

Review Article

KSHV-Encoded MicroRNAs: Lessons for Viral Cancer Pathogenesis and Emerging Concepts

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The human genome contains microRNAs (miRNAs), small noncoding RNAs that orchestrate a number of physiologic processes through regulation of gene expression. Burgeoning evidence suggests that dysregulation of miRNAs may promote disease progression and cancer pathogenesis. Virus-encoded miRNAs, exhibiting unique molecular signatures and functions, have been increasingly recognized as contributors to viral cancer pathogenesis. A large segment of the existing knowledge in this area has been generated through characterization of miRNAs encoded by the human gamma-herpesviruses, including the Kaposi's sarcoma-associated herpesvirus (KSHV). Recent studies focusing on KSHV miRNAs have led to a better understanding of viral miRNA expression in human tumors, the identification of novel pathologic check points regulated by viral miRNAs, and new insights for viral miRNA interactions with cellular ("human") miRNAs. Elucidating the functional effects of inhibiting KSHV miRNAs has also provided a foundation for further translational efforts and consideration of clinical applications. This paper summarizes recent literature outlining mechanisms for KSHV miRNA regulation of cellular function and cancer-associated pathogenesis, as well as implications for interactions between KSHV and human miRNAs that may facilitate cancer progression. Finally, insights are offered for the clinical feasibility of targeting miRNAs as a therapeutic approach for viral cancers.

1. Introduction

MicroRNAs (miRNAs) are small (19–24 nucleotides in length), noncoding RNAs that bind both untranslated and coding regions of target mRNAs, marking them for degradation or posttranscriptional modification. The biogenesis of miRNAs begins in the nucleus where RNA polymerase II generates primary miRNA (pri-miRNA) transcripts. Subsequently, pri-miRNAs are processed by the RNase III enzyme Drosha, generating precursor miRNAs (pre-miRNAs). Nuclear pre-miRNAs are then transported to the cytoplasm by exportin/Ran-GTP where they are cleaved by the cytoplasmic RNase III enzyme Dicer, generating mature miRNAs which are incorporated into the RNA-induced

silencing complex (RISC). This directs RISC to the target mRNA based on sequence complementarity, resulting in gene silencing [1, 2]. miRNAs are encoded by many different organisms and regulate a variety of cellular processes, including cell proliferation, apoptosis, differentiation, and development [3].

Viruses encode miRNAs whose sequences and functions are unique from human miRNAs and miRNAs encoded by human herpesviruses have been increasingly well characterized [4]. Herpesviruses are enveloped, double-stranded DNA viruses, and the human gamma-herpesviruses, Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesviruses (KSHV), are the etiologic agents of several forms of cancer. As with other herpesviruses, the KSHV lifecycle involves

TABLE 1: Overview of KSHV miRNAs regulatory functions and targets.

| Functions | KSHV miRNAs | Validated targets | References |
|--|---------------------|-----------------------|--------------|
| KSHV entry | miR-K12-1 | — | [20] |
| | miR-K12-9 | — | [20] |
| | miR-K12-11 | BACH-1 | [20, 22, 23] |
| Induction of reactive nitrogen species (RNS) | miR-K12-1, 9 and 11 | — | [20] |
| Endothelial cell reprogramming | miR-K12-6 and 11 | MAF | [24] |
| | miR-K12-7 | RTA | [37] |
| KSHV gene expression | miR-K12-9 | RTA/BCLAF1 | [38, 39] |
| | miR-K12-4 | Rbl2 | [40] |
| | miR-K12-1 | I κ B α | [41] |
| | miR-K12-3 | Nuclear factor I/B | [42] |
| | miR-K12-5 | BCLAF1 | [39] |
| | miR-K12-11 | IKK ϵ | [43] |
| | miR-K12-10 and 12 | — | [18] |
| Cytokine secretion | miR-K12-3 and 7 | — | [44] |
| | miR-K12-11 | C/EBP β | [45] |
| | miR-K12-10 | TWEAKR | [46] |
| Immune escape | miR-K12-7 | MICB | [47] |
| Cell survival | miR-K12-10 | TWEAKR | [46] |
| | miR-K12-1 | p21 | [48] |

two distinct phases: latent and lytic. During latency (the predominant phase in the majority of infected cells) only a limited number of viral genes are expressed. Provocation by a variety of stimuli induces lytic replication, resulting in virion assembly and release of infectious viral particles [5]. Existing data suggest that the oncogenic potential of KSHV is largely dependent upon genes expressed during latency, although “low level” replication occurring in a small minority of cells is also critical for infection of naïve cell targets, maintenance of the KSHV reservoir, and tumor pathogenesis [6–8]. Cancers caused by KSHV, including multicentric Castleman’s disease (MCD), primary effusion lymphoma (PEL), and Kaposi’s sarcoma (KS), arise preferentially in the setting of immune suppression as seen with HIV infection and provision of immunosuppressive medications in the context of solid organ transplantation [9–11].

Thus far, 12 KSHV pre-miRNAs, encoding 18 mature miRNAs, have been identified [12–14]. Within the KSHV genome, miRNAs are located in the KSHV latency-associated region (KLAR). Other proteins encoded within the KLAR are critical for maintenance of the viral episome and KSHV oncogenesis, including the latency-associated nuclear antigen (LANA), virus-encoded Cyclin (vCyclin), viral FLICE inhibitory protein (vFLIP), and kaposin (K12). 10 of 12 miRNAs (miR-K12-1~9 and 11) are located within the intron of K12; miR-K12-10 is located within the open reading frame of K12 A/C and the 3’UTR of K12 B, and miR-K12-12 is located within the 3’UTR of K12 [12–14]. Given their location within the KLAR, it follows that KSHV miRNAs facilitate maintenance of latent viral gene expression and the oncogenic potential of these genes. This paper will summarize recent findings regarding the expression of KSHV miRNAs and their regulatory functions and elaborate on

emerging mechanistic concepts in this field. We will also review several recently published studies offering insight into the feasibility of targeting miRNAs for therapeutic purposes. For an overview of KSHV miRNA targets and their putative functions, see Table 1.

