 IFNs upon recognition of cytosolic DNA. Typically, this pathway is activated by viral DNA; however, high genomic instability and DNA damage in cancers can produce nucleic acids in the cytosol, stimulating this pathway in a tumor suppressive manner [210]. When cytosolic DNA binds to cGAS, it is converted to cGAMP which then binds to and activates STING. STING translocates to from the ER to

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the Golgi where it interacts with TBK1. TBK1 activates STING and the transcription factor interferon regulatory factor 3 (IRF3). Phosphorylated IRF3 promotes transcription of Type-I IFNs and may stimulate nuclear factor-κB (NF-κB) signaling [211].

Our group has recently shown that overexpression of *miR-181a* induces genomic instability, mitotic defects, and nuclear rupture in HGSC by directly inhibiting the tumor suppressor gene *RB1* [98]. This increased *miR-181a* expression transforms fallopian tube secretory cells (FTSEC) and promotes early HGSC development through *RB1* inhibition. Further, our group showed that *STING* targeting by *miR-181a* enabled cancer cells to bypass interferon-mediated cell death [98]. Supporting this work, a recent study found that *miR-181a* is a crucial regulator of the STING pathway in BRCA-mutated triple-negative breast cancer (TNBC) and OC. They observed that *miR-181a* overexpression in TNBC and OC downregulates the STING pathway and promotes resistance to the PARP inhibitor, Olaparib [71]. Altogether, this research suggests a novel connection between *miR-181a* and the STING pathway **(Figure 5B).**

8.9.1. NF-κβ

NF-κβ is a family of inducible transcription factors that regulate immune and inflammatory responses, and cell survival and growth. It comprises five structurally related members: NF-κβ1 (p50), NF-κβ2 (p52), RelA (p65), RelB, and c-Rel, which mediate target gene transcription [212]. NF-κβ signaling can be activated through Toll-like receptors (TLRs), interleukin-1 receptors (IL-1R), tumor necrosis factor receptors (TNFR), growth factors receptors (GFRs), and protein kinase C (PKC). Additionally, there is proposed crosstalk between STING and the NF-κβ signaling network [213]. STING, along with aIκβ kinase epsilon (IKKε), drives NF-κβ signaling by activating the IKK complex, which leads to a pro-inflammatory cytokine response [213].

In CRC, increased *miR-181a* expression is correlated with elevated IL-1β levels through activation of the NF-κβ pathway [214]. In this context, *miR-181a* directly suppresses *PTEN*, resulting in IL-1β induction and enhanced cell viability. These findings established an IL-1β/NF-κβ/*miR-181a*/*PTEN* pathway that may contribute to CRC progression [215]. In addition, another independent study in ABC-like subgroups of DLBCL demonstrated that *miR-181a* suppressed the constitutive activity of NF-κβ signals in vitro and in vivo, thus regulating cellular proliferation and survival [68]. Furthermore, in hypopharyngeal squamous cell carcinoma, a pathway reporter assay revealed that downregulation of *miR-181a* results in decreased NF-κβ levels [216]. Overall, *miR-181a* plays a crucial role in modulating NF-κβ signaling across multiple cancer lineages, influencing inflammation, cell survival, and tumor progression.

10. Immune Microenvironment

The tumor microenvironment (TME) is a complex ecosystem comprised of extracellular matrix components, stroma, and diverse immune cell populations, which regulate cancer progression. Recent research highlights *miR-181a's* involvement in the TME. In liver metastases of CRC, *miR-181a*-rich extracellular vesicles lead to the reprogramming of the TME and the formation of pre-metastatic niches through the IL6/STAT3 signaling pathway [217]. Extracellular vesicles from HNSCC cancer cell lines containing *miR-181a*, among other miRNAs can disrupt dendritic cell function by decreasing cytokine production [218].

miR-181a also interacts with the NK cells, T-cells, and other immune cells within the TME. For instance, Amado et al. showed that increased *miR-181a-5p* in murine CD4+ and CD8+ T-cells leads to decreased IFNγ production [219]. γδ T-cells are a subgroup of T-cells that have IFNγ-associated type 1 inflammatory and cytokine properties and also express a variety of critical immune determinants like NK cell receptor NK2GD. Studies by Gordino et al. demonstrate that *miR-181a* prevents γδ T-cells' differentiation into TNF-α + effectors by regulating MAP3k2 and Notch2 signaling, inhibiting NK2GD functions in both differentiating γδ T-cells and circulating T-cells in PrCa [48]. Similarly, in MM, *miR-181a* overexpression decreases several inflammatory and adhesion factors including, IL-1, TNFα, IFNγ, and Muc-1, ICAM-1, VCAM-1 [206]. These studies revealed a novel role of *miR-181a* in

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regulating NK cell functions, which is particularly relevant as diverse strategies are currently being adopted to manipulate NK2GD to improve next-generation immunotherapies.

10.1. Immune Checkpoints

Immune checkpoints are a grouping of inhibitory pathways responsible for maintaining self-tolerance and modulating the duration and amplitude of immune responses [220]. One such immune checkpoint, programmed cell death (PD-1) and its ligands (PD-L1 and PD-L2), play a crucial role in maintaining peripheral tolerance [221]. These co-inhibitory proteins are expressed on the surface of antigen-stimulated T-cells and, when activated, can inhibit T-cell proliferation, survival, cytokine production, and other effector functions [222]. Cisplatin-induced *miR-181a* expression negatively regulates PD-L1 expression via the c-FOS subunit of the transcription factor AP-1. Since cisplatin induces negative feedback regulation of PD-L1 expression, increased *miR-181a* expression could lead to a downregulation of PD-L1 [223]. Similarly, *miR-181a* regulates PDL-1 expression through the INF-γ pathway [224,225]. The circular RNA *circHMGB2* also promotes immunosuppression and resistance to anti-PD-1 therapy in lung adenocarcinomas and squamous cell carcinomas through the *miR-181a-5p*/CARM1 axis [226].

