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REVIEW

Dual Regulation and Clinical Application of miR-34 in Virus-Related Tumors Through Anti-Viral Immunity and Tumor Suppression

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Abstract: miR-34, as an important class of microRNA, plays a dual regulatory role in host antiviral immunity and tumor suppression. Its unique mechanism targeting both viruses and tumors demonstrates significant potential for synergistic therapeutic applications. During viral infection, miR-34 enhances host immune responses by regulating interferon signaling pathways to target IRF3 phosphorylation and NF-κB activation, which leads to the viral replication suppression. In tumor prevention and treatment, miR-34 acts as a downstream effector of the p53 signaling pathway, inducing cell cycle arrest and apoptosis by inhibiting Cyclin D1 and promoting Bax expression, exhibiting the tumor-suppressive roles. Additionally, miR-34 plays a key role in the interactions between viruses and hosts, as well as tumors and the microenvironment, by balancing the expression of inflammatory factors (eg, IL-6, TNF-α). Although miR-34 has shown significant potential in preclinical studies, its clinical application still faces challenges such as low drug delivery efficiency, off-target effects, and safety concerns. Notably, miR-34 mimics have demonstrated potential in tumor trials to restore tumor suppressor functions, offering the promising and novel strategies for combined anti-viral and anti-tumor therapies. In the future, through multi-omics integration, the development of novel nano-delivery systems, and multicenter clinical trials, miR-34 is expected to become a crucial target for viral prevention and precision tumor therapy.

Keywords: MiR-34, viral infection, tumor suppression, immune regulation, precision therapy

Introduction

Research Topic and Purpose Statement

MicroRNAs (miRNAs), as a class of small non-coding RNA molecules, play a crucial role in suppressing target mRNAs' translation or promoting their degradation through binding to the 3' untranslated region (UTR), leading to regulate various biological processes including cell growth, differentiation, apoptosis and immune responses.[1] The miR-34 family is an important member of miRNAs and plays a key role in antiviral immunity and tumor suppression.[1] The family influences the defense mechanisms against viral infections and the occurrence and development of tumors by regulating key signaling pathways in host cells, such as the p53 signaling pathway and cell cycle regulation.[2,3] For example, during viral infection, miR-34 may suppress viral replication by targeting host factors that viruses depend on, thereby reducing the viral proliferation rate.[4] In tumors, miR-34 may exert its tumor-suppressive effects by inducing apoptosis and inhibiting the proliferation and metastasis of tumor cells.[3,5]

However, the function of miR-34 is not always singular. In certain cases, miR-34 may also promote viral immune evasion or play a dual role in the transformation of chronic inflammation into tumors.[6,7] For instance, scientists

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discovered that miR-34a can directly target multiple critical host factors throughout the viral life cycle, effectively inhibiting viral replication. This dual regulatory function positions miR-34a as a bridge connecting tumor biology with virology research, holding particular significance in understanding mechanisms and developing therapeutic strategies for virus-associated cancers such as HCV-related liver cancer.[8] Therefore, miR-34 has a "dual role" in viral infections and tumor management, and understanding these different mechanisms of action is crucial for developing miR-34-based therapeutic strategies. Given the complex regulatory role of miR-34 in various diseases, in-depth research into its mechanisms of action will facilitate its development as a potential therapeutic target.[1,9]

Viruses can evade immune surveillance by altering the host miRNA expression profile.[6,10] After viral infection, the miRNA expression patterns of host cells undergo significant changes. Certain viruses can interfere with the host immune response by upregulating or downregulating specific miRNAs, including miR-34, thereby promoting their own replication and spread.[6,11] MiR-34, as a downstream target of p53, plays an important role in the p53 signaling pathway.[3] p53 is a tumor suppressor protein that regulates processes such as the cell cycle, apoptosis, and DNA damage repair. The p53 signaling pathway can directly regulate the transcription of miR-34, thereby affecting the physiological state of cells.[3] During viral infection, changes in miR-34 expression and its impact on the p53 signaling pathway may significantly influence viral fate and host immune responses. For example, certain DNA viruses may promote their own proliferation by inhibiting the p53-miR-34 axis, leading to persistent and aggravated infections.[6,7]

Therefore, restoring the function of miR-34 may become a potential antiviral therapeutic strategy. By regulating the expression of miR-34, it can enhance the host's immune defense capabilities, inhibit viral replication, and ultimately clear viral infections.[1,5] Further research into the mechanisms of miR-34 in viral infection and immune response will contribute to the development of more effective antiviral treatments.

Research Significance and Background Introduction

Dysregulation of miRNA expression is closely associated with various human diseases, particularly infectious diseases and cancers.[12–14] In infectious diseases, miRNAs can regulate the host immune response and influence viral replication and transmission; in cancers, miRNAs can act as tumor suppressors or oncogenes, modulating tumor cell proliferation, metastasis, and drug resistance.[5–7] Therefore, in-depth research into the mechanisms of miRNAs in disease development is crucial for developing novel diagnostic and therapeutic approaches.

The miR-34 family plays a crucial role in maintaining cellular homeostasis.[3,15] Members of this family influence cell proliferation and apoptosis by targeting and regulating genes related to the cell cycle and apoptosis, such as Cyclin D1 and Bcl-2, to prevent excessive cell proliferation and eliminate abnormal cells.[3]

In immune responses, miR-34 is involved in regulating the interferon signaling pathway, NF-κB activation, and the expression of other antiviral-related factors.[1,4] It can enhance interferon production and signaling, improving cellular antiviral capacity; MiR-34 also modulates the extent of inflammatory responses by regulating the NF-κB signaling pathway, preventing excessive inflammatory damage.[1,5,16] It balances the relationship between pro-inflammatory factors (such as IL-6, TNF-α) and anti-inflammatory factors, thereby maintaining inflammatory homeostasis. Dysregulation of miR-34 led to uncontrolled inflammatory responses, subsequently triggering various diseases, including chronic inflammation, autoimmune disorders, and cancer.[5,6,16]

During the transformation from chronic inflammation to tumors, miR-34 may exert dual effects.[7,17] On one hand, miR-34 can inhibit tumor cell proliferation and metastasis, acting as a tumor suppressor; on the other hand, it also promote inflammatory responses, creating a favorable environment for tumorigenesis and progression.[5,7] Therefore, in-depth research into the mechanisms of miR-34 in disease pathogenesis will contribute to the development of more precise therapeutic strategies.

Clinical Examples and an Overview of Preliminary Research Findings

Clinical cases have demonstrated the application value of miR-34 in viral infections and tumor therapy. For example, during influenza virus infection, the expression of miR-34 undergoes significant changes, thereby affecting the host's immune response.[11,18] Studies have found that influenza virus infection can lead to downregulation of miR-34, thereby suppressing the host's antiviral immune response and promoting viral replication and spread.[11,18,19] During human

cytomegalovirus (HCMV) infection, the expression of miR-34 is also regulated by the virus to promote immune evasion.[6] HCMV can suppress the activation and function of immune cells by modulating the miRNA expression profile of host cells, including miR-34, thereby evading clearance by the immune system.[6] MRX34 is a miR-34-based nanoparticle drug that has shown certain anti-tumor activity in early clinical trials.[20,21] However, MRX34 also has some limitations, such as low delivery efficiency and off-target effects. Nevertheless, the clinical trial results of MRX34 indicate that miR-34-based therapeutic strategies have certain application prospects, but further improvements and optimizations are still needed.

There are still many issues and controversies in miR-34 research, such as the mechanisms of miR-34 in different diseases, its delivery efficiency and safety. Further in-depth study of miR-34's functions will help overcome these challenges and lay the foundation for developing more effective treatments.[1,9]

The Basic Biology of miR-34

Sequence Conservation and Genomic Distribution of the miR-34 Family

The miR-34 family is a highly conserved miRNA family, with its core members including miR-34a, miR-34b, and miR-34c. These three miRNAs exhibit extremely high conservation in the seed region and cross-species sequence alignment reveals almost no variation in their sequences (Table 1). This conservation indicates that the miR-34 family plays a critically important role in cellular biological functions during evolution, with its key regulatory targets and signaling networks demonstrating similar modes of action across different organisms.[22,23]

At the genomic level, the distribution of different members also exhibits distinct variability and specific patterns. Specifically, miR-34a typically exists as an independent gene, with its coding region located in relatively stable chromosomal regions in certain species, whereas miR-34b and miR-34c are often co-transcribed within the same gene cluster, distributed in another chromosomal region. This distribution not only reflects their cooperative relationship in transcriptional regulation but also suggests that they may share partial functional overlap while each possessing specialized regulatory properties within the cell.[24] Furthermore, secondary structure predictions of precursor miRNAs reveal that the precursors of the miR-34 family generally adopt a typical hairpin structure, which is sequentially processed by Drosha and Dicer into mature functional miRNAs. The highly conserved secondary structure provides the molecular basis for their proper loading into the RNA-induced silencing complex (RISC). These structural features, along with their precise genomic localization, lay a solid foundation for further research into the role of miR-34 in cellular biology.

Members of miR-34 Family Exhibit Tissue-Specific Expression Patterns

Numerous in vitro and in vivo experiments and clinical sample analyses have shown that the expression of miR-34 family members exhibits significant specificity and spatiotemporal dependence in different tissues (Figure 1). Studies have found that miR-34a has a relatively high basal expression level in various organs, including the heart, liver, lungs, and brain, suggesting its broad role in maintaining organ homeostasis, cell cycle control, and apoptosis regulation. In contrast, miR-34b and miR-34c display more pronounced tissue specificity. For instance, miR-34b/c expression is particularly abundant in respiratory epithelium, the digestive system, and certain immune cells, a pattern often closely associated with localized regulatory demands and defense responses[25] (Figure 1).

Table 1 Sequence Conservation and Genomic Localization of miR-34 Family Members in Different Species

Species	miR-34a Genomic Locus	miR-34b/34c Genomic Locus	Conservation Features	Ref.
Human (H. sapiens) Mouse (M. musculus) Rat (R. norvegicus)	1p36.22 4qE2 5q32	11q23.1 (clustered) 9qA5.2 (clustered) 8q24 (clustered)	Seed sequence (5'-GGCAGUG-3') perfectly conserved High homology in mature sequence (>90%) Highly similar precursor stem-loop structures	[22,23]

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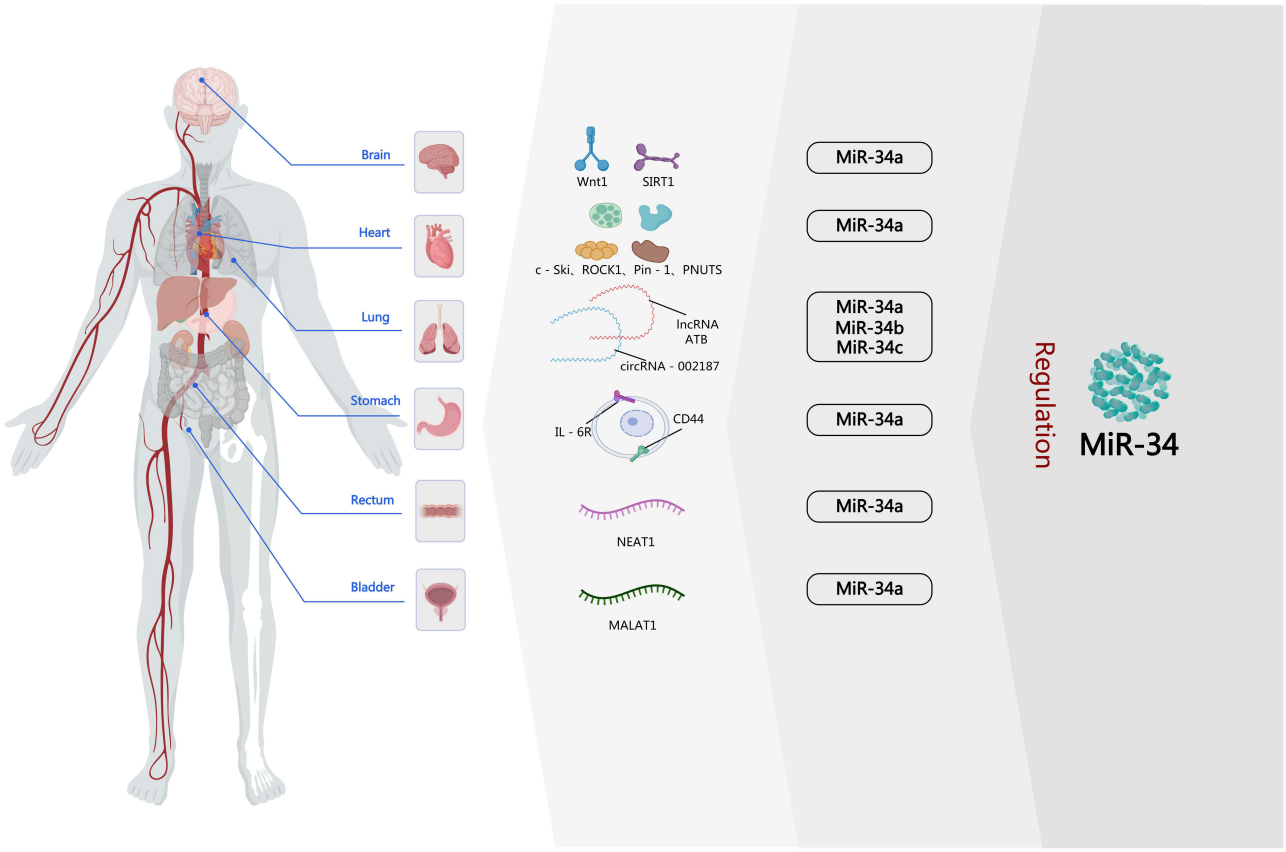

Figure 1 Key molecules involved in the regulation of the miR-34 family in various organs.