2. Expression Patterns for KSHV miRNAs

Expression of KSHV miRNAs has been demonstrated within latently infected primary human cells and KSHV-infected PEL cells [12–16]. PEL cell lines exhibit significant conservation (~99.6%) of KSHV-miRNA expression [17], although one group recently reported that miR-K12-9 may be mutationally inactivated in different PEL lines [18]. Moreover, expression levels for individual KSHV miRNAs vary considerably [13]. Phylogenetic analyses of KSHV miRNA sequences from clinical samples of KS and MCD patients of divergent geographic backgrounds reveal the existence of 2 major sequence clusters, referred to as the major (A/C) and variant (B/Q) clusters [17]. Further analyses of the pre-miRNA sequences show that some KSHV miRNAs are highly conserved (such as miR-K12-1, 3, 8, 10, 11, and 12), while others (including miR-K12-2, 4, 5, 6, 7, and 9) exhibit sequence alterations likely affecting their processing and function, although this hypothesis requires additional confirmation [17]. In addition, one study found distinct polymorphisms within pri-miRNAs, pre-miRNAs, or mature miRNAs encoded by circulating KSHV in a European patient cohort, and some of these polymorphisms may affect mature miRNA processing and associate with KS risk [19]. Collectively, these data indicate that individual KSHV miRNAs may regulate KSHV pathogenesis in a disease-specific manner, and that they may exhibit cell type-specific functions within

the tumor microenvironment. Identification of viral and cellular factors governing these differences should illuminate additional mechanisms and help determine whether screening for miRNA polymorphisms can be used to quantify one's risk for developing KSHV-associated tumors.

3. KSHV miRNA Regulation of Virus Entry

We have reported that miR-K12-1, 9, and 11 increase macrophage and endothelial cell (EC) susceptibility to KSHV entry and latent gene expression through upregulation of xCT [20], an inducible amino acid exchanger and fusion-entry receptor for the virus [21]. One mechanism for these observations involves upregulation of xCT through miR-K12-11 repression of BACH-1, a negative transcription regulator of xCT [20]. These findings are consistent with earlier reports revealing direct targeting of BACH-1 by miR-K12-11 [22, 23]. Mechanisms for regulation of xCT expression by miR-K12-1 and 9 have not yet been elucidated. One related report noted involvement of KSHV miRNAs in endothelial cell reprogramming through repression of the cellular transcription factor Maf (cMaf) [24]. cMaf also serves as a negative transcription regulator for xCT, so we have speculated that multiple KSHV miRNAs, through cooperative mechanisms, facilitate KSHV entry [20]. Whether KSHV miRNAs regulate expression and/or function of other cellular receptors for KSHV, including DC-SIGN and integrins [25–29], has not been established. Increased cell permissiveness for KSHV entry following initial infection and miRNA expression may represent an evolutionary mechanism for KSHV promotion of its own persistence. Supporting this hypothesis, several reports have shown that a significant proportion of KSHV-infected tumor cells contain multiple viral clones [30–32]. Moreover, downregulation of MHC Class I (MHC-I) in KSHV-infected cells is directly proportional to intracellular KSHV episome copy number [33], implying that an increase in intracellular viral copies reduces KSHV epitope presentation to CD8⁺T cells. In addition, precedence exists for human miRNAs regulation of virus entry. For example, one group has demonstrated that several human miRNAs regulate monocyte/macrophage susceptibility to HIV infection [34, 35]. Another group reported that miR-23b inhibits Rhinovirus 1B (RV1B) entry through targeting of the very low density lipoprotein receptor [36]. Therefore, it is plausible that KSHV and human miRNAs cooperatively regulate surface determinants of cell targeting by KSHV and other viruses. Furthermore, it is likely that KSHV and other viral miRNAs regulate secretion of microenvironmental factors by infected cells that influence susceptibility of neighboring cells to virus entry. We have shown that KSHV miRNA induce secretion of reactive nitrogen species (RNS), and that inhibition of the enzymatic generation of RNS reduces cell susceptibility to KSHV infection [20]. Given that both BACH-1 and cMaf are negative transcription regulators for genes containing antioxidant response elements (AREs) in their promoters, and since several genes involved in production of reactive nitrogen- and oxygen-based species (RNS and ROS, resp.) contain AREs, we hypothesize that KSHV miRNAs regulation of BACH-1 and cMaf influences

a more complex network of genes to generate tumor-promoting RNS and ROS while simultaneously protecting KSHV-infected cells from oxidative damage inflicted by these species [20]. These data have implications for development of therapeutic strategies to reduce KSHV infection in the tumor microenvironment and, therefore, KS progression [6–8].

4. KSHV miRNA Regulation of Viral Gene Expression

Maintenance of latent KSHV infection, coordinated with lytic reactivation within a small subset of infected cells, is critical for simultaneous promotion of KSHV persistence and dissemination. Studies published recently indicate a role for KSHV miRNAs in the regulation of this latent-lytic “switch”. miR-K12-7 and 9 bind and repress transcription of the KSHV immediate-early gene ORF50 which encodes the replication and transcription activator (RTA) [37, 38]. RTA activation is critical for the initiation of lytic replication of the virus [37, 38]. miR-K12-4 represses expression of the retinoblastoma (Rb)-like protein 2 (Rbl2), a known repressor of DNA methyl transferases (DNMT)-1, -3a and -3b. Increased activity of these DNMTs maintains methylation of the RTA promoter and suppresses its expression [40]. Furthermore, miR-K12-1 targeting of I κ B α , an inhibitor of NF- κ B complexes, promotes NF- κ B-dependent viral latency and cell survival [41]. miR-K12-3 also promotes KSHV latency through targeting of nuclear factor I/B, an activator of the RTA promoter [42]. Conversely, miR-K12-5 and 9 repress the Bcl-2-associated factor (BCLAF1), resulting in an increase in lytic replication, albeit through mechanisms that have yet to be defined [39]. One recent study indicates that miR-K12-11 targets and downregulates IKK ϵ , a signaling intermediate shown previously to facilitate lytic reactivation of KSHV from latently infected cells [43]. Another report revealed upregulation of two miRNAs, miR-K12-10, and 12, during chemical induction of KSHV lytic reactivation [18], but whether these miRNAs play an active role in regulation of the lytic switch for KSHV remains to be determined. Collectively, these data support the notion that KSHV miRNAs function primarily to maintain viral latency, congruous with their location within the KLAR. This is also supported by recent work revealing that cells infected with KSHV deletion mutants lacking KSHV miRNAs exhibit increased expression of lytic viral genes, including ORF50 [40, 41].