11. Cell cycle & DNA Repair Regulation

11.1. TP53

The tumor suppressor p53 is activated in response to DNA damage and hypoxia. Upon activation, it induces cell cycle arrest and apoptosis. *TP53* is widely mutated and dysregulated across cancer lineages. The interaction of *TP53* and *miR-181a* has been well summarized by the Baradaran group [154]. More recent studies have shown that *miR-181a* is significantly downregulated in CLL patients with attenuated *TP53* compared to patients with wild-type *TP53* [94] . Upregulation of *miR-181a* has also been linked to p53 through the modulation of its transcriptional targets, Bax and Bcl-2 in CRC [227]. Knarr et al. report that the upregulation of *miR-181a* in HGSC is part of an early mechanism contributing to tumor development. We found that small regions of histologically normal FTSECs carrying a *TP53* mutation also have *miR-181a* upregulation [98]. Additionally, in NSCLC, Rezaei et al. report that the overexpression of *TP53* upregulates *miR-181a*. This correlation suggests that *miR-181a* could be involved in G1 phase cell cycle regulation via the p53 pathway [154].

11.2. G1/S Regulation

Cell cycle control mechanisms and their role in cancer have been well-characterized and exploited in cancer treatments. More recently, *miR-181a* has been connected to several cell cycle regulatory pathways, including the G1/S control. In NSCLC, *miR-181a* is downregulated and plays a tumor-suppressive role by regulating *CDK1* expression and downregulating Cyclin D1 and Cyclin B1 [228]. Additionally, *miR-181a-5p* targets *E2F7* in NSCLC, another important cell cycle modulator of Cyclin B1 and Cyclin D1 [124].

mi-181a has also been linked to the RB1 pathway, which drives the progression from the G1 to the S phase of the cell cycle. In thyroid cancer and HGSC, where *miR-181a* is significantly upregulated, *miR-181a* directly represses *RB1*, promoting unregulated cell cycle progression and preventing apoptosis [97,98]. Further, *miR-181a* targeting of *RB1* in FTSEC promotes transformation and increased genomic instability [98]. In uveal melanoma, *miR-181a* is overexpressed and targets the 3'UTR of *CTDSPL*, inhibiting its translation. This inhibition leads to increased phosphorylation of RB1 and accumulation of the downstream cell cycle effector E2F1, ultimately negatively regulating cell cycle progression [229]. A similar relationship exists in multiple myeloma, where *miR-181a* is upregulated in tumor tissue. When *miR-181a* was silenced in vitro, there was a significant decrease in S-phase cells, a decrease in proliferative and invasive tendencies, and a return of apoptotic function [230]. Collectively these studies indicate *miR-181a*'s oncogenic role in cell cycle regulation, but more research is needed to understand the mechanisms behind this phenotype.

Furthermore, researchers found that in AML where *miR-181a* upregulated, *miR-181a* overexpression drives a decreased ratio of G1-phase cells and an increased ratio of S-phase cells. To

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assess this mechanism, the authors found that *miR-181a* directly targets Ataxia-telangiectasia mutated (*ATM*), which plays an important role in DNA damage control [231]. *miR-181a* also promotes G1/S transition and cell proliferation in clear cell renal cell carcinoma by directly binding and suppressing the antitumor protein *KLF6* [232]. Corroborating this finding, when *miR-181a* was inhibited in ALL cells, a G1/S cell cycle arrest was observed. Together these findings suggest that *miR-181a* is involved in inhibiting the G1/S phase regulation and cell cycle promotion across cancer types.

11.3. G2/M Regulation

In addition to *miR-181a*'s role in G1/S cell cycle regulation, research across several cancer types suggests that *miR-181a* also regulates G2/M cell cycle progression. In non-malignant FTSECs, overexpression of *miR-181a*, showed an increased in the G2/M subpopulation suggesting increased proliferation [98]. In BC and GC, *miR-181a* targeting of *ATM* was shown to regulate DNA repair and cell cycle regulation at the G2M checkpoint [104,233]. In GC, *miR-181a* inhibition of *ATM* led to increased proliferation and inhibited apoptosis [233]. In BC, increased levels of *miR-181a* also decrease ATM and BRAC1 functions in tumors while also blocking G2/M transition [104].

12. Apoptosis

Apoptosis is a naturally occurring process by which a cell experiences programmed death. This can include both extrinsic and intrinsic pathways, both of which are regulated by *miR-181a*.

12.1. BCL-2

BCL-2 (B-cell lymphoma 2) is an outer mitochondrial membrane protein that regulates cell death by blocking apoptosis. BCL-2 can inhibit pro-apoptotic signals and promote chemoresistance when overexpressed in cancer cells. BCL-2 expression is also linked to *miR-181a* activity in several cancers. In CLL patient samples, treatment with a *miR-181a* mimetic reduces the expression of multiple apoptotic genes, including *BCL-2, MCL-2, XIAP,* and *TCL-1* by directly binding to the 3'UTR of *BCL-2*, *MCL-1*, and *XIAP* [94,234]. Treatment with a *miR-181a* mimic in CLL models was found to sensitize cells to fludarabine treatment and induce apoptosis [94]. Furthermore, treatment with a *miR-181a* mimic in daunorubicin (DNR) resistant CLL lines sensitized them to DNR via direct inhibition of *BCL-2* [234]. These findings suggest that *miR-181a* plays an tumor suppressive important role in cancer pathogenesis and sensitivity to apoptosis-inducing agents through its action on BCL-2.

miR-181a has also been linked to chemosensitivity in BC by targeting *BCL-2* [235]. Additional work confirms *miR-181a*'s direct inhibition of *BCL-2* in BC, inducing apoptosis [94]. Majzoub et al. screened the antitumor activity of different thiosemicarbazone derivatives in TNBC. They found that combined Cisplatin + thiosemicarbazone compound 4 treatment increased *miR-181a-5p* expression, resulting in the downregulation of *BCL-2* [236]. Interestingly, treatment with a *miR-181a* mimic in low-invasion BC cells resistant to Adriamycin increased chemosensitivity in a BCL-2-dependent manner, juxtaposing previous studies [235]. Taken together, these studies demonstrate how the efficacy of many BC chemotherapeutic agents relies upon increased *miR-181a* expression to facilitate apoptosis in a BCL-2-dependent manner.