Moreover, systematic comparisons of miR-34 expression levels across different tissue samples using public databases such as the Gene Expression Omnibus (GEO) and extensive experimental data indicate that under certain physiological or pathological conditions, the expression of the miR-34 family undergoes significant changes depending on tissue type, developmental stage, and stress environment. During viral infection or early tumorigenesis, cells upregulate miR-34 expression to induce cell cycle arrest and apoptosis as an intrinsic defense mechanism, whereas under normal conditions, miR-34 expression remains relatively stable This dynamic and tissue-specific expression pattern not only highlights the multifaceted roles of miR-34 in regulating cell fate but also provides a theoretical basis for its potential application as a biomarker in clinical diagnosis and treatment.[26] In summary, the distinct expression profiles of miR-34a, miR-34b, and miR-34c in various organs reflect their specialized roles in the fine-tuned regulation of cellular functions across multiple systems and levels (Figure 1).

Regulation of the miR-34 Promoter Region and the Activating Effect of p53

The transcriptional regulation of miR-34 plays a pivotal role in cellular stress responses, with the direct involvement of the p53 transcription factor being one of its most central regulatory mechanisms. As the "guardian of the genome", p53 is rapidly activated when cells encounter DNA damage, oxidative stress, or other internal/external stimuli, directly binding to specific sites in the miR-34 promoter region to stimulate transcriptional initiation. Experimental results using a dual-luciferase reporter system demonstrate that enhanced p53 activity significantly increases miR-34 promoter activity, clearly validating the direct regulatory relationship between p53 and miR-34.[27]

This regulatory mechanism has been repeatedly validated not only in in vitro cell models but also demonstrated consistency across various animal models and clinical samples. Following p53-induced miR-34 expression, miR-34 further regulates multiple downstream target genes, participating in critical biological processes such as inducing cell cycle arrest, promoting apoptosis, and triggering cellular senescence. Specifically, p53 transcriptionally activates key

effectors including p21 (mediating cell cycle arrest) and PUMA (promoting apoptosis), while miR-34 contributes to these processes by directly suppressing the oncogene MYCN, thus forming a coordinated tumor-suppressive network.[28,29] The p53-miR-34 regulatory axis plays an irreplaceable role in maintaining genomic stability and preventing abnormal cell proliferation, serving as a key pathway for cells to activate self-protective mechanisms upon genotoxic stress. In fact, the high evolutionary conservation of this regulatory relationship indicates that p53-mediated activation of miR-34 constitutes an indispensable component of the cellular stress response network across mammals and other higher organisms (Figure 2).

The Impact of Epigenetic Factors on miR-34 Expression

In addition to direct regulation by transcription factors, epigenetic mechanisms also play a crucial role in the regulation of miR-34 gene expression. Studies have shown that the promoter region of miR-34 is often tightly regulated by DNA methylation and histone modification states (Figure 3). In certain tumor cells, high levels of DNA methylation are frequently detected in the miR-34 promoter region (Figure 3A and C). This methylation modification leads to a more compact chromatin structure, thereby inhibiting the binding of transcription factors such as p53 and ultimately causing a significant decrease in miR-34 expression[27] (Figure 3A). Similarly, the acetylation, methylation, and phosphorylation states of histones can directly influence chromatin openness, with different histone modification patterns either positively or negatively regulating the transcriptional activity of miR-34 (Figure 3).

In experiments, the downregulated expression of miR-34 due to epigenetic silencing can be partially restored by using DNA demethylating agents or histone deacetylase inhibitors, a finding that provides a feasible basis for restoring miR-34 function through epigenetic intervention strategies (Figure 3B and D). Notably, under pathological conditions such as viral infection and tumors, the epigenetic modification status of the miR-34 promoter region is often closely related to changes in biological function: hypermethylation not only enables cells to evade p53-mediated apoptotic signals but also reduces their defense capability against foreign pathogens, thereby promoting the progression of pathological processes. Therefore, restoring the normal expression of miR-34 by regulating the epigenetic state may become a novel strategy to

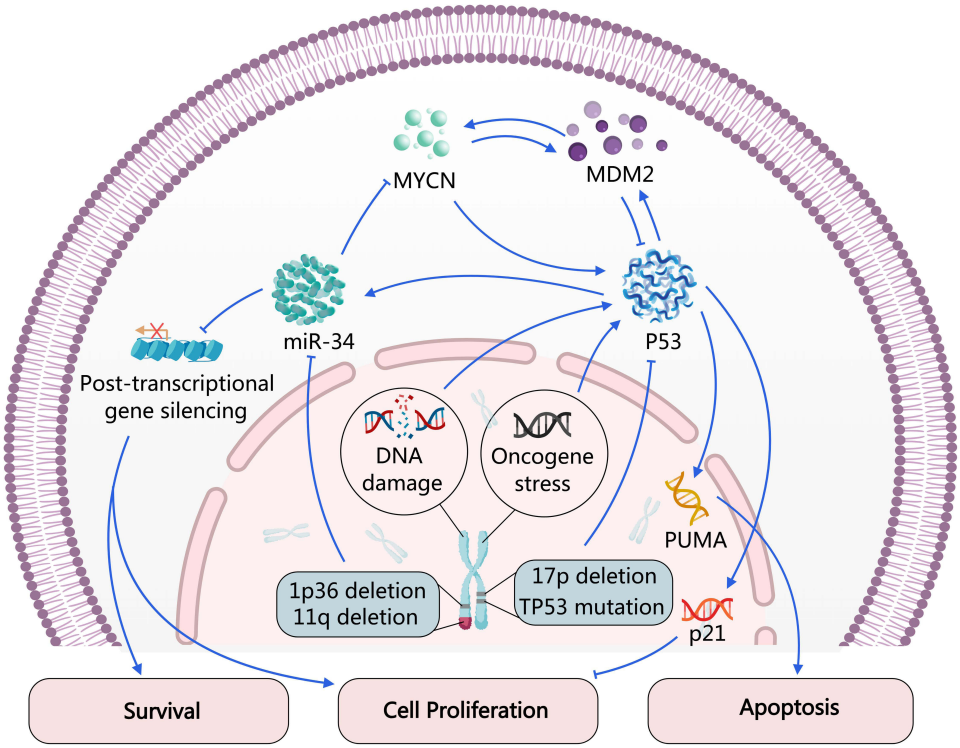

Figure 2 Regulation of the miR-34 promoter region and the activating effect of p53.

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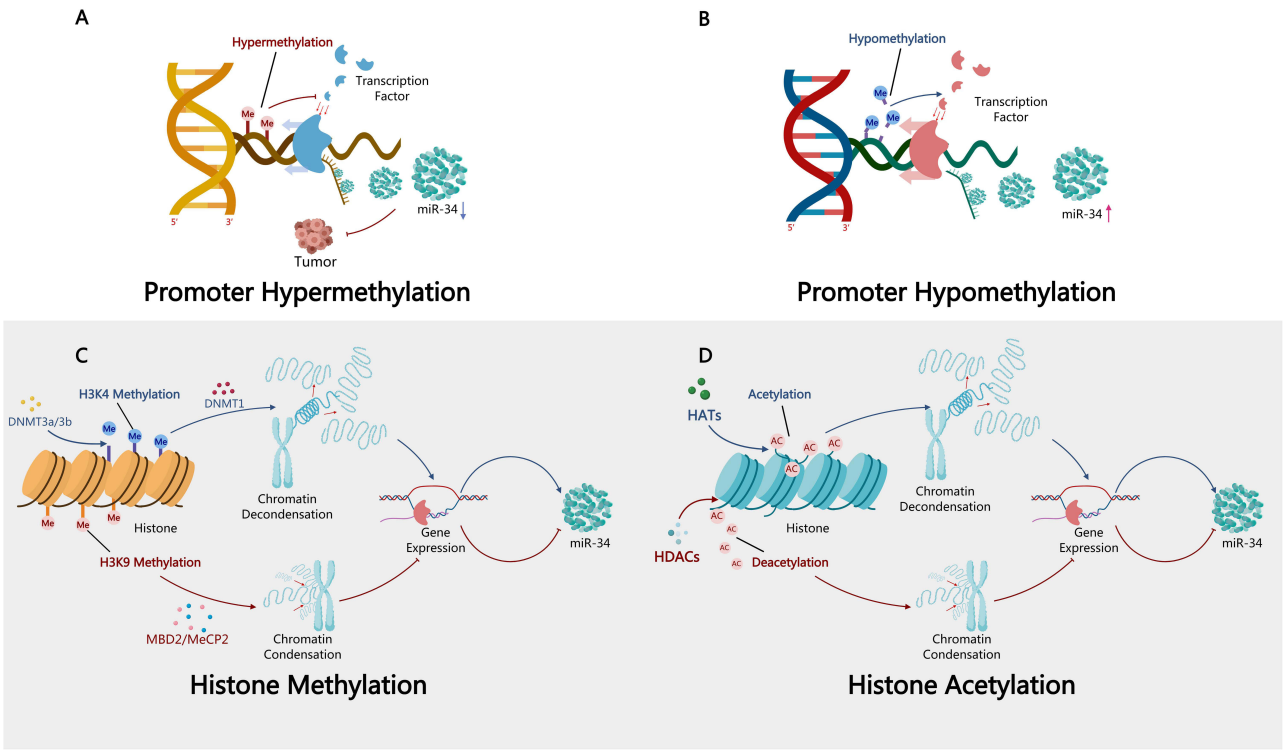

Figure 3 The impact of epigenetic factors on miR-34 expression. (**A** and **B**) The effects of promoter hypermethylation or hypomethylation on miR-34 expression; (**C** and **D**) The effects of histone methylation or acetylation modifications on miR-34 expression.

improve antiviral and antitumor therapeutic efficacy. Overall, DNA methylation and histone modifications, as critical layers in the regulation of miR-34 expression, reveal a multi-level and multi-dimensional fine regulatory network within cells, providing new perspectives for understanding cellular stress responses and disease development (Figure 3).

The Key Direct Regulatory Targets of miR-34

The miR-34 family effectively recognizes and regulates multiple key signaling molecules by binding to their conserved seed sequences during cellular physiological processes. Research indicates that Cyclin D1 is one of the important target genes of miR-34. Cyclin D1 plays a critical role in promoting the G1-S phase transition during cell cycle regulation, while miR-34 downregulates Cyclin D1 expression by directly binding to its 3′-UTR region, thereby inducing cell cycle arrest and allowing cells sufficient time for DNA repair or programmed apoptosis.

In addition to Cyclin D1, the pro-apoptotic molecule Bax is also a key target of miR-34 regulation. Upregulation of miR-34 enhances Bax expression, promoting the progression of cells toward apoptosis, which plays a crucial role in eliminating damaged or abnormally proliferating cells. Furthermore, SIRT1, a deacetylase involved in regulating cellular stress responses and metabolic balance, indirectly releases p53 activity when its expression is reduced, further amplifying apoptotic signals. miR-34 enhances p53-mediated apoptosis mechanisms by suppressing SIRT1 expression. On the other hand, the IL-6 receptor (IL-6R) plays a pivotal role in cytokine signaling and inflammatory responses. By regulating IL-6R expression, miR-34 not only influences cellular sensitivity to inflammatory signals but may also exert a unique role in mediating immune responses and inflammatory balance.[24,46] This multi-target regulatory pattern positions miR-34 as a central molecule integrating multiple cellular signaling pathways, demonstrating broad and profound biological functions in cell fate determination, tumor suppression, and antiviral defense.

From a functional perspective, the various targets regulated by miR-34 do not exist independently but form a complex network with synergistic effects and feedback regulation. For example, the downregulation of Cyclin D1 not only directly affects the cell cycle progression but may also indirectly influence the proliferative state of cells through multiple signaling branches; meanwhile, the upregulation of Bax expression is closely associated with the activation of the

