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Review

MicroRNA-34a: a potential therapeutic target in human cancer

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MicroRNAs (miRs) are small noncoding RNAs that negatively regulate gene expression by binding to the three untranslated regions of their target mRNAs. Deregulations of miRs were shown to play pivotal roles in tumorigenesis and progression. Recent research efforts have been devoted to translating these basic discoveries into applications that could improve the therapeutic outcome of patients with cancer. *MiR-34a* is a highly conserved miR throughout many different species. In humans, there are three homologs (*hsa-miR34a, hsa-miR-34b* and *hsa-miR-34c*). Early studies have shown that miR-34a acts as a tumor-suppressor gene by targeting many oncogenes related to proliferation, apoptosis and invasion. In this review, we provide a complex overview of *miR-34a*, including regulating its expression, its known functions in cancer and future challenges as a potential therapeutic target in human cancers.
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Facts

- Deregulations of microRNAs were shown to play pivotal roles in tumorigenesis and progression.
- MiR-34a was reported as a tumor suppressor in multiple types of cancer through the suppression of multiple targets.
- In human liver cancer, miR-34a has already entered phase I clinical trial (NCT01829971).

Open Questions

- Although the improvements in cancer treatment are observed, intrinsic or acquired drug resistance remains a major obstacle to successful treatment, leading to ultimate cancer death.
- One of the greatest challenges in miR research is the identification and functional analysis of their targets. Identification of the downstream pathway of *miR-34a* will help to further understand the role of *miR-34a* in cancer development.
- Currently, there is no report regarding the *in vivo* pharmacokinetics of miRs, and the effective delivery of synthetic therapeutic miRs to the desired target tissues will be a challenge.

MicroRNAs (miRs) are ~22-nucleotide noncoding RNAs that have key roles in fundamental biological processes by regulating the levels of multiple proteins. An miR (~22 nt) originates from a long primary transcript (pri-miR) containing a hairpin structure. In the nucleus, pri-miR is cleaved with RNase III Drosha to liberate the hairpin-shaped precursor miR that is transported from the nucleus with exportin-5 protein. In the cytoplasm, the RNase III Dicer removes the terminal loop to generate a miR/miR* duplex, containing the functional miR strand and the passenger strand (miR/miR*). The miR/miR* duplex then binds to argonaut proteins to create a complex called RNA-induced silencing complex. The functional miRs induce mRNA degradation and/or translational inhibition by base-pairing to the target mRNAs.[1,2] However, the miR can also act in a RNA-induced silencing complex-independent manner on the transcriptional level by interaction with ribonucleoprotein[3] or direct binding to DNA.[4]

Here, we review the role of *miR-34a* in human cancer. *MiR-34a*, a highly conserved miR has recently emerged as a key tumor suppressor in multiple malignancies through the suppression of multiple targets. These fascinating discoveries raise many questions regarding its regulation pathway, mechanisms of activity and advances in miR-based therapeutic strategies.

The *MiR-34* Family

The *miR-34* family members comprise three processed miRs that are encoded by two different genes: *miR-34a* is encoded by its own transcript, whereas *miR-34b* and *miR-34c* share a

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Abbreviations: miRs, MicroRNAs; TF, transcription factor; SIRT1, sirtuin type 1; MAGE-A, melanoma antigen-A; ZEB1, zinc finger E-box-binding homeobox 1; T3, thyroid hormone 3,3,5-triiodo-L-thyronine; E2, estradiol; Hes-1, hairy and enhancer of split 1; PCa, prostate cancer; CSC, cancer stem cell; BCL2, B-cell lymphoma/leukemia-2; CDK6, cyclin-dependent kinase 6; NB, neuroblastoma

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common primary transcript (Figure 1). In the human genome, the *miR-34a* is encoded on chromosome 1, and the homologs *miR-34b* and *miR-34c* are encoded on chromosome 11. The *miR-34* family is highly conserved among vertebrates. These homologs code for different mature sequences of miRs, which contain the same seed sequence and therefore might suggest that they may have the same targets and show analogous functions.