Transient *miR-181a* overexpression in GBM cells downregulates BCL-2 and sensitizes glioma cells to radiation treatment. This may indicate that *miR-181a* could modulate radiosensitivity and be a molecular driver of cellular instability through BCL-2 signaling in GBM models [214]. In melanoma research, *miR-181a* has been linked to *lncRNA-LHFPL3-AS1-long's* regulation of CSC apoptosis. Zhang et al. found that *miR-181a-5p* is sponged by *LHFPL3-AS1-long,* thereby preventing *miR-181a-5p*'s degradation of *BCL-2* and restoring apoptosis [237]. In B-cell lymphoma pathogenesis, Kozoski et al. identified a less direct relationship between *miR-181a* and BCL-2. They previously found that *miR-181a* directly targets *BCL-2*; however, upon completing a rescue experiment in which they transfected a plasmid lacking the 3'UTR of *BCL-2*, *BCL-2* did not alleviate the effect of *miR-181a* on cell proliferation or viability. Thus, further investigation is needed to draw conclusions between *miR-181a* and *BCL-2* in this context[68].

12.2. Other Apoptotic Pathways

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miR-181a is linked to various apoptotic pathways across several cancers. In leukemia research, Zhou et al. investigated the effect of *miR-181a* overexpression on cell apoptosis and DNA damage post-chemotherapy. They observed that *miR-181a* overexpression increased several DNA damage markers, such as caspase-3 and γH2Ax, and enhanced Poly [ADP-ribose] polymerase 1 (PARP1) acetylation by downregulating histone deacetylase *SIRT1*, suggesting a role in repairing DNA damage and PARP1-mediated cell death [238].

In acute promyelocytic leukemia, PML/RARα oncogenic fusion protein upregulates *miR-181a*, increasing colony formation and resistance to apoptosis. Given that *RASSF1A* is downregulated in this cancer, it was hypothesized that *miR-181a* interacts with *RASSF1A*. They show that *miR-181a* binds to the 3'UTR of *RASSF1A*, downregulating its expression and inhibiting cell proliferation and apoptosis [239]. Similarly, in GC, *miR-181a* drives carcinogenesis through direct interaction with *RASSF1A*, promoting cell proliferation and reducing apoptosis [239,240].

Other studies suggest that *miR-181a-5p* promotes apoptosis in BC via Bax and Caspase-9 [241]. In GBM, *miR-181a* increases sub-G1 cell cycle arrest, inducing apoptosis by regulating several genes, including, *caspase-9*, *Bcl-2*, and *SIRT1* [53]. *miR-181a* also directly targets *PDCD4*, a known marker of programmed cell death often lost in BC cases [242,243]. In vitro studies by Gu et al. demonstrate that *miR-181a* suppresses BC cell growth, viability, and has a pro-apoptotic role [241]. Elevated *miR-181a* expression in cryogenically normal acute myeloid leukemia (CA-AML) is also correlated with increased apoptosis, decreased cell viability, delayed leukemogenesis, favorable outcomes, and improved overall survival by directly targeting the 3'UTR of the homeobox gene *PBX3*, a transcription factor that drives poor prognosis in CA-AML [244]. A similar relationship is seen in cervical cancer, where *miR-181a-5p* overexpression inhibits apoptosis by targeting inositol polyphosphate-5-phosphatase A (*INPP5A*) [245]. Alternatively, in lung adenocarcinoma, Wang et al. found that *miR-181a* in exosomes derived from M1 macrophages promotes apoptosis via the *miR-181a-5p*/ETS1/STK16 axis [246].

13. Angiogenesis

Angiogenesis is a tightly regulated process by which new blood vessels are formed from pre-existing microvasculature. Pro-angiogenic factors like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) (along with their receptors VEGFR, FGFR, and EGFR) modulate hypoxia and normoxia in tumors [247]. The enzyme Src, linked to VEGF-induced angiogenesis with malignant activation, is present across many cancers, including BC, CRC, PaCa, OC, HNSCC, lung, bladder, neuronal tumors, chronic myeloid leukemia (CML), and MM. *miR-181a* regulates VEGF-induced angiogenesis via Src activation [248]. Studies have shown that *miR-181a* directly targets the 3'UTR of Src-related tumor suppressor factor SRC kinase signaling inhibitor 1 (*SRCIN1*) in CRC. Inhibition of *SRCIN1* by *miR-181a* activates Src, triggering VEGF secretion and leading to angiogenesis [222]. Furthermore, in BC, Taylor et al. found that increased *miR-181a* expression enhances Src phosphorylation and activation [136]. *miR-181a* is also involved in angiogenesis in chondrosarcoma and clear cell renal cell carcinoma. When *miR-181a* expression is increased, mRNA and protein expression levels of VEGF are upregulated, contributing to increased tumor microvasculature [249].

Transitioning to matrix metalloproteinases (MMPs), *miR-181a* MMPs, which mediate angiogenesis through modulation of anti- and pro-angiogenetic factors. In BC, *miR-181a* directly targets *MMP-14*, decreasing cell migration, invasion, and angiogenesis [161]. *miR-181a* was also found to target *MMP-14* in supportive study in human fibrosarcoma and BC models [162]. In CRC, *miR-181a* directly binds to reversion-inducing cysteine-rich protein with Kazal motifs (*RECK*), driving MMP-9 upregulation and phosphorylation of SMAD2/3 [163]. In summary, *miR-181a* plays a critical role in angiogenesis across various cancers by modulating VEGF signaling, Src activation, and the expression of MMPs, thereby influencing tumor growth and progression.