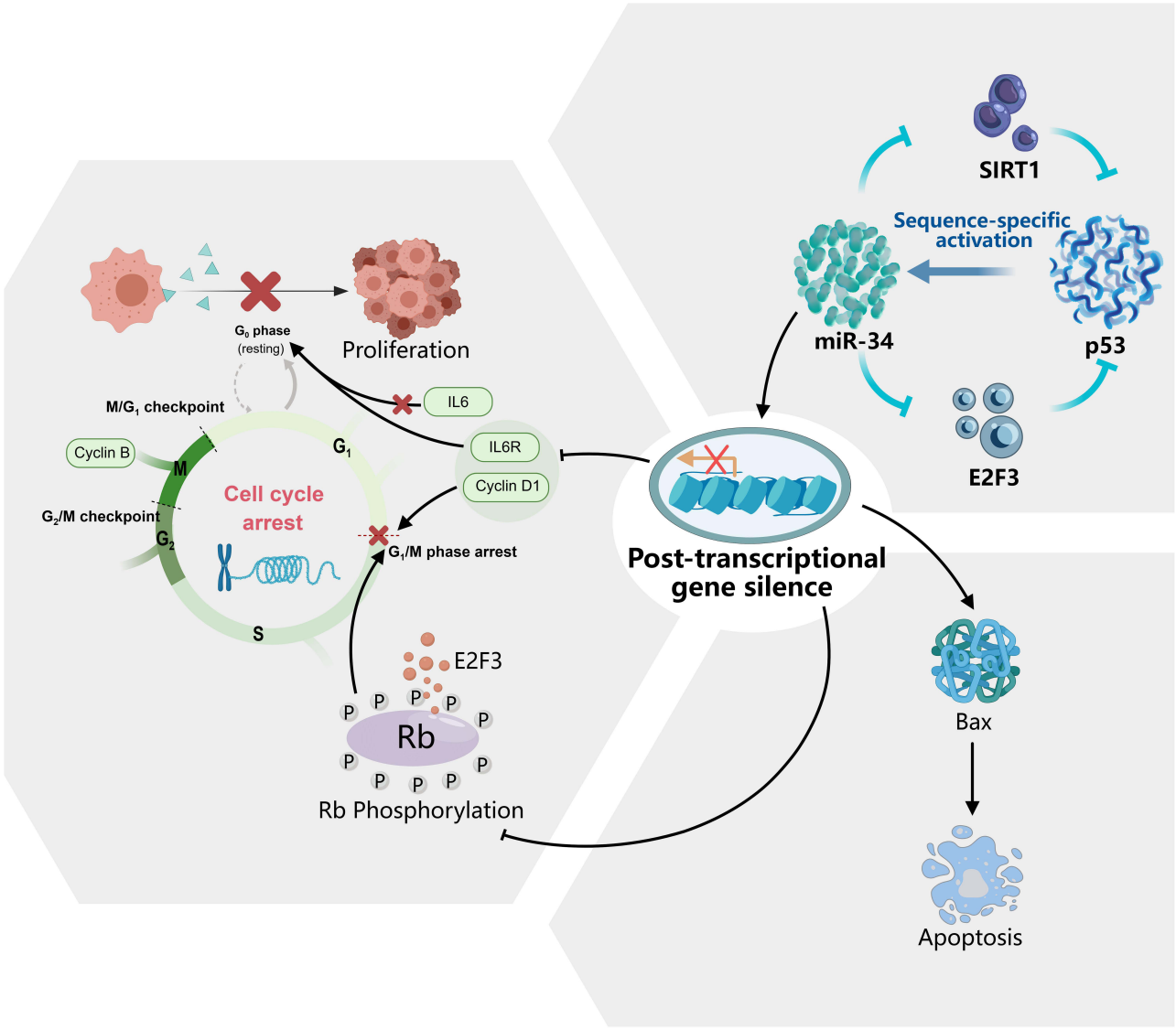

Figure 4 Integration of miR-34 family target genes and signaling pathways. The p53 gene activates miR-34 by recognizing specific sequences. Post-transcriptional gene silencing by miR-34 acts on cell cycle-related molecules (such as CDK4/6, Cyclin, etc.) to arrest the cell cycle, and also regulates c-myc, BCL-2, Bax, etc, affecting cell proliferation and apoptosis. miR-34 can form a positive feedback loop with E2F3 and SIRT1, reducing their inhibition of p53 by suppressing the expression of E2F3 and SIRT1.

mitochondrial pathway, further accelerating the apoptotic process. It is precisely due to these multi-layered regulatory mechanisms that miR-34 can exert precise and efficient regulatory effects in various pathological conditions (Figure 4).

Experimental Validation of miR-34 Target Genes and Regulatory Network

To elucidate the direct regulatory relationship between miR-34 and its target genes, researchers employed multiple experimental validation methods, with the dual-luciferase reporter gene assay being the most intuitive and commonly used approach. By constructing reporter plasmids containing the 3′-UTR sequences of candidate target genes and co-transfecting them with miR-34 mimics or inhibitors into cells, the inhibitory effect of miR-34 on target gene expression could be directly observed through changes in fluorescence signal intensity, thereby validating the likelihood of their direct binding.

Meanwhile, the application of whole-transcriptome sequencing (RNA-seq) technology enables researchers to systematically monitor the global changes in gene expression in cells before and after miR-34 regulation. By comparing expression differences, not only can potential direct target genes be screened, but also indirectly regulated signaling

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networks can be revealed, further constructing a complex and dynamic miR-34 regulatory map. To ensure the accuracy of the results, subsequent secondary validation of the screened candidate target genes is often performed using methods such as quantitative real-time PCR (qPCR) and protein detection.

Recently, with the advancement of bioinformatics and systems biology, researchers have progressively integrated RNA-seq data with proteomics and epigenetics data. By constructing regulatory network models, they have further elucidated the interactions and feedback regulatory mechanisms of miR-34 among various signaling pathways in cells. This comprehensive strategy not only reveals the multifaceted role of miR-34 as a central regulator in gene expression regulation but also provides robust data support for exploring its precise functions in viral infections, tumorigenesis, and other pathological processes.[24,30] Overall, the adoption of a multi-technique experimental strategy has strongly supported the construction of multi-layered regulatory networks encompassing transcriptional regulation, post-translational modifications, and feedback regulation, while also opening new research directions for the future development of combined therapeutic strategies based on miR-34 function.

The Mechanism of miR-34 in Viral Prevention and Treatment

Dynamic Changes in miR-34 Expression After Viral Infection

Viral infection induces significant changes in the expression levels of miR-34 in host cells, which are dependent on time and virus type. At different stages of viral infection, miR-34 expression exhibits dynamic alterations, reflecting its complex regulatory role in immune responses (Table 2).

Time series analysis: After viral infection, the expression level of miR-34 changes over the course of infection. For example, in the early stages of infection, host cells may upregulate the expression of miR-34 to inhibit viral replication and spread, thereby initiating immune defense mechanisms. However, as the infection progresses, the virus may downregulate the expression of miR-34 by interfering with the host cell's signaling pathways to evade clearance by the immune system. Therefore, conducting time series analysis of miR-34 expression during viral infection helps reveal its role in antiviral immune responses.

Effects of multiple viruses: Different types of viruses may have varying impacts on miR-34 expression. Some viral infections may lead to upregulation of miR-34 expression, while others may cause its downregulation. For instance, RNA viruses and DNA viruses differ in their replication mechanisms and interactions with host cells, which could result in distinct regulatory patterns of miR-34 expression. Therefore, comparing the effects of different viral infections on miR-34 expression helps elucidate the role of miR-34 in broad-spectrum antiviral immunity.

Differences in cell types: Variations in miR-34 expression may also differ among different host cell types. Immune cells and non-immune cells play distinct roles in antiviral immune responses, and their reactions to viral infections may also vary. For example, immune cells (such as macrophages, dendritic cells, and lymphocytes) may rapidly initiate miR-34 expression after viral infection to enhance their antiviral functions. In contrast, non-immune cells (such as epithelial and endothelial cells) may exhibit changes in miR-34 expression only during later stages of infection. Therefore, comparing miR-34 expression changes across different cell types helps elucidate its antiviral effects in various tissues and organs.

miR-34 in Regulating Interferon, IRF3 Phosphorylation and NF-κB Signaling Pathway

miR-34 plays a critical role in host immune responses by regulating key immune signaling pathways such as interferon, IRF3 phosphorylation, and the NF-κB pathway, thereby influencing the intensity and duration of antiviral immune responses.

Interferon signaling:[1] Type I interferons play a central role in antiviral immunity. miR-34 can regulate antiviral responses by influencing the production and signaling of type I interferons. Specifically, miR-34 may modulate interferon activity by targeting key molecules in the interferon signaling pathway, such as interferon receptors or signal transduction proteins. This regulation can affect viral replication and spread, as well as the survival and apoptosis of host cells.

IRF3 Phosphorylation:[1] IRF3 is a member of the interferon regulatory factor family, activated upon viral infection and involved in the expression of type I interferons. miR-34 may interact with IRF3, influencing its phosphorylation status and transcriptional activity. Some studies suggest that miR-34 can promote IRF3 phosphorylation, thereby

Table 2 Relationship Between Different Viral Infections and miR-34 Expression

Virus Type	miR-34 Related Members	Expression Change	Validation Method	Function and Mechanism	Ref.
West Nile Virus (WNV)	miR-34c	Downregulated	Microarray, RNA-Seq	Inhibits viral replication	[1,30]
Japanese Encephalitis Virus (JEV)	miR-34c	Downregulated	Microarray, RNA-Seq	MiR-34c reduces the secretion of inflammatory cytokines by regulating the Notch signaling pathway, thereby inhibiting inflammatory responses	[1,30]
Influenza A Virus	miR-34a miR-34c	miR-34a downregulated miR-34c upregulated	qRT-PCR	MiR-34a induces cell apoptosis by targeting Bax; miR-34c promotes viral replication	[18,19,30]
Epstein-Barr Virus (EBV)	miR-34a	Downregulated	qRT-PCR	EBV alters immune checkpoint PD-L1 expression by downregulating miR-34a in B-cell lymphomas	[31,32]
Respiratory Syncytial Virus (RSV)	miR-34b-5p, miR-34c-5p	Downregulated	qRT-PCR Microarray	RSV infection reduces their expression, and induces MUC5AC production by activating c-Jun, promoting mucus secretion	[33–35]
White Spot Syndrome Virus (WSSV)	Shrimp miR-34	Downregulated	Northern Blot qRT-PCR	Its expression increases in WSSV-infected shrimp, which has antiviral activity, inhibits WSSV replication, and reduces shrimp mortality	[36]
Avian Leukosis Virus Subgroup J (ALV-J)	miR-34b	Upregulated	qRT-PCR	miR-34b inhibits MDA5 (an activator of the interferon signaling pathway), promotes ALV-J replication, and enhances cell proliferation and migration	[37]
Kaposi's Sarcoma-Associated Herpesvirus (KSHV)	miR-34a-5p	Downregulated	qRT-PCR Northern Blot	miR-34a-5p inhibits the proliferation and cell cycle of KSHV-positive cells, and reduces the expression of pathogenic genes RTA and v-GPCR	[38,39]
Dengue Virus (DENV)	miR-34a miR-34c	miR-34a downregulated miR-34c downregulated	Microarray, RNA-Seq	miR-34a inhibits the Wnt signaling pathway to promote the interferon signaling pathway and suppress viral replication; downregulation of miR-34c can inhibit viral replication	[40]
Hepatitis B Virus (HBV)	miR-34a miR-34c	miR-34a downregulated miR-34c downregulated	qRT-PCR, Western Blot	Downregulation of miR-34a promotes cell growth by regulating MAP4K4; downregulation of miR-34c promotes viral replication and cell proliferation; miR-449a enhances HBV replication by targeting CREB5 and regulating FXRα	[41,42]
Human Papillomavirus (HPV16, HPV18)	miR-34a	Downregulated	Northern Blot	E6 protein of HPV disrupts the stability of the tumor suppressor p53, which is a binding transcription factor for the miR-34a gene promoter, leading to decreased miR-34a expression and promoting cell proliferation MiR-34a inhibits viability and invasion of HPV-positive Cervical Cancer Cells by Targeting E2F3 and Regulating Survivin	[43–45]

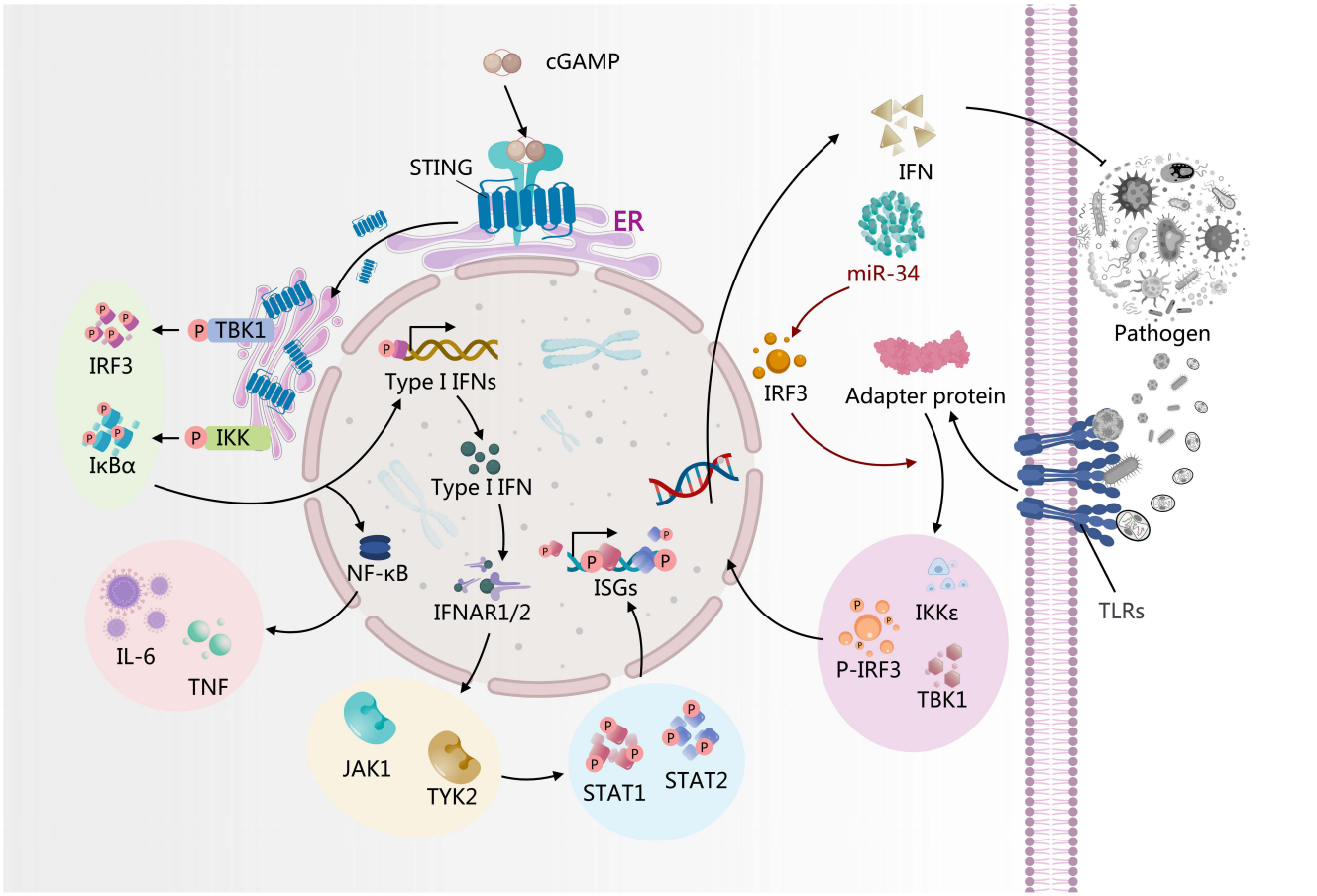

Figure 5 The role of miR-34 in the interferon and IRF3 phosphorylation pathway. Pathogens act on TLRs on the cell membrane, activating intracellular adaptor proteins, with miR-34 participating in regulation to inhibit or promote IRF3 phosphorylation (P-IRF3) and NF–κB activation mediated by TBK1 and IKKε. After entering the nucleus, it initiates interferon (INF) production, demonstrating the regulatory role of miR-34 in the interferon and IRF3 phosphorylation pathway.

enhancing interferon expression and antiviral immune responses. Others indicate that miR-34 may inhibit IRF3 phosphorylation, weakening interferon expression and immune responses. These conflicting results may reflect the diverse roles of miR-34 in different viral infections and cell types (Figure 5).