The *miR-34* gene promoters contain p53-binding sites that are conserved among human, and, thus, leaves room for speculation of a p53-dependent regulation of the *miR-34* family.[5] *MiR-34a* is located in the second exon of a 33-kb transcript (EF570049). The promoter region contains a prominent CpG island and p53-binding sites. The CpG island in the *miR-34b*/c gene is a bidirectional promoter,[6] and its methylation is associated with the silencing of both *miR-34b/c* and B-cell translocation gene 4, which is seen as a novel tumor suppressor.[7]

Regulators of *MiR-34a* Expression

Currently, the expression of the *miR-34a* can be regulated mainly including genomic loss, epigenetic modification, transcriptional regulation and other molecules (Figure 2).

MiR-34a maps to the distal region of chromosome 1p, and genomic loss of this chromosomal region has been reported in many types of tumors.[8–10] Therefore, loss of *miR-34a* gene, which functions as a tumor suppressor in these tumors, is not surprising.

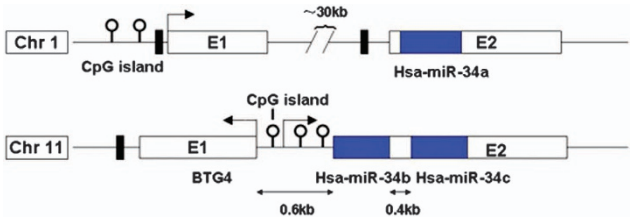

Figure 1 Structure of genomic loci of the human *miR-34a* and *MiR-34b*/c genes. White and blue boxes represent exons and miRNA hairpins, respectively. Black boxes indicate p53-binding sites; the positions of the CpG islands are indicated

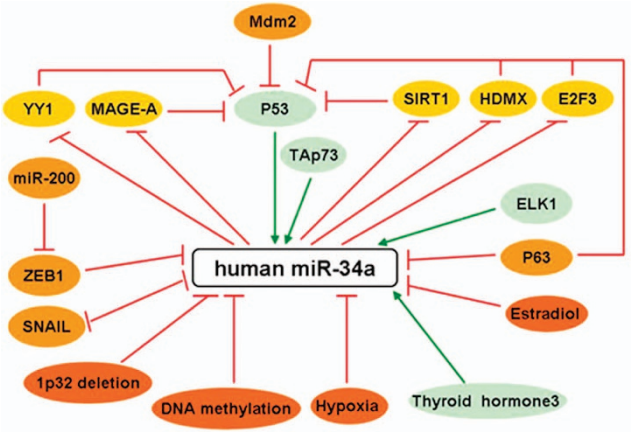

Figure 2 The figure summarizes how *miR-34a* expression is regulated. Red arrows indicate inhibition and green arrows indicate activation

Although the mechanism underlying *miR-34a* dysregulation in human cancer is not yet fully understood, much evidence suggests that an epigenetic mechanism is involved. Transcriptional silencing by CpG methylation represents an important mechanism responsible for the inactivation of tumor-suppressive genes. Similar to genomic loss, inactivation by CpG methylation may promote clonal growth with a selective advantage during tumor progression. Hypermethylation of CpG islands in the *miR-34a* gene promoter region in a variety of solid tumors including breast, lung, colon, kidney, bladder and pancreatic carcinoma correlated with silenced expression of *miR-34a*.[11] Interestingly, after treatment with 5-aza-2'deoxycytidine, which is an inhibitor of DNA methyltransferases, a reduction in the level of CpG methylation of *miR-34a* and the *miR-34a* gene is reactivated. Analysis of the promoter region more distal to the transcription start site revealed uniformly dense CpG methylation. Therefore, silencing of *miR-34a* expression is presumably mediated by CpG methylation of the region 100–500 base-pairs upstream of the *miR-34a* transcription start.[11] However, in chronic lymphocytic leukemia, *miR-34a* was upregulated, despite same DNA methylation levels of the *miR-34a* promoter CpG islands,[12] and other molecular mechanisms may contribute to deregulated *miR-34a* expression in hematological malignancy.