14. Hormonal Signaling

In BC, *miR-181a-5p* plays a role in both ERα and ERβ downregulation [69,70]. This is unsurprising given that the 3'UTR of estrogen receptor 1 (*ESR1*) contains a binding site for the *miR-181a* seed sequence [178]. *miR-181a* also directly targets the 3'UTR of progesterone receptor (*PGR*)

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[250]. Gilam et al. validate this finding and show that *miR-181a* negatively regulates PGR in BC [251]. Further, Maillot et al. suggest that *pri-miR-181a/b* are primary targets of ER-mediated transcriptional activity. Consequently, estradiol reduces *miR-181a/b* expression, while anti-estrogen treatment increases *miR-181a* expression. Although *miR-181a*'s effects on estradiol and BC progression are not fully explored, it is known that *miR-181a* can abrogate the estradiol-dependent increase in cell proliferation via downregulation of *PR* [250]. *miR-181a* also reduces *PGRMC1* expression, thereby attenuating progestin-induced cell growth in BC [241].

15. *miR-181a* as a Non-Toxic Targetable oncomiR

As emerging studies highlight *miR-181a's* role as an oncogenic driver in particular contexts and explore ways to inhibit its activity, it is important to note that the knockout of this gene is not lethal or toxic to the host, making it a promising therapeutic target. For example, in an HCC model, knockout of *miR-181a* did not change the number of hematopoietic or endothelial cells, suggesting a non-toxic effect on rapidly dividing cells, despite *miR-181a's* role in development [252]. These findings suggest a favorable safety profile for *miR-181a* targeting. In another study, Paterson et al. developed a *miR-181a* knockout C57BL/6J mouse model and found minor phenotypic effects, such as elevated blood pressure, increased mean arteriole blood pressure, and increased salt sensitivity. However, there was no difference in food or water intake, mouse weight, and only slightly larger spleens in the *miR-181a* knockout group compared to the control [253]. Another group showed that germline *miR-181a1/b1* or *miR-181a2*/b2 knockout did not cause gross phenotypic abnormalities in growth, development, or survival [254]. These studies establish proof of concept, suggesting that *miR-181a* inhibitory treatments could be safe and potentially have no significant toxicities.

Given *miR-181a's* abundance and role in T-cell development activation, and function, it was imperative to investigate the effects on T-cells following *miR-181a* deletion [255]. Kim et al. reviewed *miR-181a* regulatory pathways in T-cell differentiation and aging, noting that *miR-181a/b* modulates T-cell tolerance by controlling T-cell selection and peripheral T-cell function, indicating a correlation between *miR-181a/b* and the active immune system [46]. They showed that *miR-181a-1/b-1* murine germline deletion increases in the intrinsic reactivity of naïve T-cells to self-antigens without causing spontaneous autoimmunity [256]. The Krueger lab found that knockout of *miR-181a* is not detrimental to T-cell function [257]. Although whole-body murine knockout of *miR-181a* results in impaired T-regulatory cell development, total frequency, and diversity in T-regulatory cells in the periphery are not changed due to homeostatic expansion [257]. These findings were corroborated by Fragoso et al., who showed that germline knockout of *miR-181a1/b1* did not change the cellularity of hematopoietic organs, including bone marrow, spleen, thymus, and peripheral blood [174]. Additionally, the Krueger lab showed show that whole body *miR-181a/b* knockout does not result in a substantial compensatory takeover by other miRNAs in the thymus, suggesting that cellular functions remain stable in the absence of *miR-181a* and further supporting the positive developing safety profile of *miR-181a* targeting [16]. In work on allogeneic hematopoietic stem cell transplantation therapy, Sang et al. found that *miR-181a* affects the differentiation of naive CD41+ T-cells by targeting IFN-γ production [224]. Further research showed that *miR-181a/b-1* deficient mice display aberrant double positive thymocyte development and a complete block in early invariant natural killer T-cell development, resulting in impaired T-cell receptor signaling [254,258]. This study suggests that loss of *miR-181a* could compromise TCR signaling and the immune system; however, as stated earlier, an abundance of studies suggest that T-cell function is not harmed despite impaired development. Future studies clarifying how loss of *miR-181a* affects immune function in the context of cancer will be critical to understanding the regulatory role of this miRNA. Collectively, these studies establish *miR-181a* as a promising therapeutic target with a potentially favorable safety profile. The non-toxic effects observed across various knockout models and organs, combined with *miR-181a's* crucial role in lymphocyte regulation, underscore the potential for developing *miR-181a*-targeted therapies.

16. Genetically Engineered *miR-181a* Murine Models

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In more recent studies, genetically engineered mouse models (GEMMs) have been created to highlight the role of *miR-181a* across cancers. Valencia et al. created GEMMs of KRAS-driven lung and pancreatic cancers. After identifying *miR-181a/b* as a miRNA cluster upregulated by oncogenic KRAS, they used several *miR-181a-KRAS* GEMMs to evaluate the loss of function of *miR-181a/b*. They demonstrated the key role of *miR-181a/b* in tumorigenesis, and further defined the need for *miR-181a* targeted therapies in KRAS-mutant cancers [259]. Similarly, in HCC, a *miR-181a*-altered GEMM was created to elucidate *miR-181a* expression in combination with TGF-β treatment to combat hepatocyte EMT. Here, *miR-181a* was significantly upregulated in response to TGF-β treatment, and aberrantly expressed in HCC and chronic liver disease [134]. These studies underscore the importance of *miR-181a* in cancer development. Continued development of GEMMs is crucial for further contextually characterization *miR-181a* as an oncomiR in pan-cancer models.

17. Nanoparticles as a Delivery Platform for *miR-181a*

Despite increasing knowledge of *miR-181a,* and the use of complementary oligos, sponges, and mimics to ascertain the relevance of its genetic manipulation in disease-based contexts, hurdles pertaining to the delivery of antagomiRs and mimics still exist and limit the clinical application of microRNA-based therapeutics. AntagomiRs are synthetic oligos that complementarily bind and sequester miRNAs, thereby preventing them from interacting with MREs and target mRNA networks. Nanoparticles (NP) have been employed for several decades to deliver miRNAs and antagomiRs across various cancer types, providing an efficient and safe mechanism for miRNA-driven diseases.