NF-κB pathway:[47] The NF-κB signaling pathway plays a crucial role in regulating immune and inflammatory responses. miR-34 can influence the expression of inflammatory factors and the activity of immune cells by modulating key molecules in the NF-κB signaling pathway, such as NF-κB inhibitory proteins or NF-κB target genes. Some studies suggest that miR-34 can suppress the NF-κB signaling pathway, thereby alleviating inflammatory responses and autoimmune diseases. Other studies indicate that miR-34 may activate the NF-κB signaling pathway, enhancing antiviral immune responses. These divergent findings may reflect the distinct roles of miR-34 in different diseases and cell types (Figure 5).

miR-34 Targets Virus-Dependent Host Factors to Reduce Viral Proliferation Rate

miR-34 can effectively reduce the viral proliferation rate by targeting virus-dependent host factors. After infecting host cells, viruses rely on certain specific host factors to complete replication and transcription processes. miR-34 can interfere with the viral life cycle and reduce its proliferation capacity by inhibiting the expression of these host factors.

Target description: Virus-dependent host factors include various types of molecules, such as certain cytokines, receptors, and host proteins involved in viral replication and transcription. For example, some viruses require specific cytokines to facilitate their entry into host cells or specific receptors to mediate their binding to host cells. miR-34 can inhibit viral infection by targeting these cytokines or receptors. Additionally, some viruses rely on certain protein factors

from host cells to complete their replication and transcription processes. miR-34 can inhibit viral replication and transcription by targeting these protein factors.

Functional experimental results: Relevant studies have confirmed that miR-34 can reduce viral proliferation rates by targeting virus-dependent host factors. For example, in certain viral infection models, researchers found that miR-34 can significantly decrease viral load. These studies typically employ functional validation experiments, such as data demonstrating viral load suppression, to confirm the antiviral effects of miR-34.

Comparison of Regulatory Patterns of miR-34 in Different Viruses

RNA viruses and DNA viruses exhibit significant differences in their replication mechanisms, which may lead to distinct patterns of miR-34 regulation in the context of these two viral infections.

Characteristics of different viruses: RNA viruses typically have higher mutation rates and shorter replication cycles, replicating directly in the cytoplasm of host cells. DNA viruses, on the other hand, usually exhibit lower mutation rates and longer replication cycles, requiring replication in the nucleus of host cells. These differences may lead to varying impacts of RNA and DNA viruses on the host cell's immune response, thereby influencing the regulatory role of miR-34 (Table 2).

Comparative studies suggest that in RNA virus infections, miR-34 may primarily inhibit viral replication and spread by targeting viral RNA or virus-dependent host factors. In DNA virus infections, miR-34 may mainly clear the virus by regulating the host cell's immune response. Additionally, different viruses may employ distinct mechanisms to induce miR-34. Some viruses may upregulate miR-34 expression by activating host cell signaling pathways, while others may downregulate it by suppressing these pathways.

miR-34 Regulates the Expression of Inflammatory Factors

miR-34 plays a crucial role in regulating inflammatory responses by modulating the expression of various inflammatory factors, such as IL-6 and TNF-α, thereby influencing the intensity and duration of inflammation.

Analysis of specific inflammatory factors: IL-6 and TNF-α are two important pro-inflammatory cytokines that play a key role in inflammatory responses. miR-34 can inhibit their translation by targeting the mRNA of IL-6 and TNF-α, thereby reducing their expression levels. Additionally, miR-34 can indirectly regulate their expression levels by influencing the transcription of IL-6 and TNF-α.

Mechanism analysis: The mechanism by which miR-34 affects the transcription and expression of inflammatory factors may involve multiple aspects. For example, miR-34 may influence the transcription of inflammatory factors by targeting and regulating transcription factors. Additionally, miR-34 may also regulate the expression of inflammatory factors by affecting mRNA stability.

Virus-Induced miR-34 Dysregulation Promotes Immune Escape and Regulates Homeostasis

Viral infection can induce the downregulation of host cell miR-34 expression, which helps the virus evade immune system clearance and promotes its persistent infection (Table 3).

Downregulation mechanisms: Viruses can downregulate the expression of host miR-34 through various molecular mechanisms. For example, some viruses can encode proteins that directly inhibit the transcription or processing of miR-34. Others may indirectly suppress miR-34 expression by activating signaling pathways in host cells.

Regulatory balance strategy: miR-34 plays a crucial role in balancing the regulation between viruses and hosts. In the early stages of viral infection, host cells may upregulate the expression of miR-34 to inhibit viral replication and spread. However, as the infection progresses, viruses may downregulate miR-34 expression to evade clearance by the immune system. Therefore, maintaining the balance of miR-34 between viruses and hosts is essential for controlling viral infections. Strategies targeting the regulatory balance of miR-34, such as restoring its expression through drugs or gene therapy, may provide new approaches for treating viral infections.

Table 3 MiR-34 Mediates the Core Pathway of Viral Infection Replication and Tumorigenesis

Pathway Type	Core Mechanism	Involved Viruses/ Tumors	Specific Role of miR-34	Expression Pattern (Tissue/ Cancer)[c]	Validation Method	Ref.
Virus Infection-related Pathways	AP-1 pathway regulation	RSV	RSV infection downregulates miR-34b/c-5p, relieves inhibition on c-Jun, activates AP-1, and promotes MUC5AC mucus secretion	(miR-34b/c) Downregulated upon RSV infection in respiratory epithelium	Western blot, ELISA	[33–36]
	PI3K/Akt/mTOR pathway regulation	KSHV-related tumors	MiR-34a-5p inhibits the pathway by targeting, reducing proliferation of KSHV-positive cells and expression of viral genes	(miR-34a) Downregulated in KSHV-associated malignancies	Functional assay (CCK-8)	[38,39]
	TGF-β/Smad pathway regulation	HBV related tumors	MiR-34a inhibits the TGF-β/Smad4 pathway to reduce migration of cholangiocarcinoma cells; miR-34b downregulates TGF-βR1, etc, to inhibit invasion of prostate cancer cells	(miR-34a) Downregulated in HBV-CCA (vs normal bile duct); (miR-34b) Downregulated in prostate cancer (vs normal prostate)	Transwell assay, WB, qPCR	[40–42]
	NF-κB signaling pathway regulation	EBV, HPV16/18	EBV upregulates miR-34a through LMP-1-mediated activation of NF-κB to promote cell growth; E6 protein of HPV16/18 degrades p53, downregulates miR-34a, and promotes cell proliferation	(miR-34a) EBV: Upregulated in EBV+ lymphomas (vs normal B cells); HPV: Downregulated in HPV+ cancers (vs normal cervix)	ChIP, Luciferase assay, qPCR	[43–45]
	Notch pathway regulation	HCV related to CRC	MiR-34a targets Notch1/Jagged1 to inhibit CRC metastasis; miR-449a targets Notch1 to regulate HCV-related inflammation	(miR-34a) Downregulated in CRC tissue (vs matched adjacent normal)	Luciferase assay	[48]
	EMT pathway regulation	Colorectal cancer, breast cancer, etc.	Targets the 3'UTR of EMT transcription factors such as SNAIL and ZEB, inhibits their expression, and reduces migration and invasion of tumor cells	(miR-34a) Downregulated in metastatic tumor tissues (vs primary tumors or normal)	Luciferase assay, WB, Wound healing	[49–51]
Tumorigenesis-related Pathways	p53 pathway synergistic regulation	Leukemia, etc.	Serves as a downstream target of p53, forming a positive feedback loop with p53 to inhibit tumor cell proliferation; low expression of miR-34a/c in leukemia is associated with p53 and Rab27b abnormalities	(miR-34a/c) Frequently downregulated in leukemias and HCC (vs corresponding normal tissues)	Co-IP, RNA-seq, Functional assay	[52,53]
	Cell cycle regulation	Breast cancer, Lung cancer, Osteosarcoma, etc.	Targets CCND1, CDK6, etc, induces G0/G1 phase arrest; promotes apoptosis and cell cycle arrest in osteosarcoma	(miR-34a) Downregulated in breast and lung cancer (vs normal breast/ lung tissue)	Flow cytometry, WB, qPCR	[23,51,52,54,71]

Evaluation of the Application of miR-34 in Viral and Tumor Prevention and Treatment

Antiviral Trials and Preclinical Studies of miR-34 Replacement Therapy

miR-34, as a class of tumor-suppressing miRNAs, demonstrates potential application value in the prevention and treatment of viral infections. By targeting and regulating key molecules during viral infection, miR-34 can influence viral replication, transmission, and host immune responses, thereby exerting antiviral effects.

In antiviral experimental design, researchers have explored various delivery methods for miR-34, including viral vectors, liposomes, and novel nanoparticles. These delivery systems aim to enhance the stability and transfection efficiency of miR-34 in target cells, thereby boosting its antiviral activity. In vitro experiments typically employ cell models, such as cell lines infected with specific viruses, to evaluate the impact of miR-34 on viral replication and cell survival. Animal models, often using mice, simulate the in vivo process of viral infection to investigate the effects of miR-34 on disease progression, immune responses, and histopathology.

Currently, preclinical research on miR-34 mainly focuses on the following aspects:

1. Inhibition of viral replication: Studies have shown that miR-34 can directly or indirectly target viral genes or virus-dependent factors in host cells, thereby reducing the efficiency of viral replication. For example, in certain viral infection models, the introduction of miR-34 can significantly decrease the expression of viral RNA or proteins, thereby mitigating the severity of infection.
2. Regulation of immune response: miR-34 plays a crucial role in modulating host immune responses. It can influence interferon production, cytokine release, and immune cell activity, thereby enhancing the body's ability to clear viruses. Preclinical studies indicate that miR-34 promotes the expression of certain key immune factors and activates immune signaling pathways, thereby improving the intensity and durability of antiviral immune responses.
3. Balance of inflammatory response: Viral infections are often accompanied by inflammatory responses, and excessive inflammation may lead to tissue damage and disease progression. miR-34 has been found to regulate the expression of inflammatory factors such as IL-6 and TNF-α, thereby reducing the severity of the inflammatory response and protecting tissues from damage. Preclinical studies have shown that the introduction of miR-34 can lower the levels of inflammatory factors and improve disease prognosis.

Although miR-34 has shown promising results in preclinical studies for viral prevention and treatment, further in-depth research is still needed to elucidate its mechanisms of action, targets, and potential side effects, laying the foundation for future clinical applications.

miR-34 Regulation of Host Immune Strategies and Their Limitations

miR-34 plays a multifaceted role in regulating host immune responses by influencing the expression of key immune factors such as interferons and cytokines, thereby modulating both innate and adaptive immune responses. For instance, miR-34 may regulate interferon production by affecting the activity of interferon regulatory factors (IRFs) or influence the release of inflammatory cytokines by modulating the NF-κB signaling pathway. Through these mechanisms, miR-34 can enhance the body's immune defense against viruses.

However, the strategy of using miR-34 to regulate host immunity also faces some challenges and limitations:

(1) Specificity issue: miR-34 has multiple target genes, and its impact on the immune system may not be entirely specific to viral infections, potentially affecting other physiological processes simultaneously. This lack of specificity could lead to deviations in immune regulation or even trigger autoimmune responses.
(2) The issue of efficacy: The effective delivery and targeting of miR-34 are key factors affecting its immunomodulatory effects. Current delivery technologies may not ensure that miR-34 effectively reaches target cells and maintains sufficient intracellular concentrations, thereby impacting its immunomodulatory efficacy.

(3) Off-target effects: miR-34 may bind to non-target genes, producing off-target effects that interfere with normal cellular functions. Such off-target effects may lead to adverse reactions, limiting its application in immune regulation.
(4) Safety concerns: Long-term or high-dose miR-34 therapy may have potential toxic effects on the body. For example, miR-34 may affect cell growth, differentiation, and apoptosis, leading to tissue damage or organ dysfunction.

To overcome these limitations, future research can proceed from the following aspects:

(1) Enhancing specificity: By optimizing the design of miR-34, such as using miR-34 analogs that target specific immune cells or signaling pathways, its specificity in immune regulation can be improved, reducing off-target effects.
(2) Enhancing efficacy: Developing novel delivery systems, such as targeted nanoparticles or exosomes, can improve the delivery efficiency and targeting of miR-34, thereby enhancing its immunomodulatory effects.
(3) Safety assessment: Conduct a comprehensive safety evaluation, including toxicity tests, immunogenicity tests, and long-term follow-up studies, to assess the safety of miR-34 therapy and provide assurance for clinical application.

Through continuous research and optimization, miR-34 is expected to become an effective immune regulation tool, providing new strategies for the prevention and treatment of viral infections.