Various transcription factors (TFs) have been shown to regulate *miR-34a* expression. One of the strongest inducers of *miR-34a* is p53.[13–16] The p53 protein regulates multiple cellular pathways in response to genotoxic stress and recent studies have shown p53-dependent upregulation of *miR-34a* in human and mouse cells as a consequence of DNA damage. The p53 acts through positive feedback loops, which adding robustness to this network have been described, one of them involving sirtuin type 1 (SIRT1) protein.[17,18] Decreased SIRT1 stimulates p53 expression, which leads to the activation of *miR-34a*. An increase in *miR-34a* leads to SIRT1 inhibition, an important target of *miR-34a*. Other significant players are also involved in this regulatory mechanism between p53 and *miR-34a*: murine double minute 4 (HDMX),[19] melanoma antigen-A (MAGE-A)[20] and Yin Yang-1.[21,22] These findings were not confirmed in primary human fibroblasts and CLL, where *miR-34a* is regulated independently of p53.[23,24] Whether this regulation is p53-dependent or -independent, it still remains to be determined. Similar to p53, the p53-family member TAp73 is another TF that drives the transcript expression of miR-34a, by acting on the p53-binding sites present in the miR-34a promoter, indicating a possible TAp73/p53-independent mechanism in miR-34a regulation.[25] The p53 family can participate in this regulation, and this finding indicates that miR-34a can be involved in different pathways, depending on the stimuli and the cellular context.

In addition to the above-mentioned p53 and TAp73, ETS-like protein 1 has also been shown to increase *miR-34a* expression.[26] Negative regulation of *miR-34a* is carried out via p63, another p53 family member, which is associated with cell cycle progression by directly repressing transcription of *miR-34a* gene. In the absence of p63, increased levels of *miR-34a* was observed in epidermal cell via directly binding to p53-consensus sites in *miR-34a* regulatory regions and inhibited their activity.[27]

Furthermore, a 'double-negative feedback mechanism' has been reported, in which the TFs SNAIL and zinc finger E-box-binding homeobox 1 (ZEB1) could co-repress transcription of *miR-34a* gene via binding to E boxes in the *miR-34a* promoter. Conversely, Ectopic *miR-34a* down-regulated expression of SNAIL and ZEB1 by a conserved *miR-34a* seed-matching sequence in the SNAIL/ZEB1 3'UTR, resulting in a SNAIL (ZEB1)/*miR-34a* double-negative feedback loop.[28]

Last but not least, the effect of tumor microenvironment on *miR-34a* expression needs to be mentioned. Hypoxia is a common microenvironment for multi-pathophysiologic progress, including tumorigenesis.[29] Increasing evidence has shown that miR is involved in tumorigenesis, and angiogenesis driven by hypoxia.[28] *miR-34a* was identified as being significantly downregulated in hypoxic renal tubular epithelial cells, and hypoxia-mediated downregulation of *miR-34a* could promote renal tubular cell epithelial–mesenchymal transition by modulating the Notch signaling pathway.[30] Interestingly, the thyroid hormone 3,3,5-triiodo-L-thyronine (T3) has also been shown to induce the expression of *miR-34a*.[31] Additional hormones to be considered in *miR-34a* regulation are estradiol (E2), as shown in human breast cancer.[32] These results indicate that tumor microenvironment imbalance is needed for *miR-34a*'s expression and this basal expression may be reduced if these stimuli are either withdrawn (T3) or increased (E2).

MiR-34a as a Tumor-Suppressor Gene

Cancer cells are characterized by hypermethylation of trascription-associated CpG islands of tumor-suppressor genes, resulting in transcriptional repression and gene inactivation. *MiR-34a* was shown to be hypermethylated in breast, colon and lung cancers, and ectopic *miR-34a* induces a G1 cell cycle arrest, senescence and apoptosis, thereby demonstrating the tumor-suppressive role of *miR-34a*.[11] Consistent with this observation, in hematological malignancies, *miR-34a* is hypermethylated and hence 5-aza-2′-deoxycytidine demethylation treatment resulted in demethylation of *miR-34a* promoter and re-expression of the pri-*miR-34a*.[33]