5. KSHV miRNA Regulation of Cytokine Responses, Immune Recognition, and Cell Survival

Several factors secreted by KSHV-infected cells (and other cells found within the tumor microenvironment), including VEGF, IL-8, IL-6, IL-10, IL-1 β , and TNF- α , support KSHV-associated pathogenesis through complimentary mechanisms involving interference or augmentation of cellular functions relevant to cancer pathogenesis [49]. More

specifically, IL-6 and IL-10 collectively promote growth of KSHV-infected tumor cells, angiogenesis and suppression of T-cell activation [50–53]. We have demonstrated that KSHV-miRNAs induce IL-6 and IL-10 secretion by murine macrophages and human myelomonocytic cells, and that this is accomplished, in part, through miR-K12-3 and 7 repression of a dominant-negative isoform of *C/EBP β* which serves as a transcriptional repressor of IL-6 and IL-10 [44]. However, it remains unclear whether this effect results from direct targeting of *C/EBP β* by these miRNAs or an indirect effect within a more complex regulatory network. Furthermore, and whether these events occur following *de novo* infection of human primary cells is unknown. A more recent study reported that miR-K12-11 induces splenic B-cell expansion and KSHV-associated lymphomagenesis through direct targeting of *C/EBP β* [45]. It is plausible that lymphomagenesis in this model is dependent on miRNA regulation of cytokine responses through targeting of *C/EBP β* , congruous with existing clinical data suggesting a role for cytokines in PEL pathogenesis [54]. Another study reveals that KSHV-encoded ORF57 competes with human miRNAs for binding of transcripts for both human IL-6 (hIL-6) and the KSHV-encoded viral homolog for IL-6 (vIL-6) [55]. In doing so, ORF57 impairs hIL-6 and vIL-6 RNA association with human miRNA-specified RISCs, thereby stabilizing IL-6 RNA.

Existing data further indicate that KSHV miRNAs may facilitate conditional *suppression* of cytokine responses and immune recognition. miR-K12-10 repression of the tumor necrosis factor-like weak inducer of apoptosis receptor (TWEAKR) in primary human ECs results in decreased expression of IL-8 and monocyte chemoattractant protein 1 (MCP-1) which are normally induced following TWEAKR interactions with its cognate ligand, TWEAK [46]. In addition, one group has found that KSHV miRNAs repress expression of the stress-induced natural killer (NK) cell ligand, MICB, thereby permitting escape of KSHV-infected cells from NK cell recognition and killing [47]. It seems likely that KSHV miRNA regulation of cytokine responses and immune evasion is a finely coordinated effort hinging on intracellular and/or exogenous microenvironmental signals that are cell type-specific.

Maintenance of viability for KSHV-infected cells depends, in part, on KSHV regulation of cellular pathways promoting cell survival and antiapoptotic signaling. Several studies indicate that KSHV miRNAs are involved in this process. Microarray analyses using cells stably expressing KSHV-encoded miRNAs revealed that 3'UTRs of select cell proliferation/apoptosis-associated genes, including SPP1, S100A2, and PRG1, are likely targeted by multiple KSHV miRNAs [56]. However to our knowledge, specific target sequences within the 3'UTRs for these genes have not yet been validated, and functional correlates for KSHV miRNAs targeting these genes have not been determined. As mentioned previously, miR-K12-10 represses TWEAKR, and cells transfected with miR-K12-10 are more resistant to TWEAK-induced apoptosis [46]. Another group showed that expression of the cellular cyclin-dependent kinase inhibitor p21, a key inducer of cell cycle arrest, is repressed

through its direct targeting by miR-K12-1 [48]. Ectopically expressed miR-K12-1 strongly attenuated cell cycle arrest induced during p53 activation through repression of endogenous p21. In summary, KSHV miRNA support of anti-apoptotic signaling, coupled with their regulation of cytokine responses and their putative role in suppression of immune recognition, suggests that KSHV miRNAs invoke cooperative mechanisms critical for survival of KSHV-infected cells.

6. Future Directions

6.1. Establishing Biologic Assays for Identification of KSHV miRNAs Targets. Online miRNA databases (<http://www.mirbase.org/>) and bioinformatics programs have been developed to predict virus-encoded miRNAs targets [22, 57–59]. Several groups have utilized these programs for identifying putative targets of KSHV miRNAs [14, 20, 22, 24, 40, 44], although the use of seed sequence matching as the principal predictive tool for these programs is too stringent given that many valid targets of miRNAs will not meet predetermined sequence matching criteria [60]. This has led to interest in developing screening tools involving more direct assessment of viral miRNAs regulation of potential targets. One group published their use of a tandem array-based screening approach: first, they quantified expression of host genes under conditions of either KSHV miRNA overexpression or inhibition of single KSHV miRNAs in latently infected cells; second, they identified targets using stringent criteria including seed sequence complementarity at positions 2–8 which, although not required for targeting, has been associated with more reliable prediction of target downregulation [39]. Through this effort, they identified one gene targeted by miR-K12-5 (BCLAF1). As noted by the authors, limitations for this approach are its labor-intensive nature and lack of all-inclusiveness in target identification. Another group performed immunoprecipitation of RISCs followed by microarray analysis of the RISC-bound miRNA targets (RIP-Chip) of KSHV miRNAs, EBV miRNAs, and human miRNAs using latently infected or stably transduced human B-cell lines [61]. Two targets were validated for EBV miRNAs, and transcript half-life of human and viral miRNA targets correlated inversely with recruitment to RISC complexes, indicating that RIP-Chip may offer a quantitative estimate of viral miRNA function [61]. Furthermore, two putative targets exhibited miRNA binding sites within their coding sequences, not within 3'UTRs. Additional studies should clarify whether these and other methods are ultimately cost-effective and yield more reliable identification of viral miRNA targets relative to bioinformatics screens.

6.2. KSHV Regulation of Human miRNAs. Although the majority of published work has thus far focused on defining KSHV miRNA targets and functional correlations, data published more recently also suggest that KSHV-encoded proteins regulate cellular machinery by virtue of their regulation or interference with cellular miRNA functioning. In KS and PEL tumors, tumor-suppressor miRNAs, including miR-221, miR-222, and let-7 family members, are underrepresented

[62]. Furthermore, pre-miRNA signatures may define the stages of EC transformation following KSHV infection [63]. More specifically, the loss of miR-221 expression marks the transition from immortalization to tumorigenicity for these cells [63]. Since the publication of these studies, several groups have identified specific mechanisms for KSHV regulation of cellular miRNAs. KSHV-encoded vFLIP represses expression of the chemokine receptor CXCR4 through NF- κ B-mediated upregulation of miR-146a [64]. Since KSHV encodes redundant mechanisms for NF- κ B upregulation, and since multiple cellular miRNAs have NF- κ B binding sites within their promoters, this study illuminates an important mechanism for KSHV regulation of the cellular miRNA machinery. Another elegant study recently confirmed that KSHV induces EC migration through regulation of cellular transcription factors, and the authors identified two complementary mechanisms for this effect [65]. First, they found that the transcription factors ETS2 and ETS1 are downstream targets of cellular miR-221 and miR-222, respectively. They confirmed that two KSHV-encoded latent proteins, LANA, and Kaposin B, downregulate the miR-221/miR-222 cluster through direct interactions with the miR-221/miR-222 promoter resulting in upregulation of ETS1/2-induced EC mobility [65]. Second, they found that KSHV upregulates EC expression of miR-31, thereby repressing expression of the tumor suppressor FAT4. They confirmed the presence of miR-31 binding sites within the coding region of FAT4, and that KSHV/miR-31-induced suppression of FAT4 results in enhanced EC mobility. The same group published additional data suggesting that the minor variant of KSHV-encoded K15 induces cell migration and invasion through induction of miR-31 [66]. KSHV regulation of cellular miRNAs may also influence immune evasion and immunopathogenesis. KSHV infection induces expression of miR-132, thereby reducing expression of interferon (IFN)-stimulated genes and facilitating viral replication in EC [67]. And as previously mentioned, KSHV-encoded ORF57 competes with cellular miRNAs for binding of transcripts for IL-6, thereby stabilizing IL-6 RNA [55]. These studies further underscore the complex regulatory network of viral and human miRNAs that contribute to tumor pathogenesis, and future studies will confirm whether inhibition of KSHV regulation of human miRNAs offers a viable therapeutic strategy for KSHV-associated diseases.