Evaluation of miR-34 Application in Tumor Prevention and Treatment

miR-34, as a tumor-suppressive microRNA, exhibits significantly reduced expression levels in various tumors, and its expression is closely associated with tumor grade, metastasis, and patient prognosis.[55–58]

(1) Tumor grading: In various tumor types, including ovarian cancer[57] and prostate cancer,[56] the expression level of miR-34 is negatively correlated with tumor grade. This means that the higher the tumor grade, the lower the expression level of miR-34. miR-34 can inhibit cell proliferation and induce apoptosis in tumor cells, thereby delaying tumor progression. When miR-34 expression decreases, tumor cells lose this protective mechanism, leading to abnormal proliferation of tumor cells and promoting malignant tumor development.
(2) Tumor metastasis: The expression level of miR-34 is also closely related to the metastatic ability of tumors.[56] During the process of tumor cell metastasis, cells need to acquire motility and invasive capabilities. miR-34 can inhibit tumor cell metastasis by targeting and regulating genes associated with cytoskeletal remodeling, cell adhesion, and extracellular matrix degradation. When miR-34 expression decreases, the metastatic ability of tumor cells increases, making distant metastasis more likely to occur.
(3) Prognosis: The expression level of miR-34 is closely related to the prognosis of cancer patients.[56,59,60] Generally, patients with higher miR-34 expression levels have longer survival periods and better prognoses. This may be because miR-34 can inhibit the proliferation, metastasis, and drug resistance of tumor cells, thereby improving treatment efficacy and prolonging patient survival.

It should be noted that the expression patterns and mechanisms of miR-34 may vary across different types of tumors. For instance, in some tumors, miR-34 primarily functions by regulating genes related to the cell cycle and apoptosis, while in others, it may be more involved in modulating the tumor microenvironment and immune responses. Therefore, when evaluating the potential applications of miR-34 in tumor prevention and treatment, the tumor type and molecular characteristics must be thoroughly considered.

miR-34 in Targeted Therapy, Synergistic Effects with Combination Chemotherapy and Immune Checkpoint Inhibitors

miR-34 has potential applications in tumor therapy, especially when combined with targeted therapy, combination chemotherapy, and immune checkpoint inhibitors, where it may produce synergistic effects.[55,61,62]

(1) Targeted therapy: Targeted therapy refers to the design and development of drugs that specifically target molecular targets unique to tumor cells, such as oncogenes and growth factor receptors, to selectively kill tumor cells. miR-34 can enhance the efficacy of targeted drugs by regulating genes related to tumor cell growth, proliferation, and survival. For example, miR-34 can inhibit tumor cell drug resistance, making tumor cells more sensitive to targeted drugs; or, miR-34 can regulate the tumor microenvironment, improving the permeability and distribution of targeted drugs.
(2) Combination chemotherapy: Chemotherapy is a common method for tumor treatment, utilizing cytotoxic drugs to kill tumor cells. However, while chemotherapy drugs target tumor cells, they also damage normal cells, leading to adverse effects. miR-34 can enhance the efficacy of chemotherapy drugs and reduce their toxic side effects by regulating processes such as the cell cycle, apoptosis, and DNA repair. For example, miR-34 may increase chemotherapy sensitivity by promoting cancer cell apoptosis.[63]
(3) Immune checkpoint inhibitors: Immune checkpoint inhibitors are a novel class of tumor immunotherapy drugs that block immune checkpoint molecules, thereby lifting the suppression of tumor cells on the immune system and activating the patient's own immune cells to kill tumor cells. miR-34 can enhance the efficacy of immune checkpoint inhibitors by regulating the tumor microenvironment and the activity of immune cells. For example, miR-34 can promote the activation and proliferation of tumor-infiltrating lymphocytes, improving the ability of immune cells to kill tumor cells; or, miR-34 can inhibit the immune escape mechanisms of tumor cells, making them more easily recognized and eliminated by the immune system.

By combining with targeted therapy, combination chemotherapy, and immune checkpoint inhibitors, miR-34 is expected to become a versatile tumor treatment tool, bringing better therapeutic outcomes for patients.

Detection of Circulating miR-34 in Plasma and Exosomes and Its Potential for Early Diagnosis

Circulating miR-34, as a non-invasive biomarker, has the potential for early diagnosis through detection in body fluids such as plasma and exosomes.[64–66]

(1) Detection methods: Currently, the main techniques for detecting circulating miR-34 include quantitative reverse transcription polymerase chain reaction (qRT-PCR) and next-generation sequencing (NGS). qRT-PCR is a commonly used detection method with advantages such as high sensitivity, strong specificity, and simple operation, making it suitable for small sample size detection. NGS technology offers benefits like high throughput and high resolution, enabling simultaneous detection of multiple miRNA expression levels, making it suitable for large-scale screening and discovery research.
(2) Potential for early diagnosis: Studies indicate that during the early stages of various diseases, including tumors, cardiovascular diseases, and neurodegenerative disorders, the expression levels of circulating miR-34 undergo significant changes. For example, in the early stages of tumors, tumor cells release large amounts of miR-34 into the bloodstream, leading to elevated plasma miR-34 levels. Detecting plasma miR-34 levels can enable the early identification of tumors. Exosomes are tiny vesicles secreted by cells and are rich in miRNA. Exosomal miR-34 can reflect the physiological state and pathological changes of cells. Measuring exosomal miR-34 levels allows for more accurate disease diagnosis.

Case studies on the application of circulating miR-34 as an early diagnostic biomarker:

(1) Non-alcoholic fatty liver disease (NAFLD): A study showed that serum miR-34 levels were significantly higher in NAFLD patients compared to healthy individuals, and miR-34 was associated with the severity of NAFLD.[64]
(2) Male infertility: Studies have shown that miR-34 expression in treated and untreated infertile male rats is associated with sperm parameters and can serve as a diagnostic marker.[65]
(3) Nasal inflammatory diseases and tumors: Studies have shown that the expression profiles of miRNAs are altered in chronic rhinosinusitis (CRS), allergic rhinitis (AR), and sinonasal tumors, indicating that miRNAs have diagnostic potential in nasal diseases.[66]

miR-34 in the Exploration of Strategies for Prognostic Monitoring and Real-Time Efficacy Evaluation

Regular monitoring of miR-34 levels is of great significance for understanding disease progression and prognosis. The expression levels of miR-34 can reflect the degree of disease progression, response to treatment, and risk of recurrence. By regularly monitoring miR-34 levels, treatment plans can be adjusted in a timely manner to improve therapeutic outcomes and enhance patient prognosis.

In clinical practice, miR-34 can serve as an efficacy evaluation tool for real-time monitoring of treatment effects. For example, during tumor therapy, the inhibitory effect of therapeutic drugs on tumor cells can be assessed by regularly measuring the expression levels of miR-34 in patient plasma or tumor tissues. A significant increase in miR-34 expression levels indicates effective treatment, while no significant change suggests poor therapeutic outcomes, necessitating adjustments to the treatment plan.

The vision for future applications of biomarkers to monitor treatment efficacy includes:

(1) Establish standardized detection methods: To ensure the accuracy and reliability of detection results, it is necessary to establish standardized miR-34 detection methods, including sample collection, processing, detection, and data analysis.
(2) Development of rapid and convenient detection techniques: To meet clinical needs, it is essential to develop fast and convenient miR-34 detection technologies, such as point-of-care testing (POCT) techniques, enabling physicians to promptly obtain test results and guide clinical decision-making.
(3) Develop individualized prognostic prediction models: Integrate patients' clinical information, pathological characteristics, and genomic data to establish personalized miR-34 prognostic prediction models, providing patients with more precise treatment plans.

In summary, by utilizing miR-34 as a biomarker for early disease diagnosis, prognosis monitoring, and treatment efficacy evaluation, personalized precision medicine can be achieved, leading to better therapeutic outcomes for patients.

The Clinical Application Prospects of miR-34

miR-34 in Non-Invasive Clinical Monitoring and Diagnostic Applications

Circulating miR-34 has emerged as a highly promising noninvasive biomarker due to its stable molecular structure and detectability in plasma and exosomes. Advanced techniques such as qRT-PCR, next-generation sequencing (NGS), and microfluidic chips enable highly sensitive and specific detection of miR-34 expression levels. These methods not only allow quantitative measurement of circulating miR-34 but also facilitate comprehensive profiling of its expression patterns, providing critical insights for early identification of pathological conditions such as viral infections (eg, influenza virus, HCMV infection) and tumors (eg, liver cancer, lung cancer).[16]

Recent studies have shown that the expression level of miR-34a-5p in plasma is closely associated with systemic inflammatory states and immune dysregulation. For instance, in certain chronic viral infections and immunodeficiency diseases, dynamic monitoring of circulating miR-34 levels can not only assess disease activity but also serve as

a predictive marker for therapeutic efficacy, reflecting patient responses to antiviral or antitumor treatments.[16] Unlike traditional biomarkers, which are often limited by operator dependency, high invasiveness, and lengthy detection cycles, miR-34-based liquid biopsy offers significant advantages such as speed, simplicity, and reproducibility, making it highly promising for clinical monitoring and early diagnosis.

Moreover, with the development of new technologies such as microfluidic chips, precise detection can be achieved with only minimal sample volumes, further reducing patient trauma. In practical applications, by monitoring the dynamic changes in miR-34 expression before and after treatment, a closed-loop management system of "detection-treatment-monitoring" can be established, providing clinicians with real-time, quantifiable feedback on disease progression. In the future, by integrating multi-omics data and other clinical parameters, sensitivity and specificity can be further optimized to build a comprehensive diagnostic platform for early disease screening, prognosis assessment, and treatment efficacy monitoring, thereby providing more reliable support for precision medicine.[16]

Design and Improvement of Novel Nanocarriers in miR-34 Delivery Platforms

To achieve the clinical translation of miR-34 replacement therapy, the design of an efficient delivery system is of paramount importance. Currently, novel delivery systems such as lipid nanoparticles (LNP), exosomes, and polymeric nanoparticles have become research hotspots. These platforms leverage the dual advantages of physical encapsulation and chemical modification to achieve effective protection and targeted delivery of miR-34. Specifically, PEGylation modifications on the surface of nanocarriers can extend circulation half-life, while antibody conjugation or cell-penetrating peptide modifications significantly enhance the carrier's recognition and affinity for specific target cells, thereby enabling miR-34 enrichment in targeted lesion areas.[67]

In recent years, some studies have successfully delivered miR-34 by constructing polymer nanocomplexes in tumor models such as triple-negative breast cancer, observing high-efficiency enrichment in tumor tissues and low off-target distribution effects, providing strong data support for clinical trials.[67] Meanwhile, novel delivery platforms also emphasize environmentally responsive designs, such as utilizing pH-responsive release mechanisms. When carriers enter the tumor microenvironment or virus-infected areas, they can respond to local acidic conditions, triggering controlled release of miR-34 to achieve highly efficient and precise therapeutic effects.

In addition, the carrier design process must fully consider parameters such as particle size, surface charge, and encapsulation efficiency, as these factors directly affect the distribution and retention time of drugs in the body. Overall, the optimized miR-34 nano-delivery system employing multiple strategies not only achieves breakthroughs in targeting and release rate but also plays a key role in reducing toxicity to normal tissues, providing solid technical support for viral prevention and tumor management applications.[67]

Problems Faced by miR-34 Delivery Systems

As a nucleic acid molecule, miR-34 is highly susceptible to degradation by RNase in vivo and may lose its bioactivity due to clearance by the immune system. Therefore, when constructing a miR-34 delivery system, certain chemical modification strategies, such as locked nucleic acid (LNA) modification and 2′-O-methylation, must be employed to significantly enhance its resistance to degradation and stability in the bloodstream.

Meanwhile, nonspecific distribution remains a major challenge affecting the therapeutic efficacy of miR-34. Conventional nanocarriers are often recognized by the mononuclear phagocyte system in vivo, leading to significant accumulation in organs such as the liver and spleen, thereby triggering toxic reactions. Therefore, by rationally regulating the particle size, surface charge, and coating materials of nanoparticles, the risk of uptake by non-target organs can be effectively reduced. Additionally, strategies utilizing tissue-specific promoters and cell-penetrating peptide modifications can further enhance the targeted capture efficiency of carriers, enabling miR-34 to concentrate more in lesion areas rather than distributing randomly throughout the body.[67]

Moreover, the latest research indicates that stealth modifications to the surface of nanocarriers, such as polyethylene glycol (PEG) coating, can effectively evade complement activation and immune system recognition, thereby preserving the integrity and bioactivity of miR-34 during delivery. Animal model experiments also demonstrate that these optimized

delivery systems significantly reduce nonspecific distribution, prolong circulation time in vivo, and increase drug concentration in target tissues, laying the foundation for further safety evaluations and large-scale clinical trials.[67]

Disease Classification Based on Patient miR-34 Expression Profiles

With the continuous development of precision medicine, personalized treatment models based on molecular subtyping are gradually becoming a clinical focus. As a key regulator of cell cycle, apoptosis, and immune response, miR-34 exhibits significant expression variability among patients, providing a theoretical basis for patient stratification using miR-34 expression profiles. By detecting miR-34 expression levels in patient blood or tumor tissues, patients can be categorized into high-expression or low-expression groups, thereby predicting their sensitivity to conventional chemotherapy, immunotherapy, or targeted therapy. For instance, some studies suggest that patients with high miR-34 expression may respond better to chemotherapy regimens, while the low-expression group might benefit more from combined immunotherapy or other novel targeted therapies.

To achieve this goal, building a comprehensive detection process and data analysis platform is crucial. Currently, dynamic expression data of miR-34 can be obtained in real time through RNA sequencing of biopsy samples and liquid biopsy techniques, combined with comprehensive analysis of other molecular markers (such as p53 mutation status, PD-L1 expression levels, etc.) to form a multidimensional classification system. This not only helps more accurately define disease subtypes but also provides quantitative evidence for developing personalized treatment plans, making therapeutic strategies more targeted and effective.