Genomic analyses of human cancers have uncovered deletions encompassing 1p36, thereby providing an extensive body of literature supporting the idea that a potent tumor suppressor resides in this interval.[34] *MiR-34a* gene is in chromosome 1p36, which is commonly deleted in neuroblastoma (NB) and was the first miR identified as a tumor-suppressive factor in NB.[35,36] In addition, *miR-34a* was reported as a tumor suppressor in multiple types of cancer, including leukemias,[33] hepatocellular carcinoma,[37] pancreatic,[38] glioblastoma,[39] lung[40] and colon.[41] Furthermore, *miR-34a* was found to inhibit cancer stem cells (CSCs) self-renewal[42] and invasion,[43] promoting their sensitivity to chemo- and radiotherapy,[42] providing evidence that *miR-34a* may function as an anti-oncogene. These findings collectively indicate that identification of *miR-34a* as a potent tumor suppressor is a highly significant finding with respect to the development of potential therapeutics for cancer. Although it is well accepted that miR-34a is a tumor-suppressor gene, the miR-34a KO mice are not tumor prone.[44] The tumor-suppressive function of miR-34a might be restricted to specific tissues and loss of miR-34a might cooperate with specific oncogenic lesions.

Role of *miR-34a* in Human Cancer

Although the improvements in cancer treatment are observed, intrinsic or acquired drug resistance remains a major obstacle to successful treatment, leading to ultimate cancer death. The underlying mechanisms of chemoresistance are still poorly understood. So full understanding of drug resistance mechanisms may offer promise for improving current chemotherapy regimens and revising the resistance. In recent years, growing evidence demonstrate *miR-34a* has a key role in tumor cell responses to chemotherapeutic agents and may serve as an effective antitumor therapic target.

Breast Cancer

Breast cancer is the most common malignant disease in women in Western countries and the incidence rate is increasing dramatically in China in recent years. Understanding the molecular mechanisms underlying in breast cancer drug resistance is crucial to provide better therapeutic strategies for breast cancer patients.

A direct role for *miR-34a* in breast cancer is thought to exist owing to the common loss of 1p36[45,46] and CpG methylation of the *miR-34a* promoter,[11] leading to an downexpression of the *miR-34a* in comparison with normal tissues. Underexpression of *miR-34a* was shown to facilitate invasion *in vitro* and metastasis *in vivo* through suppressing a potential oncogene fos-related antigen 1 in breast cancer (Figure 3).[47] Our previous studies have shown that ectopic overexpression of miR-34a significantly increased the sensitivity of MCF-7 adriamycin-resistant cells to adriamycin by directly inhibiting its target, Notch 1,[48] one notch receptor involved in Notch signaling, which has a pivotal role in the regulation of many fundamental cellular processes, such as proliferation, stem cell maintenance and differentiation during embryonic and adult development. There are four Notch receptors (Notch 1–4) and five Notch ligands (delta-like-1, -3 and -4 and Jagged1 and 2) in mammals, and after specific ligand binding, the intracellular part of the Notch receptor is cleaved off and