6.3. Regulation of KSHV miRNA Expression. Numerous studies have focused on defining the regulatory functions of miRNAs. Less well understood are mechanisms for transcriptional and posttranscriptional regulation of miRNAs themselves, including viral miRNAs, although burgeoning data suggest that these processes are important for cancer pathogenesis [68, 69]. miRNAs are under the control of a wide range of transcription factors, including some tumor suppressors and oncogenes [70–72], and recent reports reveal that certain environmental conditions like hypoxic stress influence miRNA expression. miR-210 is induced by hypoxia-inducible factor-1 alpha (HIF-1 α) to promote cell survival and adaptation to hypoxic environmental conditions [73], and HIF-1 α alters miR-101 expression in a

prostate cancer model [74]. Interestingly, HIF-1 α is highly expressed in HIV-associated KS lesions [75], and KSHV-encoded IFN regulatory factor 3 (vIRF3) stabilizes HIF-1 α , thereby inducing vascular endothelial growth factor (VEGF) expression [76]. KSHV-encoded LANA also functions both as an inhibitor of a HIF-1 α suppressor, the von Hippel-Lindau protein, and as an inducer of nuclear accumulation of HIF-1 α during latent KSHV infection [77, 78]. These data would support additional work to determine whether KSHV regulation of HIF-1 α dysregulates human miRNA expression and tumor pathogenesis. Other factors regulate miRNA expression at the posttranscriptional level, including Drosha and its interactional protein DGCR8 [79–81]. One study also noted that a single nucleotide polymorphism within the miR-K12-5 precursor stem-loop reduces Drosha processing and inhibits mature miR-K12-5 expression in BCBL-1 cells [82]. This implies that mutations within miRNA genes themselves may arise during the transformation of an infected cell and differential expression of KSHV miRNAs which favor specific pathogenic events. DNA methylation and histone deacetylation also contribute to regulation of miRNA transcription [83–85]. A report referenced previously found that genomic DNA from cells infected with a KSHV deletion mutant lacking 10 of the 12 mature KSHV miRNAs exhibited a striking loss of methylation [40], but whether miRNAs expression is regulated through epigenetic mechanisms, possibly involving miRNAs themselves, has not been elucidated.

6.4. Targeting Viral and Cellular miRNAs for Clinical Applications. Despite challenges in achieving efficient and selective approaches for suppressing miRNA functions *in vivo*, the concept of targeting miRNAs for therapeutic benefit has gained considerable attention with the publication of elegant studies revealing effective methods for suppressing miRNA-associated tumor progression in animal models. One of the first examples of chemical modification of oligonucleotides for miRNA inhibition was the development of antagomirs, small ribonucleotide chains whose 2'-hydroxyl on the ribose is replaced by a 2'-O-Methyl group for stability [86]. Commercially available antagomirs have additional modifications that stabilize miRNA-antagomir binding and prevent recognition of cognate mRNAs by miRNAs. Antagomirs have demonstrated utility for inhibiting KSHV miRNA-induced pathogenesis in KSHV-infected cells *in vitro* [20, 44, 56]. Intravenous delivery of antagomirs has also demonstrated utility *in vivo* [86–88], but off-target effects and excessive doses required to suppress miRNA expression have raised concerns about the utility of this approach [89].

Examples of other chemical modifications of oligonucleotides for clinical applications include morpholinos and locked nucleic acids (LNAs). Morpholinos contain six-member morpholine rings rather than five-member ribose rings, conferring resistance to nucleases [90]. Morpholinos may be further engineered to bind and protect mRNA target sequences from miRNA to confer superior target specificity [91]. Morpholinos conjugated to peptides to enhance cell penetration have demonstrated utility in animal models [92], and a modified drug based on this technology, delivered

by intramuscular injection, is undergoing evaluation in one clinical trial [93]. LNAs contain a biochemical modification where the 2'-oxygen and 4'-carbon atoms of the ribose rings are chemically bridged. This "locked" confirmation confers high thermal stability and resistance to exo- and endonucleases. An LNA-based miR-122 inhibitor is under evaluation for the treatment of hepatitis C [94]. In fact, LNAs have demonstrated utility in a number of animal model systems [95–99]. As noted previously, miR-K12-11, a KSHV-encoded ortholog of cellular miR-155, targets C/EBP β , [45], and one study indicates that silencing of miR-155 in mice using LNAs leads to derepression of C/EBP β [99]. It is interesting to speculate whether LNAs could be used to suppress KSHV-associated lymphoma progression *in vivo* using this approach to target miR-K12-11.

As we discussed previously, KSHV itself suppresses expression of human miRNAs serving as tumor suppressors, including miR-221 and miR-222. This raises the question of whether delivery of selected miRNAs would interfere with KSHV pathogenesis *in vivo*. The utility of miRNA delivery for cancer therapeutics has been supported recently through studies indicating successful suppression of tumors *in vivo* using liposomal nanoparticles containing miRNA or lipid-based delivery reagents which are commercially available [100, 101]. In one study, systemic delivery of miR-34a inhibited prostate cancer metastasis and extended survival of tumor-bearing mice, in part through targeting of CD44 [101]. CD44 is one of two well-characterized receptors for hyaluronic acid (HA) [102], and we have recently reported effective sensitization of human KSHV-infected lymphoma cells to chemotherapy using various approaches for interfering with HA-receptor interactions [103]. Whether KSHV regulation of human miRNAs initiates upregulation of HA receptors and drug resistance for KSHV-infected cells remains unknown. Regardless, this reinforces the complex interplay between viral and human miRNA, and the redundancy of viral miRNA regulatory mechanisms, and implies that viral cancer treatment approaches targeting a single miRNA would likely be limited in their clinical efficacy. Combining miRNA targeting with existing therapies for viral tumors may be a more tractable approach. Of note, *in vivo* effects of targeting multiple miRNAs simultaneously have not been defined, although simultaneous use of multiple antagonists targeting KSHV miRNAs demonstrates additive or synergistic suppression of KSHV pathogenesis *in vitro* [20, 44, 56].