Meanwhile, closely integrating patient typing with clinical follow-up data can establish a closed-loop management system of "detection-treatment-dynamic monitoring". In this system, initial test results guide the formulation of treatment plans, while dynamic monitoring of miR-34 and other key molecular changes during treatment provides real-time feedback for subsequent plan adjustments. Through the accumulation of multicenter, large-sample clinical data, typing criteria and treatment decision-making processes can be further optimized, thereby improving overall treatment efficacy and patient quality of life.[67]

Multicenter Clinical Trial Validates the Clinical Efficacy of Combined Treatment Strategies

In the process of precision therapy, combination therapy strategies have gradually become an important direction for improving treatment efficacy. miR-34, due to its involvement in regulating the cell cycle, apoptosis, and immune responses, can exert synergistic effects when combined with traditional therapeutic drugs (such as small-molecule targeted drugs, chemotherapy drugs, and immune checkpoint inhibitors). For example, miR-34 supplementation can reverse drug resistance in some patients while enhancing tumor immune cell infiltration, thereby increasing the overall response rate to treatment.

To validate the efficacy of the combination therapy strategy, multicenter randomized controlled trials are essential. The design should stratify enrollment based on patients' miR-34 expression levels, such as focusing on populations with low miR-34 expression and poor response to conventional monotherapy. The trial can set multiple key endpoints, including objective response rate (ORR), progression-free survival (PFS), and overall survival (OS), while dynamically monitoring liquid biopsy data to assess real-time changes in miR-34 expression, thereby determining whether the combination therapy can improve patient outcomes.

Moreover, multicenter trials can aggregate diverse clinical data from different institutions and regions, effectively controlling potential biases through rigorous randomization and blinding designs, thereby enhancing the scientific validity and credibility of the trial results. Current preliminary research data indicate that miR-34, when combined with chemotherapy or immunotherapy, can significantly improve treatment response, laying the foundation for large-scale, multicenter trials.[67] During the trial process, researchers should also closely monitor drug interactions, optimal timing of administration, and dose coordination to establish a standardized evaluation system and ultimately develop a comprehensive treatment decision-making pathway centered on miR-34.

Overall, integrating miR-34 expression profile-based molecular subtyping with multicenter clinical trials not only facilitates precise stratification and personalized treatment but also provides a scientific foundation and practical pathway for the clinical translation of combination therapy strategies. In the future, leveraging big data and multi-omics analysis technologies will further refine personalized precision treatment plans targeting miR-34, thereby enhancing its clinical value in viral prevention and tumor management.

Future Research Directions and Challenges

Multi-Omics Integration Constructs a Comprehensive Regulatory Model of miR-34

Multi-omics technologies play a crucial role in comprehensively understanding the miR-34 regulatory network. Transcriptomics (eg, RNA-seq) can be used to identify downstream target genes following changes in miR-34 expression, proteomics can reveal regulatory effects at the protein level, and epigenomics (eg, ChIP-seq) can analyze the regulation of the miR-34 promoter region by transcription factors such as p53.[7] For example, RNA-seq can determine the gene expression profiles affected by miR-34 after viral infection, while ChIP-seq data analysis can assess p53 binding at the miR-34 gene promoter region, thereby revealing how viral infection influences host cell function through the p53-miR-34 axis.[3,18]

Constructing an integrated regulatory model is crucial for predicting potential target genes and signaling pathways. Bioinformatics tools, such as weighted gene co-expression network analysis (WGCNA) and pathway enrichment analysis, can integrate multi-level data to identify key nodes in the miR-34 regulatory network.[13] Additionally, machine learning algorithms, including support vector machines (SVM) and neural networks, can be employed to predict miR-34 target genes and identify critical regulatory factors within the network.[12] These approaches enable researchers to gain a more comprehensive understanding of miR-34's functions and uncover potential therapeutic targets.

However, current model construction also has certain limitations. For example, many studies lack dynamic change data, making it difficult to reflect real-time changes in miR-34 regulation. Additionally, insufficient cross-species validation is another major issue, as the sequence and function of miR-34 may vary across species, limiting the generalizability of research findings.[15] Future studies need to enhance the collection of dynamic data and cross-species validation to improve the accuracy and reliability of models.

Study on the Interaction of miR-34 with Other miRNAs and ceRNA Networks

miR-34 has complex interactions with other miRNAs, such as competitively binding to the same target, known as the ceRNA (competing endogenous RNA) mechanism.[13] ceRNAs competitively bind to miRNAs, thereby relieving the inhibitory effect of miRNAs on their target genes. During viral infection or tumorigenesis, the ceRNA network significantly influences the regulatory effects of miR-34.[68] For example, certain lncRNAs or circular RNAs (circRNAs) may act as ceRNAs, competitively binding to miR-34 and affecting its regulation of target genes (such as Cyclin D1, SIRT1, etc).[5,6]

The ceRNA network may contain positive and negative feedback loops, further complicating the regulatory effects of miR-34. For example, a ceRNA can competitively bind miR-34, upregulating the expression of a target gene, which in turn may promote the expression of the ceRNA, forming a positive feedback loop. Conversely, if the target gene suppresses ceRNA expression, a negative feedback loop is formed. These complex molecular interactions render the regulatory effects of miR-34 highly dynamic and context-dependent.

To elucidate these complex molecular interactions, researchers can employ experimental validation methods such as the Luciferase reporter system, RNA immunoprecipitation (RIP), and RNA pull-down.[38] The Luciferase reporter system can be used to validate the direct binding between miRNA and target genes, while RIP and RNA pull-down can identify RNA molecules that interact with miRNA, thereby discovering novel ceRNAs.

Future research should focus on the characteristics of ceRNA networks in specific disease contexts to enhance the specificity of targeted therapies. For instance, in certain tumors, specific ceRNAs may significantly influence the regulatory effects of miR-34, making targeting these ceRNAs a potential effective therapeutic strategy. Additionally,

studying ceRNA networks can help us understand how viruses evade immune surveillance and achieve persistent infection by modulating host miRNAs and ceRNA networks.[23]

Validate the Function of miR-34 Using CRISPR/Cas9 and Transgenic Models

CRISPR/Cas9 technology, as a powerful gene-editing tool, provides a novel approach for studying the function of miR-34. Using CRISPR/Cas9, researchers can precisely knock out or overexpress the miR-34 gene to investigate its roles in cells and animal models.[15] For instance, by knocking out the miR-34 gene with CRISPR/Cas9, changes in cell cycle regulation, apoptosis, and inflammatory responses can be observed, thereby revealing the role of miR-34 in these processes.

Transgenic mouse models are of great value in studying the function of miR-34, particularly in simulating viral infections or tumor microenvironments.[12] By constructing mouse models with miR-34 overexpression or knockout, the role of miR-34 in viral infections and tumorigenesis can be investigated. For example, in influenza virus infection models, changes in miR-34 expression levels and their effects on viral replication and immune responses can be observed.[11,18] In tumor models, the impact of miR-34 on tumor growth, metastasis, and angiogenesis can be studied.[3,5]

When selecting models, species differences and physiological relevance must be considered to avoid oversimplification or misinterpretation of experimental results. For example, the sequences and target genes of miR-34 may vary among different species, so these factors should be taken into account when choosing animal models. Additionally, animal models should simulate the physiological environment of human diseases as closely as possible to enhance the translational value of research findings.

Novel gene-editing tools, such as base editors, hold potential applications in future miR-34 research. Base editors enable precise single-base editing without inducing DNA double-strand breaks, thereby avoiding the off-target effects that CRISPR/Cas9 may cause. Using base editors, researchers can precisely modify miR-34 target genes to study their impact on cellular functions.

Cross-Species Comparative Study of miR-34 Reveals Universal and Specific Mechanisms

Cross-species comparative studies are of great significance for understanding the evolutionary conservation and functional diversity of miR-34. By comparing the sequences, expression patterns, and target genes of miR-34 in different species, the conservation and adaptive changes of miR-34 during evolution can be revealed.[15] For example, studies have found that members of the miR-34 family exhibit high sequence conservation across different species, indicating their important role in fundamental cellular functions.

Variations in miR-34 sequences across different species and their impact on function are also important aspects of cross-species research. For example, differences in miR-34 sequences between primates and rodents may lead to variations in their target genes and regulatory effects. Studying these differences can enhance our understanding of the specific functions of miR-34 in different species.

Cross-species research is of great significance for the development of broad-spectrum antiviral drugs and has implications for personalized medicine. If miR-34 exhibits antiviral effects across different species, drugs targeting miR-34 may have broad-spectrum antiviral efficacy. Furthermore, understanding the variations in miR-34 among different individuals can provide a basis for personalized medicine.

Currently, cross-species research faces several challenges, such as difficulties in sample acquisition and high technical costs. Obtaining samples from different species may be constrained by ethical and technical limitations, while cross-species comparative studies require technologies like high-throughput sequencing and bioinformatics analysis, which are costly. Future research needs to overcome these challenges to advance cross-species studies.

Exploring the Synergistic Mechanisms of miR-34 Controlled Release, Targeted Delivery, and Combination with Small Molecule Drugs

Existing drug delivery platforms (such as liposomes and nanoparticles) each have their own advantages and disadvantages in miR-34 delivery. Liposomes exhibit good biocompatibility and ease of preparation, but their stability is poor, making them prone to degradation in vivo. Nanoparticles offer higher stability and tunability, but their biocompatibility may be problematic. Therefore, continuous improvement of drug delivery platforms is necessary to enhance the delivery efficiency and safety of miR-34.[66]

The controlled-release system has the potential to prolong the efficacy of miR-34 and reduce side effects. By regulating the rate and timing of drug release, it can maintain effective drug concentrations in the body while decreasing dosing frequency and dosage. For instance, encapsulating miR-34 in biodegradable polymer microspheres enables its sustained release.

Combination therapy strategies, such as combining miR-34 replacement therapy with chemotherapeutic drugs or immune checkpoint inhibitors, can have synergistic effects. miR-34 can enhance the sensitivity of tumor cells to chemotherapeutic drugs and suppress drug resistance.[5,9] When used in combination with immune checkpoint inhibitors, it can boost the immune system's anti-tumor capacity and improve treatment efficacy.

The specific experimental design concept can validate the effectiveness of combination therapy in mouse models. For example, miR-34 replacement therapy can be combined with chemotherapy drugs in tumor-bearing mouse models to observe changes in tumor growth, metastasis, and survival rates, thereby evaluating the efficacy of the combination therapy.

Optimized Approaches to Reduce Immune Side Effects and Enhance Therapeutic Efficacy

Potential immune response issues may arise during miR-34 delivery, such as inducing the release of inflammatory factors or activating the innate immune system. To mitigate immune side effects, the following methods can be employed: modifying the carrier surface, for example, by coating nanoparticle surfaces with PEG, which can reduce interactions with immune cells and lower immunogenicity; adjusting the dosage, where optimizing the administration frequency can decrease the incidence of immune responses; and selecting appropriate delivery routes, such as local administration, which can minimize systemic immune reactions.

The optimized treatment regimen is of great significance in improving patient prognosis and quality of life. By reducing immune-related side effects, it can enhance patient tolerance and improve quality of life. By increasing treatment efficacy, it can prolong patient survival and improve prognosis.

More basic research and preclinical trials are needed to support the safety and feasibility of these optimization measures. For example, cell and animal experiments are required to evaluate the impact of modifying vector surfaces, adjusting dosages, and optimizing dosing frequency on immune responses. Additionally, preclinical trials are necessary to assess the efficacy and safety of optimized treatment regimens.

Multicenter Clinical Data Collection and Integration

Multicenter clinical studies offer the advantages of expanding sample size, enhancing statistical power, and improving the reliability of conclusions. Through multicenter collaboration, more patient samples can be collected, thereby increasing the study's statistical power. Additionally, multicenter studies can reduce the impact of regional and population differences on research outcomes, enhancing the reliability of conclusions.

During the implementation of multicenter clinical studies, ethical, technical, and economic barriers may be encountered, along with corresponding solutions. Ethically, it is necessary to ensure that all participating patients sign informed consent forms and that patient privacy is protected. Technically, a unified data collection and management platform must be established to ensure data quality and consistency. Economically, funding from governments, businesses, and social organizations should be sought to support the study. Standard Operating Procedures (SOPs) play a crucial role in

ensuring data consistency and comparability. By developing detailed operating procedures, the processes of data collection, processing, and analysis can be standardized, thereby ensuring data consistency and comparability.

Advocate for the establishment of an international cooperation network to promote data sharing and optimal resource allocation. Through international collaboration, data and resources from different countries and regions can be shared, thereby accelerating research progress. Additionally, international cooperation can facilitate communication and collaboration among different research teams, enhancing the quality of research.

Establish a Standardized Evaluation System to Promote Clinical Translation

Establishing unified standards is necessary, including miR-34 detection methods, threshold setting, and data analysis procedures. Different detection methods may lead to result discrepancies, necessitating the establishment of uniform testing criteria. Threshold setting directly affects diagnostic accuracy, requiring reasonable thresholds based on large-scale data. Standardizing data analysis procedures can enhance result comparability and reduce human error.

There are successful cases of standardized systems, such as the miRNA marker usage guidelines in certain cancer diagnosis protocols. These guidelines clearly specify miRNA detection methods, threshold settings, and clinical application scopes, providing guidance for clinicians.

It is proposed to establish a dedicated committee or working group to oversee the implementation process of the standards and to update them regularly to reflect the latest scientific research findings. The dedicated committee or working group can be responsible for developing and updating the standards, monitoring their implementation, and addressing questions from clinicians and researchers. Regular updates to the standards can ensure their alignment with the most recent scientific research outcomes.