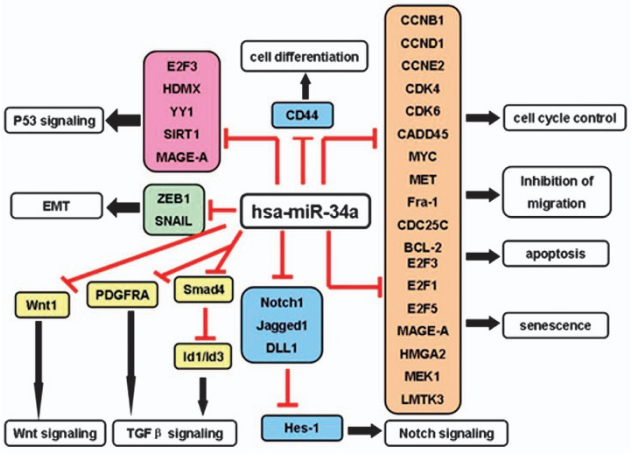

Figure 3 Experimentally confirmed cellular targets of *miR-34a*

translocates to the nucleus, where it binds to the TF and activated Notch target genes.[49] Jagged1 and its receptor Notch1 were targets of *miR-34a* and the entire signaling pathway, including *miR-34a*/Notch1/Jagged1/hairy and enhancer of split 1 (Hes-1), was suggested as an explanation of *miR-34a* repression of cell invasion (Figure 3).[42] Furthermore, the results were confirmed by *in vivo* experiment. Consistent with our observations, another study determined that *miR-34a* was downregulated in doxorubicin-resistant MCF-7 breast cancer cells. In human breast cancer samples, *miR-34a* was also downregulated in non-response group with poor prognosis and can be served to predict chemotherapy efficacy in breast cancer.[50]

Despite overwhelming evidence supporting a tumor-suppressive role of *miR-34a*, the opposite effect has also been observed. For example, MCF-7 breast cancer cell-acquired resistance was found to be associated with *miR-34a* overexpression.[51] Furthermore, inhibition of *miR-34a* enhanced response to docetaxel in MCF-7 docetaxel-resistant cells, suggesting that high *miR-34a* expression is not necessarily beneficial in terms of chemo-resistance. Taken together, these apparently contradictory functions of *miR-34a* may well be explained by an ambiguous role of *miR-34a* in regulation of the complex networks of oncogenes and tumor suppressors resulting in a cancer type-dependent outcome. Interestingly, unlike the results observed in MCF-7 tumors with estrogen receptor-positive, such association was not observed in MDA-MB-231 breast cancer cells, which are representative of the basal subtype of triple-negative breast tumors.[51] These observations indicate that *miR-34a* could be a miR that is at least in part dependent on factors like estrogen receptor alpha, and therefore need further study. Second, *miR-34a* deregulation can help to classify breast carcinomas and provide prognostic information. This study also presents some data on considering future therapeutical applications where miR profiling may be applied to identify responders as well as non-responders to chemotherapeutic treatment in breast cancer.

To examine whether overexpression of *miR-34a* is required for the DNA damage response, cell line experiments were recently performed, and the results also suggest that anti-*miR-34a* molecules might prove useful in radiosensitizing breast tumors for better treatment.[41] Together, these observations suggest a major pro-apoptotic role for *miR-34a* in breast cancer and *miR-34a* as a candidate for treatment of breast cancer.

Last but not least, miRs are released into the blood circulation and circulating miRs are remarkably stable as protected by microvesicles or exosomes.[52] Screening of circulating miRs could provide valuable information on the pathogenesis of breast cancer. Clinical trials focusing on the association between *miR-34a* and breast cancer have also been conducted, resulting in circulating *miR-34a* being identified as a potent diagnostic marker: circulating *miR-34a* are specifically elevated in the blood of breast cancer patients with metastases from healthy women;[53] *miR-34a* also provides information about the prognosis of a patient: higher *miR-34a* levels in patients are considered more aggressive with a poorer prognosis.[53,54] This discrepancy could be explained by the relatively smaller sample, and prospective studies on larger cohorts of patients will be required to substantiate the diagnostic role of the miR-34a. Next, we wanted to determine the role of miR34a in survival of breast cancer patients using a publically available bioinformatics tool MiruMir.[55] Interestingly, high levels of expression for miR-34a correlated with better survival rates for breast cancer patients. These results suggest that miR-34a is an important prognostic marker.

Prostate Cancer (PCa)

PCa is the most frequent tumor and the second leading cause of cancer-related death in the United States.[56] Similar to breast cancer, the observation suggesting a direct role for *miR-34a* in PCa is its silence by aberrant CpG methylation of its promoter, and downregulation of *miR-34a* has been observed in prostate tumors compared with normal tissues.[11] The expression levels varied depending on the methodologies used for expression and controls.[57–59] There are contradictory findings regarding the increased levels of *miR-34a* achieved in PCa samples compared with benign counterparts,[60] which remains to be elucidated by more extensive studies. Indeed, a major limitation of these studies is the relatively small sample. Hence, it is unclear whether *miR-34a* has an oncogenic role in PCa. As malignancy is a heterogeneous disease, the role of *miR-34a* needs to be further examined in the specific subtypes of PCa.