When *miR-181a* functions as a tumor suppressor, lower levels of *miR-181a* are associated with poorer outcomes in cancer. Treatment with *miR-181a* mimetics can counteract these cancer-driving phenotypes in vitro but not in vivo. In AML, Huang et al. successfully delivered *miR-181a* mimics to AML blasts via transferrin-targeted anionic lipid-based lipopolyplex nanoparticles [260]. This increased *miR-181a* expression, downregulated known targets *KRAS*, *NRAS*, and *MAPK*, reduced proliferation, impaired colony formation, and increased sensitivity to cytarabine treatment [187]. This *miR-181a* NP delivery model in murine AML represents a clinically relevant miRNA delivery platform resulting in improved survival outcomes. In RB research, *miR-181a* NP delivery has also shown success in rat xenograft models. Co-delivery of a *miR-181a* mimic along with the chemotherapeutic agent melphalan via lipid NP significantly decreased immunogenicity, and improved chemosensitivity and cytotoxicity [261]. *miR-181a* is also downregulated in GBM clinical samples. Using a hyaluronan-decorated lipid nanoparticle (HA-LNP) to deliver *miR-181a* to GBM cells in vivo and in vitro, they found that this treatment platform induced cell death and delayed tumor growth [262]. *miR-181a* has also been used as a radiosensitizer by inducing DNA damage post-radiotherapy for rectal cancer. Successful loading of *miR-181a* into a MnO_2 @ZIF-8-polyethylene glycol nanocomplex prevented miRNA degradation, reduced tumor proliferation, enhanced radiosensitivity, and improved local TME hypoxia [263]. These studies illustrate the promise of *miR-181a* mimetic treatment as a novel therapeutic approach for cancers where *miR-181a* acts as a tumor suppressor.

Other highly aggressive cancers are driven by *miR-181a* overexpression and lack effective systemic treatment options for reducing *miR-181a* expression. Recently, *anti-miR-181a* oligos loaded into Janus-based nanotubes and delivered into chondrosarcoma xenograft models, reduced *miR-181a* expression, restored *RGS16*, a known target of *miR-181a,* and improved survival outcomes [264]. Future studies confirming the use of *anti-miR-181a* oligos to systemically or organ specifically knockdown *miR-181a* in other cancer models would point to an easy and safe therapeutic delivery platform to be exploited by clinics.

18. Pharmacological Inhibition of *miR-181a*

18.1. Epigenetic Drugs Controlling miR-181a Activity

In our previous work, we developed a functional endogenous *miR-181a* biosensor for high-throughput therapeutic screens. Using this sensor, we sorted for OVCAR3 cells highly expressing

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miR-181a and screened >3,000 bioactive compounds. Notably, 50% of the hit compounds that reduced *miR-181a* activity were bromodomain and extra-terminal motif (BET) inhibitors, indicating epigenetic regulation of *miR-181a* by the BET protein family [100]. Another study demonstrated that MC3324, a hybrid lysine-specific histone demethylase inhibitor, reduced *miR-181a-5p* expression and estrogen receptor alpha (ERα) protein levels in BC [70]. The authors observed an inverse correlation between *miR-181a* and ERα, with MC3324 treatment reversing this correlation, inducing *miR-181a-5p,* and reducing ERα expression [70]. Together, these findings suggest that epigenetic modulation could be a therapeutic strategy for controlling *miR-181a* expression and oncomiR function. Further research on epigenetic regulators and their correlation with *miR-181a* expression in tissue specific contexts is needed to determine if these mechanisms could enhance treatment sensitivity.

18.2. Natural Products

In addition to synthetically derived *miR-181a* therapeutic options, several natural products have been shown to regulate the *miR-181a* family. For example, the fingerroot compound pinostrobin reduces *miR-181*b-*5p* expression and induces ATM-mediated apoptosis in acute leukemia [265]. Previous studies have implicated pinostrobin's anticancer effects in BC, GBM, OC, and leukemias. Future research might reveal that pinostrobin treatment has similar beneficial effects across *miR-181a*-driven cancer types. Similarly, all-trans-retinoic acid (ATRA), a naturally occurring derivative of vitamin A (retinal), downregulates *miR-181a*/b in acute promyelocytic leukemia. Mechanistically, *miR-181a*/b targets the 3' UTR of the ATRA-regulated tumor suppressive gene *RASSF1A*, which attenuates ATRA-induced granulocytotic differentiation through Cyclin D1 regulation [239]. New research points to an interaction between *miR-181a* and the Chinese medicinal herb astragaloside IV (AS-IV). AS-IV has been studied in NSCLC anlotinib resistance. AS-IV treatment decreases *miR-181a-3p* expression and activates endoplasmic reticulum-associated degradation (ERAD) and unfolded protein response (UPR) pathways, thereby attenuating anlotinib resistance. Thus, these authors suggest a promising new NSCLC therapy combining AS-IV treatment with inhibition of the *miR-181a-3p*/UPR-ERAD axis [266]. Curcumin, a common anti-inflammatory additive in skin care products and compound found in turmeric. Interestingly, curcumin has been shown to induce cisplatin sensitivity by increasing *ATG5* expression, a known target of *miR-181a-3p,* in OC [267,268].

Resveratrol (RV) is an active polyphenol substance found in knotweed and grapes. It is a phytoestrogen known to bind to ERs and is known for its immunoregulatory and antibacterial effects, as well as the alleviation of menopausal symptoms. In cancer, RV has known antitumor properties, including induction of apoptosis in BC and inhibition of migration and invasion in PaCa [269,270]. Although this mechanism has not been explored extensively in cancer, one study in myocardial fibrosis showed that RV treatment reduces *miR-181a* expression and inhibits TGF-β-mediated hyperproliferation [271]. Given *miR-181a's* well characterized role in TGF-β-mediated tumor suppression and oncogenic progression, further studies examining if RV treatment could reduce *miR-181a* driven invasion and metastasis would offer an innovative and accessible treatment option for *miR-181a* driven cancers. In contrast, another study showed that RV treatment increased *miR-181a* expression, thereby repressing the TRAF6/TAK1 inflammatory signaling pathway in osteoclasts [272]. Both of these studies illustrate the potential of RV as an indirect targeting agent for *miR-181a*; however, further characterization of its mechanism is crucial to determining its use and efficacy.