With the advancement of precision medicine, miR-34 is expected to become an early diagnostic and real-time monitoring tool for more diseases. By detecting the expression levels of miR-34 in patients, diseases can be identified early, and treatment plans can be adjusted based on changes in miR-34. For example, circulating miR-34 in plasma and exosomes has potential for early diagnosis.[14,16] Additionally, miR-34 can be used for prognostic monitoring and real-time efficacy evaluation.[37,69]

Conclusion

Dual Mechanisms of miR-34 in Regulating Host Antiviral Immunity and Tumor Suppression

Recent studies have comprehensively elucidated the molecular mechanisms by which the miR-34 family plays a dual regulatory role in host antiviral defense and tumor suppression. In viral infection control, host cells rapidly initiate immune responses during early infection, with miR-34 serving as a crucial regulator of interferon signaling that plays an irreplaceable role in enhancing immune responses. Specifically, miR-34 promotes phosphorylation of the interferon regulatory factor IRF3 and activates the NF-κB signaling pathway, thereby increasing the expression levels of intracellular antiviral genes, facilitating sufficient production of type I interferons, and inducing the expression of antiviral effector genes.[1] Meanwhile, certain viruses employ strategies to downregulate or suppress miR-34 expression to evade host immune surveillance. This dysregulation not only facilitates viral replication and proliferation within the host but also creates conditions for subsequent pathological changes.[18]

In tumor suppression, miR-34 closely interacts with the p53 signaling pathway, and its expression level and functional status directly influence the fate of tumor cells. Extensive research has shown that miR-34 can induce G1 cell cycle arrest and promote apoptosis by downregulating cell cycle regulators such as Cyclin D1 and directly targeting pro-apoptotic genes like Bax, thereby depriving abnormally proliferating cells of their growth advantage.[18,23] Furthermore, in many tumors, miR-34 expression is commonly reduced due to epigenetic alterations or other interfering mechanisms, which not only weakens the p53-mediated cellular stress response but also promotes tumor initiation and progression. More critically, miR-34 also exerts a shared role in viral infections and tumor progression by regulating the expression of key host factors such as SIRT1 and IL6R. This regulation can both diminish viral-dependent support for cell proliferation and survival and block the transmission of pro-tumor signals, forming a multi-layered, complementary protective

network.[6,24] Overall, while enhancing antiviral immunity, miR-34 also suppresses malignant proliferation, and its dual regulatory mechanism provides a theoretical basis and novel intervention targets for understanding viral defense and tumor management.

miR-34 Influences Pathological Processes by Regulating Cell Cycle, Apoptosis, and Inflammatory Signaling

Further studies elucidated from the cellular molecular mechanism perspective that miR-34 plays a particularly prominent role in regulating the cell cycle and apoptosis processes. In cell cycle regulation, miR-34 can inhibit multiple proteins that promote cell cycle progression, such as Cyclin D1, thereby inducing G1 phase arrest and blocking cellular responses to external proliferation signals. This regulatory process often relies on the transcriptional activation of p53, which upregulates miR-34 expression to promptly direct damaged or abnormal cells into the apoptotic program, effectively preventing potential malignant transformation.[70,71] Additionally, miR-34 directly targets the pro-apoptotic gene Bax, reducing Bax protein translation through complementary binding to its 3′UTR, thereby modulating apoptosis levels. In influenza virus-infected A549 cells, experimental data showed that upregulated miR-34a significantly reduced Bax protein expression, subsequently regulating virus-induced apoptotic responses.[18]

Moreover, inflammatory signaling plays a critical role in both viral infection and the tumor microenvironment. miR-34 regulates the inflammatory cascade by modulating the expression of inflammatory factors such as IL-6 and TNF-α. During viral infection, excessive inflammatory responses often lead to cytokine storms and tissue damage, while moderate inflammation aids in pathogen clearance. In the tumor microenvironment, persistent low-grade inflammation may promote tumor progression. miR-34 exerts regulatory effects in both pathological states by balancing inflammatory signals-suppressing pro-inflammatory factors to reduce excessive responses while maintaining sufficient immune activity when necessary.[24] Overall, miR-34 serves as a pivotal player in three key pathological processes: cell cycle arrest, pro-apoptosis, and inflammation regulation, providing a unified molecular basis for maintaining cellular homeostasis and blocking disease progression.

Drug Delivery, Safety, and Clinical Validation Limitations of miR-34

Although miR-34 has demonstrated significant antiviral and antitumor activity in preliminary basic research and animal experiments, its clinical translation still faces numerous major challenges. Firstly, in terms of drug delivery systems, current mainstream technologies such as nanocarriers, liposomes, and charge complexes have improved the stability and cellular uptake of miR-34 to some extent, but issues like nonspecific distribution, in vivo degradation, and insufficient targeting efficiency persist. For instance, novel polymer nanocomposites can effectively protect miR-34 from rapid enzymatic degradation and facilitate its entry into tumor cells in vitro.[58] However, in actual animal and human trials, the delivery efficiency and targeting capability remain inadequate to fully meet clinical requirements, leading to fluctuating efficacy and potential off-target toxicity risks.

Secondly, safety concerns also limit the further development of miR-34 replacement therapy. Some clinical trials have reported treatment interruptions due to excessive immune and cytotoxic reactions, reflecting that overly strong regulation or uneven distribution in vivo may cause severe immune side effects and nonspecific cellular damage. Additionally, miR-34 exhibits significant differences in expression profiles and microenvironments across various tissues, leading to potential variations in pharmacokinetic characteristics of the same delivery system among different individuals, further complicating safety assessments.

Moreover, there is a significant disparity between current animal models and clinical human subjects, as many notable effects observed in mouse models fail to fully replicate in human clinical trials. This discrepancy stems from multiple factors such as host genetic background, tissue-specific expression, and epigenetic regulation, leading to a lack of unified standards for evaluating the efficacy of miR-34. Meanwhile, existing biomarker detection systems have yet to establish a cross-center, standardized evaluation model, making large-scale, multi-center clinical data integration a major challenge. All these factors collectively limit the comprehensive validation of miR-34's efficacy and safety during clinical translation, necessitating breakthroughs in drug delivery platforms, dosage control, and biomarker monitoring.

Broad Prospects of miR-34-Based Precision Therapy in Viral Prevention and Tumor Management

Despite current limitations in drug delivery and safety, miR-34-based precision therapy demonstrates broad potential in viral prevention and tumor management. Future optimization of miR-34 modifications through molecular design and the development of novel targeted delivery platforms may enable precise regulation of multiple host antiviral pathways, effectively blocking viral replication-particularly by targeting the specific pathogenic mechanisms of different virus types (eg, RNA and DNA viruses). Additionally, combining interferons, cytokines, and other immunomodulatory drugs can enhance host immune responses while creating synergistic effects to further improve overall antiviral efficacy.

In the field of tumor management, with in-depth analysis of miR-34 expression profiles and its regulatory networks, future personalized treatment plans are expected to be developed based on patients' individual molecular characteristics and epigenetic backgrounds. Precision subtyping technologies integrating multi-omics data and AI-driven predictions will advance the application of miR-34 across different tumor types, enabling synergistic strategies combining chemotherapy, targeted therapy, and immune checkpoint inhibition. Meanwhile, the development of novel exosome delivery systems and controlled-release technologies provides technical support for improving the in vivo stability and targeting efficiency of miR-34, which helps reduce systemic toxicity and maximize therapeutic efficacy to some extent.[21,67]

Moreover, interdisciplinary collaboration will become a crucial direction for overcoming current limitations in the future. By deeply integrating bioinformatics, materials science, nanotechnology, and clinical medicine, further elucidating the miR-34 regulatory network and its interactions with other miRNAs and the ceRNA system will help construct a comprehensive molecular regulatory map of diseases. This will not only provide a theoretical basis for novel drug carriers and standardized evaluation systems but also promote the establishment of multicenter, large-scale clinical cohorts and unified efficacy monitoring systems, laying a solid platform for assessing the safety and effectiveness of miR-34 replacement therapy.

Despite current limitations in drug delivery, safety, and clinical validation of miR-34, its dual antiviral and antitumor effects hold promises for future breakthroughs. Through technological innovation and interdisciplinary integration, the development of precise and efficient therapeutic models will become feasible. This approach not only has the potential to establish a critical defense in viral prevention and treatment but also offers personalized and precise therapeutic strategies in tumor management. It will drive antiviral and antitumor therapies to higher levels, bringing transformative progress to numerous clinical challenges.

Abbreviations

ALV-J, Avian Leukosis Virus Subgroup J; AR, Allergic rhinitis; ceRNA, Competing endogenous RNA; CRS, Chronic rhinosinusitis; circRNAs, circular RNAs; DENV, Dengue Virus; EBV, Epstein-Barr Virus; GEO: Gene Expression Omnibus; HBV, Hepatitis B Virus; HCMV, Human cytomegalovirus; HPV. Human Papillomavirus; IL-6R, IL-6 receptor; INF, Initiates interferon; JEV, Japanese Encephalitis Virus; KSHV, Kaposi's Sarcoma-Associated Herpesvirus; LNA, Locked nucleic acid; LNP, Lipid nanoparticles; NAFLD, Non-alcoholic fatty liver disease; miRNAs, MicroRNAs; NGS, Next-generation sequencing; ORR, Objective response rate; OS, Overall survival; PEG, Polyethylene glycol; PFS, Progression-free survival; P-IRF3, IRF3 phosphorylation; POCT, Point-of-care testing; qPCR, Real-time PCR; RSV, Respiratory Syncytial Virus; RIP, RNA immunoprecipitation; SOPs, Standard Operating Procedures; SVM, Support vector machines; 3'- UTR, 3'- untranslated region; WGCNA, Weighted gene co-expression network analysis; WNV, West Nile Virus; WSSV, White Spot Syndrome Virus.

Data Sharing Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics Approval and Consent to Participate

This study did not involve human or animal subjects, and thus, no ethical approval was required. The study protocol adhered to the guidelines established by the journal.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Smith L, Jeng S, McWeeney K, et al. A MicroRNA screen identifies the wnt signaling pathway as a regulator of the interferon response during flavivirus infection. *J Virol*. 2017;91(8):e02388–16. doi:10.1128/JVI.02388-16
2. Zhao L, Duan J, Cao A, et al. Activating Wnt1/β-Catenin signaling pathway to restore Otx2 expression in the dopaminergic neurons of ventral midbrain. *Exp Neurol*. 2025;388:115216. doi:10.1016/j.expneurol.2025.115216
3. Villanueva F, Araya H, Briceño P, et al. The cancer-related transcription factor RUNX2 modulates expression and secretion of the matricellular protein osteopontin in osteosarcoma cells to promoteadhesion to endothelial pulmonary cells and lung metastasis. *J Cell Physiol*. 2019;234 (8):13659–13679. doi:10.1002/jcp.28046
4. Jia Q. Yuejun Fu. microRNA-34-5p encoded by Spodoptera frugiperda regulates the replication and infection of Autographa californica multiple nucleopolyhedrovirus by targetingodv-e66, ac78 and ie2. *Pest Manag Sci*. 2022;78(12):5379–5389. doi:10.1002/ps.7160
5. Dehghan R, Rezvan N, Azizi F, et al. A promising effect of zerumbone with improved anti-tumor-promoting inflammation activity of miR-34a in colorectal cancer cell lines. *Mol Biol Rep*. 2021;48(1):203–218. doi:10.1007/s11033-020-06035-9
6. Shi L, Fan B, Chen D, et al. Human cytomegalovirus protein UL136 activates the IL-6/STAT3 signal through MiR-138 and MiR-34c in gastric cancer cells. *Int J Clin Oncol*. 2020;25(11):1936–1944. doi:10.1007/s10147-020-01749-z
7. Hussen M, Ahmadi G, Marzban H, et al. The role of HPV gene expression and selected cellular MiRNAs in lung cancer development. *Microb Pathogenesis*. 2021;150:104692. doi:10.1016/j.micpath.2020.104692
8. Zhang L, Su W, Liu S, et al. Recent progresses in electrochemical DNA biosensors for MicroRNA detection. *Phenomics*. 2022;2(1):18–32. doi:10.1007/s43657-021-00032-z
9. Salehi M, Torogi F, Jalilian A, et al. The potential role of curcumin as a regulator of microRNA in colorectal cancer: a systematic review. *MicroRNA*. 2025;14(1):42–48. doi:10.2174/0122115366304114240904051429
10. Sheraz M, Kanak M, Hasan M, et al. Use of Flaviviral genetic fragments as a potential prevention strategy for HIV-1 Silencing. *J Infect Developing Countries*. 2016;10(8):870–879. doi:10.3855/jidc.7386
11. Preusse M, Schughart K, Pessler F. Host genetic background strongly affects pulmonary microRNA expression before and during Influenza A virus infection. *Front Immunol*. 2017;8:246. doi:10.3389/fimmu.2017.00246
12. Liang Z, Yang Y, Sun X, et al. Integrated analysis of MicroRNA and mRNA expression profiles in the fat bodies of MbMNPV-infected Helicoverpa armigera. *Viruses*. 2022;15(1):19. doi:10.3390/v15010019
13. Tanabe S, Quader S, Cabral H, et al. Master regulators of causal networks in intestinal- and diffuse-type gastric cancer and the relation to the RNA virus infection pathway. *Int J Mol Sci*. 2024;25(16):8821. doi:10.3390/ijms25168821
14. Nagandla K, Lin H, Chitra E, et al. Role of microRNAs as biomarkers of cervical carcinogenesis: a systematic review. *Obstetrics Gynecol Sci*. 2021;64(5):419–436. doi:10.5468/ogs.21123
15. Lai Y-W, Chu S-Y, Jian-Chiuan L, et al. Visualization of endogenous type I TGF-β receptor baboon in the drosophila brain. *Sci Rep*. 2020;10 (1):5132. doi:10.1038/s41598-020-61950-y