Experiments reinducing *miR-34a* in PCa cells have led to decreased cell proliferation, invasion and increased apoptosis.[61] Cell line experiments were further extended by injecting *miR-34a* into PCa mouse models, which caused a significant decrease in tumor growth via inhibiting c-Myc oncogene, implicating the tumor-suppressive role of *miR-34a* in PCa.[61]

Emerging evidence indicates that CSCs may be involved in therapy resistance, tumor progression and metastasis.[62] Recent discoveries of miRs have provided a new avenue in understanding the regulatory mechanisms in CSCs. Down-expression studies demonstrated that miR-34a provides anti-tumor and anti-metastasis effects on tumorigenic cluster of differentiation 44^+ PCa stem cells and underlying mechanisms include the cluster of differentiation 44 downregulation, a key molecular marker in CSCs, indicating a potential therapeutic target for miR-34a (Figure 3).[63] In normal prostate stem cells, miR-34a also regulates prostate stem/progenitor cells in cooperation with p53, and hence *in vivo* studies show that inactivation of miR-34a and p53 promote aberrant expansion of prostate stem cell compartment, and may eventually lead to cancer. The findings suggest that the miR-34a gene is a bona fide tumor suppressor, and p53/miR-34a jointly negatively regulate downstream target MET expression as a key component of prostate stem cell compartment regulation, providing a valuable tool for rational design of diagnostic and therapeutic approaches (Figure 4).[64] Although PCa patients with androgen receptor-positive respond well initially to androgen therapies, a significant number of patients ultimately progress to androgen resistance, developing distant metastasis and death. The first-line treatment for hormone-refractory PCa mainly contains taxane-based chemotherapy regimens. Overexpression studies in PCa cell lines demonstrated that miR-34a provides a pro-

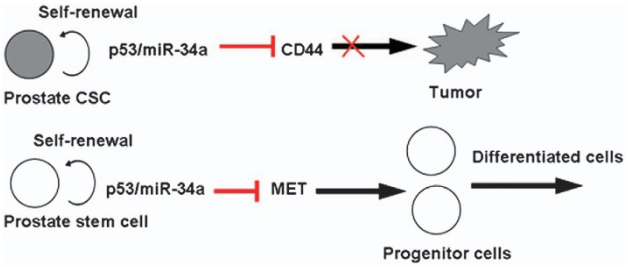

Figure 4 The function of miR-34a in prostate cancer stem cell (CSC) biology. CSCs have the capacity to self-renew and differentiate as well as the ability to regenerate tumors. In prostatic cancer, miR-34a inhibits cell migration and invasion through the inhibition of CD44 expression. In normal prostate stem cells, miR-34a also regulates prostate stem cells self-renewal through the inhibition of MET

apoptotic effect in the absence or presence of chemotherapy drugs in a P53-dependent manner. Negative correlations were observed in mRNA levels of SIRT1 and B-cell lymphoma/leukemia-2 (BCL2) controlled by miR-34a (Figure 3),[65] which may be served as an effective chemo-sensitizer in combined with standard chemotherapy for patients with tumor recurrence or metastasis.

Bladder Cancer

The current 5-year survival rate for muscle invasive bladder cancer is ~35% and the success rate for chemotherapy is only ~50%.[66] Further understanding of the mechanisms of chemotherapy failure is a critical first step for improvement in this survival rate. A finding by Vinall *et al.* is that increased *miR-34a* expression chemo-sensitizes bladder cancer cells to treatment with cisplatin, and inhibition of cyclin-dependent kinase 6 (CDK6) and SIRT1 (Figure 3), both of which are established targets of *miR-34a*, causes chemo-sensitization.[67] Undoubtedly, inhibition of CDK6 and SIRT1 has an important role in mediating *miR-34a*-induced chemo-sensitivity. Analysis of *miR-34a* expression in bladder cancer samples revealed that patients who express lower *miR-34a* expression may be associated with subsequent non-response to chemotherapy.[67]

The SNP analysis of 36 primary bladder tumors discovered allelic loss at chromosomal arm 1p[68] and loss of 1p was associated with bladder cancer progression.[69] These data implicated *miR-34a* gene as a tumor suppressor. However, a specific loss at 1p36.2, the region where the *miR-34a* gene is mapped, has not been reported in bladder cancer and future studies will determine whether loss of 1p36.2 occurs in bladder cancer.