7. Conclusion

As the etiologic agent of diverse forms of human cancer and by virtue of its tropism for a variety of human cell types, KSHV represents a model pathogen for the study of viral miRNA expression and function. Elegant studies performed recently underscore the importance of KSHV miRNAs and their interactions with human miRNA, for cancer pathogenesis, including viral biology and gene expression, cytokine responses, immune evasion, and anti-apoptotic signaling. The plasticity of these interactions and challenges inherent to miRNA targeting *in vivo* incur substantial obstacles for

development of miRNA-based therapies, but recent advances hold considerable promise for eventual clinical application of therapeutic approaches targeting viral miRNAs in the treatment of viral malignancies.

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References

- [1] U. Moens, "Silencing viral MicroRNA as a novel antiviral therapy?" *Journal of Biomedicine and Biotechnology*, vol. 2009, 2009.
- [2] D. P. Bartel, "MicroRNAs: genomics, biogenesis, mechanism, and function," *Cell*, vol. 116, no. 2, pp. 281–297, 2004.
- [3] V. Ambros, "The functions of animal microRNAs," *Nature*, vol. 431, no. 7006, pp. 350–355, 2004.
- [4] B. R. Cullen, "Viral and cellular messenger RNA targets of viral microRNAs," *Nature*, vol. 457, no. 7228, pp. 421–425, 2009.
- [5] T. F. Schulz, "The pleiotropic effects of Kaposi's sarcoma herpesvirus," *Journal of Pathology*, vol. 208, no. 2, pp. 187–198, 2006.
- [6] A. Grundhoff and D. Ganem, "Inefficient establishment of KSHV latency suggests an additional role for continued lytic replication in Kaposi sarcoma pathogenesis," *Journal of Clinical Investigation*, vol. 113, no. 1, pp. 124–136, 2004.
- [7] M. G. Aluigi, A. Albin, S. Carlone et al., "KSHV sequences in biopsies and cultured spindle cells of epidemic, iatrogenic and Mediterranean forms of Kaposi's sarcoma," *Research in Virology*, vol. 147, no. 5, pp. 267–275, 1996.
- [8] C. Lebbé, P. De Crémoux, G. Millot et al., "Characterization of *in vitro* culture of HIV-negative Kaposi's sarcoma-derived cells. *In vitro* responses to alpha interferon," *Archives of Dermatological Research*, vol. 289, no. 7, pp. 421–428, 1997.
- [9] J. Soulier, L. Grollet, E. Oksenhendler et al., "Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castlemann's disease," *Blood*, vol. 86, no. 4, pp. 1276–1280, 1995.
- [10] E. Cesarman, Y. Chang, P. S. Moore, J. W. Said, and D. M. Knowles, "Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas," *New England Journal of Medicine*, vol. 332, no. 18, pp. 1186–1191, 1995.
- [11] Y. Chang, E. Cesarman, M. S. Pessin et al., "Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma," *Science*, vol. 266, no. 5192, pp. 1865–1869, 1994.
- [12] S. Pfeffer, A. Sewer, M. Lagos-Quintana et al., "Identification of microRNAs of the herpesvirus family," *Nature Methods*, vol. 2, no. 4, pp. 269–276, 2005.
- [13] X. Cai, S. Lu, Z. Zhang, C. M. Gonzalez, B. Damania, and B. R. Cullen, "Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 15, pp. 5570–5575, 2005.