16. Nyström S, Hultberg J, Blixt E, et al. Plasma levels of mir-34a-5p correlate with systemic inflammation and low naïve CD4 T cells in common variable immunodeficiency. *J Clin Immunol*. 2023;44(1):21. doi:10.1007/s10875-023-01618-0
17. Feili X, Shuang W, Weiwei Y, et al. MicroRNA-34a-5p inhibits liver fibrosis by regulating TGF-β1/Smad3 pathway in hepatic stellate cells. *Cell Biol Int*. 2018;42(10):1370–1376. doi:10.1002/cbin.11022
18. Fan N, Wang J. MicroRNA 34a contributes to virus-mediated apoptosis through binding to its target gene Bax in influenza A virus infection. *Biomed Pharmacother*. 2016;83:1464–1470. doi:10.1016/j.biopha.2016.08.049
19. Othumpangat S, Beezhold DH, Umbright CM, Noti JD. Influenza virus-induced novel miRNAs regulate the STAT pathway. *Viruses*. 2021;13(6):967. doi:10.3390/v13060967
20. Beg MS, Brenner AJ, Sachdev J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Invest New Drugs*. 2017;35(2):180–188. doi:10.1007/s10637-016-0407-y
21. Hong DS, Kang YK, Borad M, et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *Br J Cancer*. 2020;122(11):1630–1637. doi:10.1038/s41416-020-0802-1
22. Wong MY, Yu Y, Walsh WR, et al. microRNA-34 family and treatment of cancers with mutant or wild-type p53 (Review). *Int J Oncol*. 2011;38(5):1189–1195. doi:10.3892/ijo.2011.970
23. Zhang L, Liao Y, Tang L. MicroRNA-34 family: a potential tumor suppressor and therapeutic candidate in cancer. *J Exp Clin Cancer Res*. 2019;38(1):53. doi:10.1186/s13046-019-1059-5
24. Jia Y, Miyamoto S, Soda Y, et al. Extremely low organ toxicity and strong antitumor activity of miR-34-regulated oncolytic coxsackievirus B3. *Mol Ther Oncolytics*. 2019;12:246–258. doi:10.1016/j.omto.2019.01.003
25. Huang P-S, Liao C-J, Huang Y-H, et al. Functional and clinical significance of dysregulated microRNAs in liver cancer. *Cancers*. 2021;13(21):5361. doi:10.3390/cancers13215361
26. She L, Shi M, Cao T, et al. Wolbachia mediates crosstalk between miRNA and Toll pathways to enhance resistance to dengue virus in Aedes aegypti. *PLoS Pathogens*. 2024;20(6):e1012296. doi:10.1371/journal.ppat.1012296
27. Jun H, Kwack K, Lee H, et al. Association between TP53 genetic polymorphisms and the methylation and expression of miR-34a, 34b/c in colorectal cancer tissues. *Oncol Lett*. 2019;17(5):4726–4734. doi:10.3892/ol.2019.10092
28. Perri P, Ponzoni M, Corrias MV, et al. A focus on regulatory networks linking MicroRNAs, transcription factors and target genes in neuroblastoma. *Cancers*. 2021;13(21):5528. doi:10.3390/cancers13215528
29. Wang W, Du Y, Datta S, et al. Targeting the MYCN-MDM2 pathways for cancer therapy: are they druggable ? *Genes Dis*. 2023;12(2):101156. doi:10.1016/j.gendis.2023.101156
30. Jianliang L, Zhang Z, Pan L, et al. MicroRNA-34/449 family and viral infections. *Virus Res*. 2019;260:1–6. doi:10.1016/j.virusres.2018.11.001
31. Anastasiadou E, Stroopinsky D, Alimperti S, et al. Epstein-Barr virus-encoded EBNA2 alters immune checkpoint PD-L1 expression by down-regulating miR-34a in B-cell lymphomas. *Leukemia*. 2019;33(1):132–147. doi:10.1038/s41375-018-0178-x
32. Blümke J, Bauer M, Vaxevanis C, et al. Identification and characterization of the anti-viral interferon lambda 3 as direct target of the Epstein-Barr virus microRNA-BART7-3p. *Oncoimmunology*. 2023;12(1):2284483. doi:10.1080/2162402X.2023.2284483
33. Du X, Yang Y, Xiao G, et al. Respiratory syncytial virus infection-induced mucus secretion by down-regulation of miR-34b/c-5p expression in airway epithelial cells. *J Cell Mol Med*. 2020;24(21):12694–12705. doi:10.1111/jcmm.15845
34. Liu D, Tang Z, Bajinka O, et al. miR-34b/c-5p/CXCL10 axis induced by RSV infection mediates a mechanism of airway hyperresponsive diseases. *Biology*. 2023;12(2):317. doi:10.3390/biology12020317
35. Martinez-Espinoza I, Banos-Lara MDR, Guerrero-Plata A. The importance of miRNA identification during respiratory viral infections. *J Cell Immunol*. 2021;3(4):207–214. doi:10.33696/immunology.3.101
36. Cui Y, Wan H, Zhang X. miRNA in food simultaneously controls animal viral disease and human tumorigenesis. *Mol Ther Nucleic Acids*. 2021;23:995–1006. doi:10.1016/j.omtn.2021.01.011
37. Li Z, Luo Q, Xu H, et al. MiR-34b-5p suppresses melanoma differentiation-associated Gene 5 (MDA5) signaling pathway to promote avian leukosis virus subgroup J (ALV-J)-infected cells proliferation and ALV-J replication. *Front Cell Infect Microbiol*. 2017;7:17. doi:10.3389/fcimb.2017.00017
38. Yao L, Zhang Q, Gao X, et al. Macrophage-mediated delivery of miR-34a-5p-nanoparticles for pathogenic inhibition of Kaposi's Sarcoma-associated Herpesvirus. *J Med Virol*. 2025;97(3):e70298. doi:10.1002/jmv.70298
39. Wu S, Wu Z, Xu H, et al. miR-34a-5p inhibits the malignant progression of KSHV-infected SH-SY5Y cells by targeting c-fos. *Peer J*. 2022;10:e13233. doi:10.7717/peerj.13233
40. Wong RR, Abd-Aziz N, Affendi S, et al. Role of microRNAs in antiviral responses to dengue infection. *J Biomed Sci*. 2020;27(1):4. doi:10.1186/s12929-019-0614-x
41. Mishra K, Hossain M, Umar M, et al. DDX3-mediated miR-34 expression inhibits autophagy and HBV replication in hepatic cells. *J Viral Hepatitis*. 2023;30(4):327–334. doi:10.1111/jvh.13799
42. Chen J, Wu X, Cai B, et al. Circulating microRNAs as potential biomarkers of HBV infection persistence. *Infect Genet Evol*. 2017;54:152–157. doi:10.1016/j.meegid.2017.06.023
43. Jin Y, Guan Z, Wang X, et al. ALA-PDT promotes HPV-positive cervical cancer cells apoptosis and DCs maturation via miR-34a regulated HMGB1 exosomes secretion. *Photodiagnosis Photodyn Ther*. 2018;24:27–35. doi:10.1016/j.pdpdt.2018.08.006
44. Geng D, Song X, Ning F, et al. MiR-34a inhibits viability and invasion of human papillomavirus-positive cervical cancer cells by targeting E2F3 and regulating survivin. *Int J Gynecol Cancer*. 2015;25(4):707–713. doi:10.1097/IGC.0000000000000399
45. Zhang R, Su J, Xue SL, et al. HPV E6/p53 mediated down-regulation of miR-34a inhibits Warburg effect through targeting LDHA in cervical cancer. *Am J Cancer Res*. 2016;6(2):312–320.
46. Sadri J, Shojaie L, Akhlagh A, et al. Cell death pathways and viruses: role of microRNAs. *Mol Ther Nucleic Acids*. 2021;24:487–511. doi:10.1016/j.omtn.2021.03.011
47. Wu J, Li X, Li D, et al. MicroRNA-34 Family Enhances Wound Inflammation by Targeting LGR4. *J Invest Dermatol*. 2020;140(2):465–476.e11. doi:10.1016/j.jid.2019.07.694
48. N.j S, Tiriveedhi V, Subramanian V, et al. Hepatitis C virus mediated changes in miRNA-449a modulates inflammatory biomarker YKL40 through components of the NOTCH signaling pathway. *PLoS One*. 2012;7(11):e50826. doi:10.1371/journal.pone.0050826

49. Huang Z, Kaller M, Hermeking H. CRISPR/Cas9-mediated inactivation of miR-34a and miR-34b/c in HCT116 colorectal cancer cells: comprehensive characterization after exposure to 5-FU reveals EMT and autophagy as key processes regulated by miR-34. *Cell Death Differ*. 2023;30(8):2017–2034. doi:10.1038/s41418-023-01193-2
50. Wang Y, Wu Z, Hu L. The regulatory effects of metformin on the [SNAIL/miR-34]: [ZEB/miR-200] system in the epithelial-mesenchymal transition (EMT) for colorectal cancer (CRC). *Eur J Pharmacol*. 2018;834:45–53. doi:10.1016/j.ejphar.2018.07.006
51. Tokumaru Y, Katsuta E, Oshi M, et al. High expression of miR-34a associated with less aggressive cancer biology but not with survival in breast cancer. *Int J Mol Sci*. 2020;21(9):3045. doi:10.3390/ijms21093045
52. Peng D, Wang H, Li L, et al. miR-34c-5p promotes eradication of acute myeloid leukemia stem cells by inducing senescence through selective RAB27B targeting to inhibit exosome shedding. *Leukemia*. 2018;32(5):1180–1188. doi:10.1038/s41375-018-0015-2
53. Fabbri M, Bottoni A, Shimizu M, et al. Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. *JAMA*. 2011;305(1):59–67. doi:10.1001/jama.2010.1919
54. Achari C, Winslow S, Ceder Y, et al. Expression of miR-34c induces G2/M cell cycle arrest in breast cancer cells. *BMC Cancer*. 2014;14:538. doi:10.1186/1471-2407-14-538
55. Wen L, Yunfei W, Ruifang L, et al. MicroRNA-34a: potent tumor suppressor, cancer stem cell inhibitor, and potential anticancer therapeutic. *Front Cell Develop Biol*. 2021;9:640587. doi:10.3389/fcell.2021.640587
56. Oh-Hohenhorst S, Tobias L. Role of metastasis-related microRNAs in prostate cancer progression and treatment. *Cancers*. 2021;13(17):4492. doi:10.3390/cancers13174492
57. Sarah S, Giovanna C, Irene P, et al. The biological function of MicroRNAs in bone tumors. *Int J Mol Sci*. 2022;23(4):2348. doi:10.3390/ijms23042348
58. Mahrokh D, Bahar A, Dian D, et al. miR-34 as a critical regulator in ovarian cancer. *Current Mol Med*. 2025. doi:10.2174/0115665240345216241120093846
59. Ocadiz-Delgado R, Lizcano-Meneses S, Trejo-Vazquez JA, et al. Circulating miR-15b, miR-34a and miR-218 as promising novel early low-invasive biomarkers of cervical carcinogenesis. *APMIS*. 2021;129(2):70–79. doi:10.1111/apm.13093
60. Zhu Y, Han Y, Tian T, et al. MiR-21-5p, miR-34a, and human telomerase RNA component as surrogate markers for cervical cancer progression. *Pathol Res Pract*. 2018;214(3):374–379. doi:10.1016/j.prp.2018.01.001
61. Yuki M, Kei K-M, Tsukasa O, et al. MYC causes multiple myeloma progression via attenuating TP53-induced MicroRNA-34 expression. *Genes*. 2022;14(1):100. doi:10.3390/genes14010100
62. Tikam D, Reya P, Ravi B, et al. Genetic and epigenetic regulation of non-coding RNAs: implications in cancer metastasis, stemness and drug resistance. *Pathol Res Pract*. 2024;266:155728. doi:10.1016/j.prp.2024.155728
63. Maria P, Loretta B, Barbara B. The ethanolic extract of Corylus avellana L. drives a microRNA-based cytotoxic effect on HepG2 hepatocarcinoma cells. *Natural Product Res*. 2023;39(4):674–681. doi:10.1080/14786419.2023.2287170
64. Amal M, Ahmed E-D, Eman A-H, et al. NAFLD mark: an accurate model based on microRNA-34 for diagnosis of non-alcoholic fatty liver disease patients. *J Genet Eng Biotechnol*. 2021;19(1):157. doi:10.1186/s43141-021-00257-5
65. Hadeel A-R, Sami G, Ahmad A. The potential role of circulating MicroRNAs in male rat infertility treated with Kaempferia parviflora. *Evid Based Complement Alternat Med*. 2021;2021:9622494. doi:10.1155/2021/9622494
66. Antonella L, Carlo C, Bruno F, et al. The emerging role of MicroRNAs in nasal inflammatory diseases and tumors: from bench to bedside. *Genes*. 2025;16(3):295. doi:10.3390/genes16030295
67. Djafari J, Fernández-Lodeiro J, Santos M, et al. Study and preparation of multifunctional Poly(L-Lysine)@Hyaluronic acid nanopolyplexes for the effective delivery of tumor suppressive MiR-34a intoTriple-negative breast cancer cells. *Materials*. 2020;13(23):387–398. doi:10.3390/ma13235309
68. Kumari B, Jain P, Das S, et al. Dynamic changes in global microRNAome and transcriptome reveal complex miRNA-mRNA regulated host response to Japanese Encephalitis Virus in microglial cells. *Sci Rep*. 2016;6:20263. doi:10.1038/srep20263
69. Shekhar R, Priyanka P, Kumar P, et al. The microRNAs miR-449a and miR-424 suppress osteosarcoma by targeting cyclin A2 expression. *J Biol Chem*. 2019;294(12):4381–4400. doi:10.1074/jbc.RA118.005778
70. Barbu MG, Condrat CE, Thompson DC, et al. MicroRNA involvement in signaling pathways during viral infection. *Front Cell Dev Biol*. 2020;8:143. doi:10.3389/fcell.2020.00143
71. Feng H, Ge F, Du L, et al. MiR-34b-3p represses cell proliferation, cell cycle progression and cell apoptosis in non-small-cell lung cancer (NSCLC) by targeting CDK4. *J Cell Mol Med*. 2019;23(8):5282–5291. doi:10.1111/jcmm.14404