NB

NB is one of the most common cancers in children, accounting for 15% of pediatric cancer deaths.[70] There type of genetic abnormalities containing loss of chromosome 1p, gain of 17q and MYCN amplification are strongly associated with poor prognosis.[70–72] In 2007, Welch and colleagues were the first to identify *miR-34a* as a pro-apoptotic factor in NB.[35] This has recently been confirmed by Kristina *et al.*[73] It is suggested that *miR-34a* is a suppressor of NB tumorgenesis, as it targets E2F3,[73] BCL-2[35] and MYCN (Figure 3).[35] Tivnan and colleagues further confirmed that exogenous *miR-34a* administration significantly reduces tumor growth in an *in vivo* mouse model of NB.[74] Moreover, in human NB samples, expression of *miR-34a* was also decreased in primary high-risk NB tumors with 1p36 deletion. Underlying mechanisms for *miR-34a* silenced in NB include 1p36 deletion, associated with an aggressive NB phenotype.[35,72] To examine whether underexpression of *miR-34a* is the cause of mutations in the TP53-coding region and mutations of the TP53-binding site in *miR-34a*, gene sequences were analyzed. No mutations were identified in the coding region of TP53 or in the TP53-binding site. Thus, additional work is necessary to understand the mechanism for inactivation of *miR-34a* in NB and therefore as novel therapeutic targets for patients with NB.[75]

Brain Tumor

Glioblastoma are the most common and deadly brain tumors in adults and medulloblastoma is the most common brain tumor in children. *miR-34a* downregulations have been identified in brain cancer.[76] In 2009, Li *et al.* analyzed for the first time *miR-34a* expression in human glioblastoma specimens and normal brain tissues by quantitative RT-PCR, and found that *miR-34a* expression is downregulated in glioblastoma specimen compaired with normal tissue in a p53-dependent manner.[39] Luan *et al.* further confirmed that *miR-34a* levels also deregulate in an experimental cell line in a p53-independent manner.[77] Whether or not p53 is a bona-fide TF of *miR-34a* in all cell types and species, cell line experiments further confirmed that *miR-34a* targeted multiple important molecular including the HGF/c-Met pathway, the Notch pathway and cell cycle regulator CDK6 (Figure 3). Transient transfection of *miR-34a* into glioma and medulloblastoma cell lines strongly inhibited cell proliferation, cell cycle progression, cell survival, cell invasion and *in vivo* glioma xenograft growth.[78,79] Interestingly, it was also shown that *miR-34a* expression induces glioma stem cell differentiation.[80]

In glioblastoma, TGFβ signaling pathway has been shown to act as a critical oncogenic factor in cancers[80] and TGFβ signaling is comprised of two transmembrane ser/thr kinase receptors (type I and type II) and two or three intracellular smad signal transducers. TGFβ superfamily ligands bind to the type II receptors, which phosphorylates the type I receptors. The type I receptor kinase is thereby activated and phosphorylates the receptor-regulated smads, which form heteromeric complexes with co-smads and accumulate in the nucleus to regulate gene transcription.[81] *In vitro* and *in vivo* functional experiments in mouse and human systems, both by exogenous genetic manipulation as well as by endogenous silence of *miR-34a*, show that *miR-34a* is a glioblastoma tumor suppressor and *miR-34a* loss regulates self-renewal and tumorigenesis in glioblastoma via activation of TGFβ signaling.[81] Mechanistically, *miR-34a* downregulated Smad4 and its downstream Id1 and Id3 (Figure 3), which was through direct binding to a conserved consensus region in the 3′UTR of Smad4 based on the 3′UTR luciferase reporter assay.[81] Last but not least, *miR-34a* expression level is shown to be clinical prognostic in glioblastoma, where patients with low-expressing *miR-34a* exhibited better overall survival.[81]