18.3. Drug Repurposing

Given the difficulty of developing targeted small moelcules, particularly for miRNAs, some studies have found success in repurposing drugs for targeting *miR-181a.* Perftoran, a perfluorodecalin emulsion, initially developed as an oxygen-carrying plasma additive for acute blood-loss anemia, has recently been investigated for its effect on several oncomiRs, including *miR-181a* [273]. One study analyzed its impact on the photosensitizing agent indocyanine green (ICG) used in photodynamic therapy (PDT) for lung cancer. They found that Perftoran treatment suppresses *miR-181a* and inhibits ICG/PDT-hypoxia [274]. Another study found that vincristine and prednisone treatment in naïve, but not recurrent, leukemia patients induced pro-apoptotic gene expression and suppressed exosomal

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miR-181a expression [92]. These findings highlight the potential of repurposing existing drugs to efficiently target *miR-181a* driven cancers and offer a promising avenue of developing new therapeutic strategies in miRNA-driven diseases.

19. *miR-181a* Governs Cancer Therapeutic Efficacy

19.1. miR-181a Modulates Radiosensitivity

Radiotherapy is a component of many cancers' standard of care; however, like any treatment, its efficacy is limited by radio-resistance. Dysregulation of essential genes modulating pro-apoptotic signaling pathways has been postulated to drive this therapeutic resistance, but identification of these genetic drivers still remains a challenge. In GBM models, *miR-181a* was found to be reduced by >2.6 fold upon irradiation. Upon *miR-181a* overexpression, GBM cells were sensitized to radiation therapy [214]. Similarly, in cervical cancer models, *miR-181a* was found to be upregulated in radio-resistant samples compared to radio-sensitive samples [275]. Mechanistically, the authors' postulate that *miR-181a's* direct inhibition of *PRKCD's*, a pro-apoptotic kinase, inhibits irradiation-induced apoptosis and decreases G2/M block promoting insensitivity to radiation. *miR-181a's* inhibition of *PRKCD* was further validated by Su et al. [52]. Combined, these studies suggest that *miR-181a* may be a good biomarker for identifying sensitivity to radiotherapy.

19.2. miR-181a Contributes to Chemotherapy Resistance

The interaction of *miR-181a-5p* with platinum-based therapeutics and other chemotherapeutic agents has been well characterized. *miR-181a* expression has been correlated to increased chemotherapy resistance across several cancer types. Specifically, in leukemia research, Li et al. found that *miR-181a* could play a role in developing resistance to the chemotherapeutic agent DNR. Treatment with a *miR-181a* mimic and DNR significantly reduced cell survival and increased BCL-2 expression, suggesting that *miR-181a* could sensitize leukemia cells in a BCL-2-dependent manner [234]. Similarly, in chemoresistant cervical squamous cell carcinoma, *miR-181a* enhances chemoresistance in vitro and in vivo by directly targeting *PRKCD*, an apoptotic target gene of *miR-181a* [276]. *miR-181a* expression levels are also elevated across several other cisplatin-treated models, including NSCLC, T-cell leukemia/lymphoma, and OC [76,182,223]. In contrast, in melanomas, increased *miR-181a* expression has been linked to reduced signaling down the chemoresistant pathway TFAM, leading to a restoration of chemosensitivity [58] . These findings highlight the complex role of *miR-181a* in modulating chemotherapy resistance and sensitivity.

20. Concluding Remarks

Mounting evidence has demonstrated *miR-181a's* critical role in cancer progression. Aberrant expression of *miR-181a* can cause dysregulation across numerous signaling pathways, enabling cancer cells to acquire the functional capabilities and hallmarks of cancer needed for tumorigenesis **(Figure 6)** [10]. Mechanistically, *miR-181a* targets multiple mRNA transcripts that are included in all the hallmarks of cancer [10]. Further, it is regulated by numerous ncRNAs, transcription factors, and post-transcriptional modulators that drive these cancer-relevant pathways. In some cases, the restoration of *miR-181a* expression can reinstate tumor suppressive phenotypes; however, in many cancer lineages, *miR-181a* overexpression can drive oncogenesis and chemotherapy resistance. Overall, these studies clearly define *miR-181a* as a promising, predictive biomarker with relevant clinical applications. Although therapeutic approaches to targeting miRNAs in general, and *miR-181a* specifically, remain in the early stages, current studies show promising options for targeting *miR-181a* using nanoparticles, repurposed drugs, and natural products. Given *miR-181a*'s promising safety profile, further development of *miR-181a* targeted therapies and small molecules could significantly impact oncology treatment options and increase sensitivity to current chemotherapies.