- [14] M. A. Samols, J. Hu, R. L. Skalsky, and R. Renne, "Cloning and identification of a MicroRNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus," *Journal of Virology*, vol. 79, no. 14, pp. 9301–9305, 2005.
- [15] E. Gottwein, X. Cai, and B. R. Cullen, "Expression and function of microRNAs encoded by kaposi's sarcoma-associated herpesvirus," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 71, pp. 357–364, 2006.
- [16] A. Grundhoff, C. S. Sullivan, and D. Ganem, "A combined computational and microarray-based approach identifies novel microRNAs encoded by human gamma-herpesviruses," *RNA*, vol. 12, no. 5, pp. 733–750, 2006.
- [17] V. Marshall, T. Parks, R. Bagni et al., "Conservation of virally encoded microRNAs in Kaposi sarcoma-associated herpesvirus in primary effusion lymphoma cell lines and in patients with Kaposi sarcoma or multicentric Castlemann disease," *Journal of Infectious Diseases*, vol. 195, no. 5, pp. 645–659, 2007.
- [18] J. L. Umbach and B. R. Cullen, "In-depth analysis of Kaposi's sarcoma-associated herpesvirus microRNA expression provides insights into the mammalian microRNA-processing machinery," *Journal of Virology*, vol. 84, no. 2, pp. 695–703, 2010.
- [19] V. Marshall, E. Martró, N. Labo et al., "Kaposi Sarcoma (KS)—associated herpesvirus microRNA sequence analysis and KS risk in a European AIDS-KS case control study," *Journal of Infectious Diseases*, vol. 202, no. 7, pp. 1126–1135, 2010.
- [20] Z. Qin, E. Freitas, R. Sullivan et al., "Upregulation of xCT by KSHV-encoded microRNAs facilitates KSHV dissemination and persistence in an environment of oxidative stress," *PLoS Pathogens*, vol. 6, no. 1, Article ID e1000742, 2010.
- [21] J. A. R. Kaleeba and E. A. Berger, "Kaposi's sarcoma-associated herpesvirus fusion-entry receptor: cystine transporter xCT," *Science*, vol. 311, no. 5769, pp. 1921–1924, 2006.
- [22] R. L. Skalsky, M. A. Samols, K. B. Plaisance et al., "Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155," *Journal of Virology*, vol. 81, no. 23, pp. 12836–12845, 2007.
- [23] E. Gottwein, N. Mukherjee, C. Sachse et al., "A viral microRNA functions as an orthologue of cellular miR-155," *Nature*, vol. 450, no. 7172, pp. 1096–1099, 2007.
- [24] A. Hansen, S. Henderson, D. Lagos et al., "KSHV-encoded miRNAs target MAF to induce endothelial cell reprogramming," *Genes and Development*, vol. 24, no. 2, pp. 195–205, 2010.
- [25] G. Rappocciolo, F. J. Jenkins, H. R. Hensler et al., "DC-SIGN is a receptor for human herpesvirus 8 on dendritic cells and macrophages," *Journal of Immunology*, vol. 176, no. 3, pp. 1741–1749, 2006.
- [26] G. Rappocciolo, H. R. Hensler, M. Jais et al., "Human herpesvirus 8 infects and replicates in primary cultures of activated B lymphocytes through DC-SIGN," *Journal of Virology*, vol. 82, no. 10, pp. 4793–4806, 2008.
- [27] N. Kerur, M. V. Veettil, N. Sharma-Walia et al., "Characterization of entry and infection of monocytic THP-1 cells by Kaposi's sarcoma associated herpesvirus (KSHV): role of heparan sulfate, DC-SIGN, integrins and signaling," *Virology*, vol. 406, no. 1, pp. 103–116, 2010.
- [28] S. M. Akula, N. P. Pramod, F. Z. Wang, and B. Chandran, "Integrin $\alpha 3 \beta 1$ (CD 49c/29) is a cellular receptor for Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells," *Cell*, vol. 108, no. 3, pp. 407–419, 2002.
- [29] H. J. Garrigues, Y. E. Rubinchikova, C. M. DiPersio, and T. M. Rose, "Integrin $\alpha v \beta 3$ binds to the RGD motif of glycoprotein B of Kaposi's sarcoma-associated herpesvirus and functions as an RGD-dependent entry receptor," *Journal of Virology*, vol. 82, no. 3, pp. 1570–1580, 2008.
- [30] P. S. Gill, Y. C. Tsai, A. P. Rao et al., "Evidence for multiclonality in multicentric Kaposi's sarcoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 14, pp. 8257–8261, 1998.
- [31] K. A. Staskus, W. Zhong, K. Gebhard et al., "Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells," *Journal of Virology*, vol. 71, no. 1, pp. 715–719, 1997.
- [32] E. Boulanger, R. Duprez, E. Delabesse, J. Gabarre, E. Macintyre, and A. Gessain, "Mono/oligoclonal pattern of Kaposi Sarcoma-associated herpesvirus (KSHV/HHV-8) episomes in primary effusion lymphoma cells," *International Journal of Cancer*, vol. 115, no. 4, pp. 511–518, 2005.
- [33] L. A. Adang, C. Tomescu, W. K. Law, and D. H. Kedes, "Intracellular Kaposi's sarcoma-associated herpesvirus load determines early loss of immune synapse components," *Journal of Virology*, vol. 81, no. 10, pp. 5079–5090, 2007.
- [34] X. Wang, L. Ye, W. Hou et al., "Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection," *Blood*, vol. 113, no. 3, pp. 671–674, 2009.
- [35] X. Wang, L. Ye, Y. Zhou, M.-Q. Liu, D.-J. Zhou, and W.-Z. Ho, "Inhibition of anti-HIV microRNA expression: a mechanism for opioid-mediated enhancement of HIV infection of monocytes," *American Journal of Pathology*, vol. 178, no. 1, pp. 41–47, 2011.
- [36] R. Ouda, K. Onomoto, K. Takahasi et al., "Retinoic acid-inducible gene I-inducible miR-23b inhibits infections by minor group rhinoviruses through down-regulation of the very low density lipoprotein receptor," *Journal of Biological Chemistry*, vol. 286, no. 29, pp. 26210–26219, 2011.
- [37] X. Lin, D. Liang, Z. He, Q. Deng, E. S. Robertson, and K. Lan, "miR-K12-7-5p encoded by Kaposi's sarcoma-associated herpesvirus stabilizes the latent state by targeting viral ORF50/RTA," *PLoS One*, vol. 6, no. 1, Article ID e16224, 2011.
- [38] P. Bellare and D. Ganem, "Regulation of KSHV lytic switch protein expression by a virus-encoded microRNA: an evolutionary adaptation that fine-tunes lytic reactivation," *Cell Host and Microbe*, vol. 6, no. 6, pp. 570–575, 2009.
- [39] J. M. Ziegelbauer, C. S. Sullivan, and D. Ganem, "Tandem array-based expression screens identify host mRNA targets of virus-encoded microRNAs," *Nature Genetics*, vol. 41, no. 1, pp. 130–134, 2009.
- [40] F. Lu, W. Stedman, M. Yousef, R. Renne, and P. M. Lieberman, "Epigenetic regulation of Kaposi's sarcoma-associated herpesvirus latency by virus-encoded microRNAs that target Rta and the cellular Rbl2-DNMT pathway," *Journal of Virology*, vol. 84, no. 6, pp. 2697–2706, 2010.
- [41] X. Lei, Z. Bai, F. Ye et al., "Regulation of NF- κ B inhibitor I κ B α and viral replication by a KSHV microRNA," *Nature Cell Biology*, vol. 12, no. 2, pp. 193–199, 2010.
- [42] C. C. Lu, Z. Li, C. Y. Chu et al., "MicroRNAs encoded by Kaposi's sarcoma-associated herpesvirus regulate viral life cycle," *EMBO Reports*, vol. 11, no. 10, pp. 784–790, 2010.
- [43] D. Liang, Y. Gao, X. Lin et al., "A human herpesvirus miRNA attenuates interferon signaling and contributes to maintenance of viral latency by targeting IKK ϵ ," *Cell Research*, vol. 21, no. 5, pp. 793–806, 2011.