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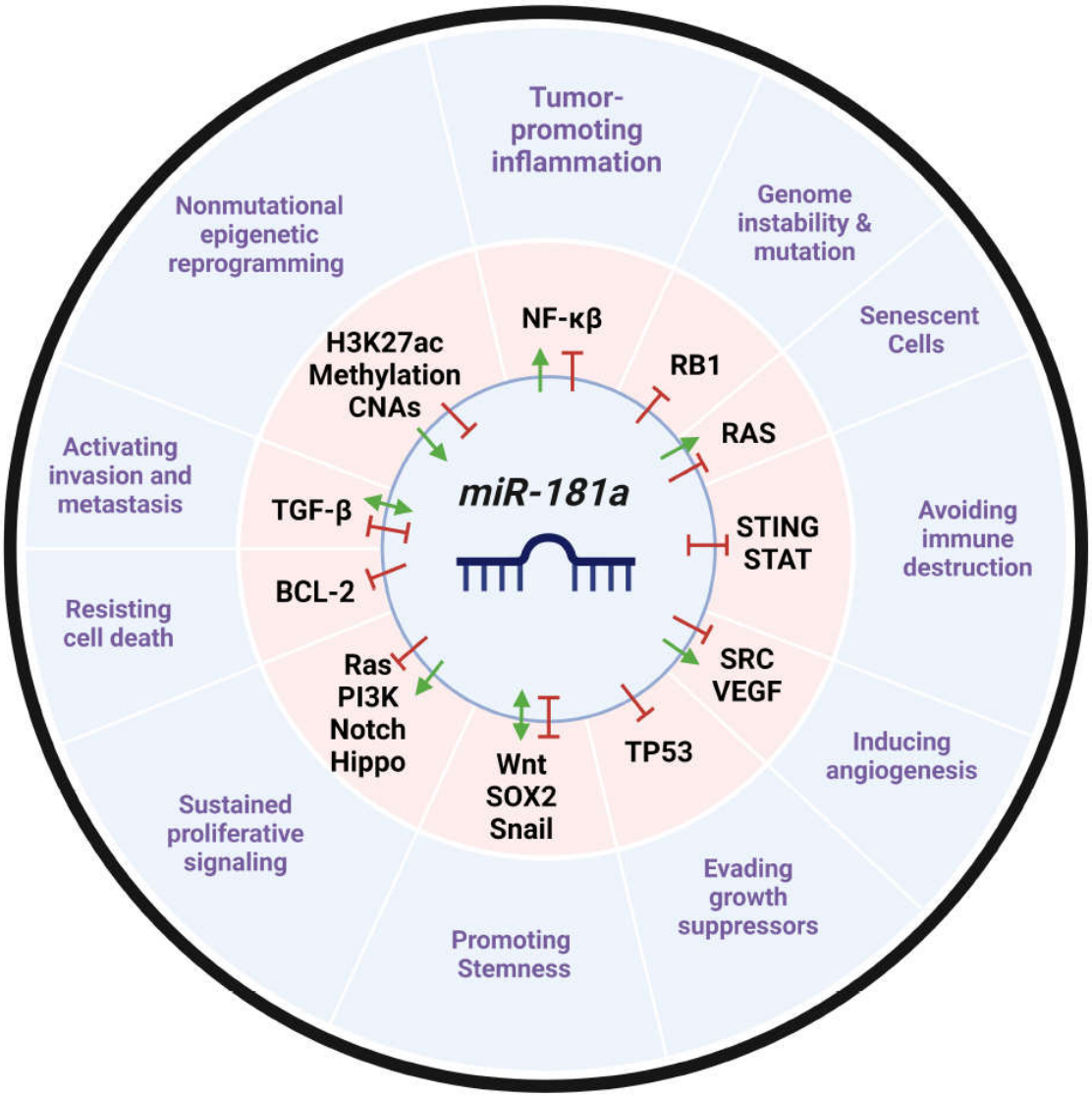

Figure 6. Schematic depicting *miR-181a's* oncogenic and tumor suppressive function across numerous signaling pathways in cancer. *miR-181a* dysregulation can enable cancer cells to acquire hallmark capabilities required by this neoplastic disease [10]. *miR-181a* can modulate the function of several key molecular pathways, including RB1, Ras, BCL-2, and SRC/VEGF, leading to genomic instability, senescent cells, apoptotic dysfunction, angiogenesis, and an evasion of growth suppressors. *miR-181a* dysregulation can also lead to the overactivation of other pathways and proteins such as the activation of Wnt, SOX2, TGF-β, and Ras/PI3K/Notch across several cancer types. This overactivation can promote stemness, activate invasion and metastasis, and increase proliferation capabilities, respectively. *miR-181a* dysfunction also impacts cancer epigenetics, causing nonmutational epigenetic reprogramming though the overactivation of H3K27ac methylation CNAs. Additionally, *miR-181a* overexpression can impact immune regulation through the inhibition of NF-κβ, STAT, and STING signaling. Figure generated using BioRender.

21. Expert Opinion

miR-181a is a novel and exciting therapeutic target that can be applied to various cancer types. It's ability to act on multiple cancer driving pathways including cell growth, developmental, angiogenic, apoptotic, and immune stimulatory pathways emphasize its multi-faceted regulatory control mechanisms. Slight variation in *miR-181a's* expression levels can significantly alter its wide breadth of regulatory reach on numerous target mRNA networks and disrupt the balance required for cancer cell maintenance and homeostasis. This altered expression profile is easily measurable and characterized in many cancers underscoring the diagnostic potential of *miR-181a* as a biomarker and

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its targetable potential. Given that most precision cancer treatments focus only on a single pathway, there is a demonstrated clinical need to develop polyvalent therapeutic approaches that can act on many molecular targets. Tailoring treatments to be directed towards *miR-181a* would offer a unique and central therapeutic approach that can achieve these goals while inhibiting tumor growth, metastasis, recurrence, and stimulating the immune system.

Indeed, the development of RNA therapeutic approaches is exploding and has the potential to revolutionize precision medicine. Since the first Phase I antagomir clinical trial in 2010 (ClinicalTrials.gov Identifier: NCT02031133), more than 22 Phase I, II, and III miRNA based clinical trials have been conducted and we expect to see *miR-181a* therapeutic approaches joining the list in the next 10 years [277]. However, the development of these miRNA-based treatments is not without its barriers. The implementation of *miR-181a* therapy in cancer requires the development of the appropriate therapeutics' platform. Such systems including nanoparticles, natural products, and repurposed drugs have been preclinically explored but not thoroughly evaluated for their clinical potential despite mounting evidence suggesting *miR-181a* is an ideal molecular target with a promising safety profile and the power to reduce tumor formation in vivo. Future studies should home in on developing robust and clinically relevant *miR-181a* therapeutic platforms.

One of the key advantages of *miR-181a* as a genetic vulnerability in cancer is that its aberrant expression has been widely characterized in normal and tumor patient samples. Furthermore, accumulating evidence validating these expression characteristics in serum samples collected from patients demonstrates the diagnostic relevance and the clinical accessibility of using *miR-181a* as a patient biomarker. Diagnostically, measuring *miR-181a* levels prior to treatment could allow clinicians to infer how patients will respond to treatment and what their prognosis will be. Future pre-clinical studies measuring *miR-181a* serum samples in normal, neo-adjuvant, adjuvant, and recurrent patients can enable clinicians to further stratify patients and their ability to respond to chemotherapeutics and future *miR-181a* targeted therapies.