- [44] Z. Qin, P. Kearney, K. Plaisance, and C. H. Parsons, "Pivotal Advance: Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded microRNA specifically induce IL-6 and IL-10 secretion by macrophages and monocytes," *Journal of Leukocyte Biology*, vol. 87, no. 1, pp. 25–34, 2010.
- [45] I. W. Boss, P. E. Nadeau, J. R. Abbott, Y. Yang, A. Mergia, and R. Renne, "A kaposi's sarcoma-associated herpesvirus-encoded ortholog of microRNA mir-155 induces human splenic B-Cell expansion in NOD/ltsz-scid IL2Rnull mice," *Journal of Virology*, vol. 85, no. 19, pp. 9877–9886, 2011.
- [46] J. R. Abend, T. Uldrick, and J. M. Ziegelbauer, "Regulation of tumor necrosis factor-like weak inducer of apoptosis receptor protein (TWEAKR) expression by Kaposi's sarcoma-associated herpesvirus microRNA prevents tweak-induced apoptosis and inflammatory cytokine expression," *Journal of Virology*, vol. 84, no. 23, pp. 12139–12151, 2010.
- [47] D. Nachmani, N. Stern-Ginossar, R. Sarid, and O. Mandelboim, "Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB to escape recognition by natural killer cells," *Cell Host and Microbe*, vol. 5, no. 4, pp. 376–385, 2009.
- [48] E. Gottwein and B. R. Cullen, "A human herpesvirus MicroRNA inhibits p21 expression and attenuates p21-mediated cell cycle arrest," *Journal of Virology*, vol. 84, no. 10, pp. 5229–5237, 2010.
- [49] E. A. Mesri, E. Cesarman, and C. Boshoff, "Kaposi's sarcoma and its associated herpesvirus," *Nature Reviews Cancer*, vol. 10, no. 10, pp. 707–719, 2010.
- [50] K. D. Jones, Y. Aoki, Y. Chang, P. S. Moore, R. Yarchoan, and G. Tosato, "Involvement of interleukin-10 (IL-10) and viral IL-6 in the spontaneous growth of Kaposi's sarcoma herpesvirus-associated infected primary effusion lymphoma cells," *Blood*, vol. 94, no. 8, pp. 2871–2879, 1999.
- [51] Y. Aoki, R. Yarchoan, J. Braun, A. Iwamoto, and G. Tosato, "Viral and cellular cytokines in AIDS-related malignant lymphomatous effusions," *Blood*, vol. 96, no. 4, pp. 1599–1601, 2000.
- [52] E. Oksenhendler, G. Carcelain, Y. Aoki et al., "High levels of human herpesvirus 8 viral load, human interleukin-6, interleukin-10, and C reactive protein correlate with exacerbation of multicentric Castleman disease in HIV-infected patients," *Blood*, vol. 96, no. 6, pp. 2069–2073, 2000.
- [53] M. Cirone, G. Lucania, S. Aleandri et al., "Suppression of dendritic cell differentiation through cytokines released by Primary Effusion Lymphoma cells," *Immunology Letters*, vol. 120, no. 1–2, pp. 37–41, 2008.
- [54] P. Gasperini, S. Sakakibara, and G. Tosato, "Contribution of viral and cellular cytokines to Kaposi's sarcoma-associated herpesvirus pathogenesis," *Journal of Leukocyte Biology*, vol. 84, no. 4, pp. 994–1000, 2008.
- [55] J.-G. Kang, N. Pripuzova, V. Majerciak, M. Kruhlak, S.-Y. Le, and Z.-M. Zheng, "Kaposi's sarcoma-associated herpesvirus ORF57 promotes escape of viral and human interleukin-6 from microRNA-mediated suppression," *Journal of Virology*, vol. 85, no. 6, pp. 2620–2630, 2011.
- [56] M. A. Samols, R. L. Skalsky, A. M. Maldonado et al., "Identification of cellular genes targeted by KSHV-encoded microRNAs," *PLoS Pathogens*, vol. 3, no. 5, article e65, 2007.
- [57] A. Lagana, S. Forte, F. Russo, R. Giugno, A. Pulvirenti, and A. Ferro, "Prediction of human targets for viral-encoded microRNAs by thermodynamics and empirical constraints," *Journal of RNAi and Gene Silencing*, vol. 6, pp. 379–385, 2010.
- [58] J.-I. Satoh and H. Tabunoki, "Comprehensive analysis of human microRNA target networks," *BioData Mining*, vol. 4, no. 1, article 17, 2011.
- [59] M. H. Radfar, W. Wong, and Q. Morris, "Computational prediction of intronic microRNA targets using host gene expression reveals novel regulatory mechanisms," *PLoS ONE*, vol. 6, no. 6, article e19312, 2011.
- [60] B. P. Lewis, C. B. Burge, and D. P. Bartel, "Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets," *Cell*, vol. 120, no. 1, pp. 15–20, 2005.
- [61] L. Dölken, G. Malterer, F. Erhard et al., "Systematic analysis of viral and cellular microRNA targets in cells latently infected with human γ -herpesviruses by RISC immunoprecipitation assay," *Cell Host and Microbe*, vol. 7, no. 4, pp. 324–334, 2010.
- [62] A. J. O'Hara, L. Wang, B. J. Dezube, W. J. Harrington, B. Damania, and D. P. Dittmer, "Tumor suppressor microRNAs are underrepresented in primary effusion lymphoma and Kaposi sarcoma," *Blood*, vol. 113, no. 23, pp. 5938–5941, 2009.
- [63] A. J. O'Hara, P. Chugh, L. Wang et al., "Pre-micro rna signatures delineate stages of endothelial cell transformation in kaposi sarcoma," *PLoS Pathogens*, vol. 5, no. 4, Article ID e1000389, 2009.
- [64] V. Punj, H. Matta, S. Schamus, A. Tamewitz, B. Anyang, and P. M. Chaudhary, "Kaposi's sarcoma-associated herpesvirus-encoded viral FLICE inhibitory protein (vFLIP) K13 suppresses CXCR4 expression by upregulating miR-146a," *Oncogene*, vol. 29, no. 12, pp. 1835–1844, 2010.
- [65] Y.-H. Wu, T.-F. Hu, Y.-C. Chen et al., "The manipulation of miRNA-gene regulatory networks by KSHV induces endothelial cell motility," *Blood*, vol. 118, no. 10, pp. 2896–2905, 2011.
- [66] Y. H. Tsai, M. F. Wu, Y. H. Wu et al., "The M type K15 protein of Kaposi's sarcoma-associated herpesvirus regulates microRNA expression via its SH2-binding motif to induce cell migration and invasion," *Journal of Virology*, vol. 83, no. 2, pp. 622–632, 2009.
- [67] D. Lagos, G. Pollara, S. Henderson et al., "MiR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator," *Nature Cell Biology*, vol. 12, no. 5, pp. 513–519, 2010.
- [68] J. Winter and S. Diederichs, "MicroRNA biogenesis and cancer," *Methods in Molecular Biology*, vol. 676, pp. 3–22, 2011.
- [69] B. C. Schanen and X. Li, "Transcriptional regulation of mammalian miRNA genes," *Genomics*, vol. 97, no. 1, pp. 1–6, 2011.
- [70] M. Dews, A. Homayouni, D. Yu et al., "Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster," *Nature Genetics*, vol. 38, no. 9, pp. 1060–1065, 2006.
- [71] X. He, L. He, and G. J. Hannon, "The guardian's little helper: microRNAs in the p53 tumor suppressor network," *Cancer Research*, vol. 67, no. 23, pp. 11099–11101, 2007.
- [72] R. Spizzo, M. S. Nicoloso, L. Lupini et al., "MiR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor- α in human breast cancer cells," *Cell Death and Differentiation*, vol. 17, no. 2, pp. 246–254, 2010.
- [73] X. Huang, L. Ding, K. L. Bennewith et al., "Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation," *Molecular Cell*, vol. 35, no. 6, pp. 856–867, 2009.

- [74] P. Cao, Z. Deng, M. Wan et al., "MicroRNA-101 negatively regulates Ezh2 and its expression is modulated by androgen receptor and HIF-1 α /HIF-1 β ," *Molecular Cancer*, vol. 9, article no. 108, 2010.
- [75] E. Long, M. Ilie, V. Hofman et al., "LANA-1, Bcl-2, Mcl-1 and HIF-1 α protein expression in HIV-associated Kaposi sarcoma," *Virchows Archiv*, vol. 455, no. 2, pp. 159–170, 2009.
- [76] Y. C. Shin, C. H. Joo, M. U. Gack, H. R. Lee, and J. U. Jung, "Kaposi's sarcoma-associated herpesvirus viral IFN regulatory factor 3 stabilizes hypoxia-inducible factor-1 α to induce vascular endothelial growth factor expression," *Cancer Research*, vol. 68, no. 6, pp. 1751–1759, 2008.
- [77] Q. Cai, K. Lan, S. C. Verma, H. Si, D. Lin, and E. S. Robertson, "Kaposi's sarcoma-associated herpesvirus latent protein LANA interacts with HIF-1 α to upregulate RTA expression during hypoxia: latency control under low oxygen conditions," *Journal of Virology*, vol. 80, no. 16, pp. 7965–7975, 2006.
- [78] Q. Cai, M. Murakami, H. Si, and E. S. Robertson, "A potential α -helix motif in the amino terminus of LANA encoded by Kaposi's sarcoma-associated herpesvirus is critical for nuclear accumulation of HIF-1 α in normoxia," *Journal of Virology*, vol. 81, no. 19, pp. 10413–10423, 2007.
- [79] R. I. Gregory, K. P. Yan, G. Amuthan et al., "The Microprocessor complex mediates the genesis of microRNAs," *Nature*, vol. 432, no. 7014, pp. 235–240, 2004.
- [80] A. M. Denli, B. B. J. Tops, R. H. A. Plasterk, R. F. Ketting, and G. J. Hannon, "Processing of primary microRNAs by the Microprocessor complex," *Nature*, vol. 432, no. 7014, pp. 231–235, 2004.
- [81] J. Han, Y. Lee, K. H. Yeom et al., "Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex," *Cell*, vol. 125, no. 5, pp. 887–901, 2006.
- [82] E. Gottwein, X. Cai, and B. R. Cullen, "A novel assay for viral microRNA function identifies a single nucleotide polymorphism that affects Drosha processing," *Journal of Virology*, vol. 80, no. 11, pp. 5321–5326, 2006.
- [83] A. Lujambio and M. Esteller, "CpG island hypermethylation of tumor suppressor microRNAs in human cancer," *Cell Cycle*, vol. 6, no. 12, pp. 1455–1459, 2007.
- [84] A. Lujambio, G. A. Calin, A. Villanueva et al., "A microRNA DNA methylation signature for human cancer metastasis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 36, pp. 13556–13561, 2008.
- [85] B. Brueckner, C. Stresemann, R. Kuner et al., "The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function," *Cancer Research*, vol. 67, no. 4, pp. 1419–1423, 2007.
- [86] J. Krützfeldt, N. Rajewsky, R. Braich et al., "Silencing of microRNAs in vivo with 'antagomirs,'" *Nature*, vol. 438, no. 7068, pp. 685–689, 2005.
- [87] L. Fontana, M. E. Fiori, S. Albini et al., "Antagomir-17-5p abolishes the growth of therapy-resistant neuroblastoma through p21 and BIM," *PLoS One*, vol. 3, no. 5, Article ID e2236, 2008.
- [88] L. Ma, F. Reinhardt, E. Pan et al., "Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model," *Nature Biotechnology*, vol. 28, no. 4, pp. 341–347, 2010.
- [89] E. E. Morrisey, "The magic and mystery of miR-21," *Journal of Clinical Investigation*, vol. 120, no. 11, pp. 3817–3819, 2010.
- [90] J. Summerton, "Morpholino antisense oligomers: the case for an RNase H-independent structural type," *Biochimica et Biophysica Acta*, vol. 1489, no. 1, pp. 141–158, 1999.
- [91] W. Y. Choi, A. J. Giraldez, and A. F. Schier, "Target protectors reveal dampening and balancing of nodal agonist and antagonist by miR-430," *Science*, vol. 318, no. 5848, pp. 271–274, 2007.
- [92] H. M. Moulton and J. D. Moulton, "Morpholinos and their peptide conjugates: therapeutic promise and challenge for Duchenne muscular dystrophy," *Biochimica et Biophysica Acta*, vol. 1798, no. 12, pp. 2296–2303, 2010.
- [93] M. Kinali, V. Arechavala-Gomez, L. Feng et al., "Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study," *The Lancet Neurology*, vol. 8, no. 10, pp. 918–928, 2009.
- [94] R. E. Lanford, E. S. Hildebrandt-Eriksen, A. Petri et al., "Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection," *Science*, vol. 327, no. 5962, pp. 198–201, 2010.
- [95] B. G. Garchow, O. B. Encinas, Y. T. Leung et al., "Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice," *EMBO Molecular Medicine*, vol. 3, no. 10, pp. 605–615, 2011.
- [96] J. Elmén, M. Lindow, A. Silahtaroglu et al., "Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver," *Nucleic Acids Research*, vol. 36, no. 4, pp. 1153–1162, 2008.
- [97] S. Obad, C. O. Dos Santos, A. Petri et al., "Silencing of microRNA families by seed-targeting tiny LNAs," *Nature Genetics*, vol. 43, no. 4, pp. 371–380, 2011.
- [98] D. M. Patrick, R. L. Montgomery, X. Qi et al., "Stress-dependent cardiac remodeling occurs in the absence of microRNA-21 in mice," *Journal of Clinical Investigation*, vol. 120, no. 11, pp. 3912–3916, 2010.
- [99] J. Worm, J. Stenvang, A. Petri et al., "Silencing of microRNA-155 in mice during acute inflammatory response leads to depression of c/ebp Beta and down-regulation of G-CSF," *Nucleic Acids Research*, vol. 37, no. 17, pp. 5784–5792, 2009.
- [100] J. F. Wiggins, L. Ruffino, K. Kelnar et al., "Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34," *Cancer Research*, vol. 70, no. 14, pp. 5923–5930, 2010.
- [101] C. Liu, K. Kelnar, B. Liu et al., "The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44," *Nature Medicine*, vol. 17, no. 2, pp. 211–216, 2011.
- [102] D. G. Jackson, "Immunological functions of hyaluronan and its receptors in the lymphatics," *Immunological Reviews*, vol. 230, no. 1, pp. 216–231, 2009.
- [103] Z. Qin, L. Dai, M. Bratova, M. G. Slomiany, B. P. Toole, and C. Parsons, "Cooperative roles for emmprin and LYVE-1 in the regulation of chemoresistance for primary effusion lymphoma," *Leukemia*, vol. 25, no. 10, pp. 1598–1609, 2011.



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