Developing a *miR-181a* inhibitory therapeutic involves determining the appropriate target. Instead of targeting the *pri-* or *pre-miR-181a* structure, some studies have tried to target its biogenesis mediators and stabilizers. Investigators have identified that *miR-181a* expression can be modulated through epigenetic drugs; however, as with many therapies in this class, off-target effects including altered expression of non-target genes and lack of precision can lead to complications and a lack of predictability. Furthermore, very few studies have examined the RNA-binding proteins that directly interact with *miR-181a* to either stabilize it or facilitate its synthesis, processing, and degradation. Future biochemical studies are crucial to clarify the protein modulators of *miR-181a* and therefore identify alternative therapeutics angles to modulating *miR-181a* expression and activity.

MiR-181a's function in cancer cells is crucial to their survival. However, mechanistically, cancer cells require a team of supporting cells in their microenvironment to propagate and metastasize. Given *miR-181a's* crucial role in lymphocyte and myeloid cell development, it is conceivable that *miR-181a* reprogramming of the immune system in the tumor microenvironment is occurring in a pro-tumorigenic fashion. Future studies characterizing *miR-181a's* immune-modulatory role as an oncogene in these stromal cells will be crucial in determining *miR-181a's* mechanism in cancer progression and further stratifying patients and their predicted therapeutic responses. Additionally, this information can inform future *miR-181a* therapeutic development, administration, and dosing when designing these therapeutic agents.

Overall, the challenges of *miR-181a* future investigations are two-fold: 1) further characterization of *miR-181a's* mechanism of action and interacting partners and 2) targeted *miR-181a* therapeutic development. More than 1,400 papers have been published discussing *miR-181a* since 2008 and we expect to see these avenues more thoroughly explored in the next 5 years with strong pre-clinical therapies rising to clinical investigation.

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Abbreviations

Abbreviation	Definition
AFP	Alpha fetoprotein
Ago	Argonaut
Akt	Protein kinase B
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AS-IV	astragaloside IV
ATM	Ataxia telangiectasia mutated protein kinase
ATRA	all-trans-retinoic acid
BC	Breast cancer
BCL-2	B-cell leukemia/lymphoma 2 protein
BET	bromodomain and extra-terminal motif
CA-AML	Cryogenically normal acute myeloid leukemia
CDX2	Caudal-type homeodomain transcription factor
ceRNAs	Competing endogenous RNAs
cGAS	Cyclic GMP AMP Synthase
cirRNAs	Circular RNAs
CLL	Chronic lymphocytic leukemia
CML	Chronic myelogenous leukemia
COAD	Colon adenocarcinoma
COFs	Cemento-ossifying fibromas
CRC	Colorectal cancer
CSC	Cancer stem cell
DACT2	Disheveled binding antagonist of beta-catenin 2

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DLBCL	Diffuse Large B-Cell Leukemia
DNMTi	DNA methyltransferase inhibitor
EGF	Epidermal growth factor
Egr1	Early growth response factor 1
EMT	Epithelial to mesenchymal transition
EOlC	Epithelial ovarian cancer
EpCAM	epithelial cellular adhesion molecule
EPDR1	Ependymin Related 1 proteinn
ER	Estrogen receptor
ERAD	endoplasmic reticulum-associated degradation
ERK2	ERK mRNA
ESR1	Estrogen receptor 1
FGF	Fibroblast growth factor
FTSEC	Fallopian tube secretory cells
GAS7	Growth arrest specific-7
GC	Gastric cancer
GEMM	Genetically engineered mouse model
HCC	Hepatocellular carcinoma
HDACi	Histone deacetylase inhibitor
HGSC	High-grade serous cancer
IFN	Interferon
IGF2BP1	Insulin Like Growth Factor2 mRNA Binding Protein 1
IKKε	IKK-inducible kinase
IM	imatinib mesylate
INPP5A	Inositol Polyphosphate-5-Phosphatase A
IRF3	Interferon regulatory factor 3
JAK	Janus kinase
kb	kilobases
lncRNAs	Long non-coding RNAs
LUSC	Lung squamous cell carcinoma

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MET	Mesenchymal to epithelial transition
MHC	Major histone compatibility complex
MIIP	Migration and invasion inhibitory protein
miR-181	microRNA 181
miRNA	microRNA
MKP-5	MAPK phosphatase 5
MMP	Matrix metallopronteinase
MRE	microRNA recognition element
mRNA	Messenger RNA
NB	Neuroblastoma
NDRG2	N-myc downstream regulated gene 2
NF-κβ	Nuclear factor kappa B
NK	Natural Killer Cells
NLK	Nemo-like kinase
NP	Nanoparticle
NSCLC	Non-small cell lung cancer
nt	Nucleotides
PaCa	Pancreatic cancer
PAM	PI3K-Akt-mTOR signaling pathway
PARP1	Poly[ADP-ribose] polymerase 1
PD-L1	Programmed cell death ligand 1
PGR	Progesterone Receptor
PI3K	phosphoinositide-3-kinase
PN	Pinostrobin
PolII	RNA polymerase II
PrCa	Prostate caner
pri-miRNA	Primary microRNA
PTC	Papillary thyroid cancer
RBP	RNA binding proteins
RISC	RNA-induced silencing complex
RTK	Receptor tyrosine kinase

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RV	Resveratrol
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variation
SRCIN1	Src kinase signaling inhibitor 1
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon gene
SYNCRIP	Synaptotagmin-binding Cytoplasmic RNA-Interacting Protein
T-ALL	T-cell acute lymphoblastic leukemia
TAZ	Transcriptional coactivator with PDZ-binding motif
TCGA	The Cancer Genome Atlas
TCR	T-cell receptor
TFs	Transcription factors
TGF-β	Canonical transforming growth factor-β
TKI	Tyrosine kinase inhibitor
T-LGL	T-cell large granular lymphocyte
TMBIM6	Transmembrane Bax Inhibitor Motif-containing 6
TME	Tumor microenvironment
TNBC	Triple negative breast cancer
T-PLL	T-cell prolymphocytic leukemia
TRIM5	Tripartite Motif Containing 5
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor
WIF1	Wnt inhibitory factor 1
XIST	X-inactivated specific transcript
YAP	Yes-associated protein

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