The above-mentioned studies clearly indicate that *miR-34a* potently inhibits brain tumor growth by targeting multiple oncogenes and might serve as a brain tumor therapeutic target. From a therapeutic point of view, *miR-34a* expression levels are positively correlated with medulloblastoma cell responsiveness to chemotherapeutic agents such as mitomycin C and cisplatin.[20] Further study identified a subset of MAGE-A genes as direct targets of *miR-34a* (Figure 3), and the repression of MAGE-A by *miR-34a* results in increased expression of p53, establishing a novel positive feedback mechanism involving *miR-34a* and p53, via direct targeting of MAGE-A. Further preclinical investigations will need to be performed to assess the feasibility of *miR-34a* replacement in combination with chemotherapy in brain cancer treatment.

Other Cancers

Apart from the widely investigated cancers mentioned above, *miR-34a* may also have important roles in other human cancers. As *miR-34a*'s potential roles in other cancers are relatively less researched, a limited number of studies are currently summarized in Table 1 and more roles for *miR-34a* in other cancers will be provided in the near future.

MiR34a-based Cancer Therapy

As the mechanism and role of miRs in human disease are gradually unraveled, recent studies also started exploring the role of miRs as therapeutics. Generally, the upregulation of miRs is achieved through administration of synthetic miRs or administration of miR-expressing vectors. The downregulation of miRs is achieved through administration of anti-sense nucleotides. *MiR-34a* acts as a tumor suppressor in multitype of cancer and emerging evidences have showed that *miR-34a*-based replacement therapy is a promising approach in cancer treatment. For instance, intratumor or systemic delivery of lipidic-formulated synthetic *miR-34a* induces anti-myeloma activity *in vivo* in mouse models of human myeloma without toxicity.[86,87] Similar results were noted that exogenous delivery of *miR-34a* to established tumors in mouse models of lung cancer resulted in dramatic suppression of tumor growth, implying the potential of miR replacement therapy in lung cancer.[85,94] Encouragingly, in human liver cancer, *miR-34a* has already entered phase I clinical trial (NCT01829971), and another miR-122-based therapy-LNA-antimir-122 (SPC3649) is successfully undergoing phase II trials.

Last but not least, the combination of miRs of the same network rather than individual miRs should be considered for assessing a biological response. As *miR-34a* and miR-15a/16 are frequently downregulated in the same tumor tissue, administrating a combination of both miRs may also potentiate their therapeutic impact.[95] Taken together, our results indicate that formulated synthetic *miR-34a* is an active agent against human tumor, which merits further investigation for clinical development in cancer disease.

Future Perspectives

In most of the above-mentioned diseases, *miR-34a* levels are downregulated, and thus lead to the recognition of this miR as a tumor suppressor. *MiR-34a*'s importance is further highlighted by the many factors known to be involved in its regulation. The complex network of regulatory mechanisms results in *miR-34a*'s tissue-specific expression as shown in various types of cancer. However, how *miR-34a* functions and how it is regulated must be determined by future studies.

These basic questions have translational perspectives. As *miR-34a* could be served as a therapeutic target, using *miR-34a* mimics may lead to its level restoration and the restoration of many target protein levels and thus to an improvement of the disease. MiR-based treatment is currently becoming a reality as there are biotech companies focusing on the use of miRs in theranostic as well as pharmaceutical

Table 1 Overview of miR-34a roles in other cancers

Cancer type	MiR-34a significance	Reference
Colon cancer	Low expression of miR-34a was detected in colon cancer tissues. Overexpression of miR-34a inhibited colon cancer cell migration and invasion by targeting Fra-1.	82
Lung cancer	Transfection of miR-34a could increase the sensitivity of both lung cancer cell lines to cisplatin in a p53-independent manner.	83
	Low levels of miR-34a expression were correlated with a high probability of relapse.	84
	Application of miR-34a into mouse model leads to significant decrease in tumor mass.	85
Myeloma	Therapeutic potential of synthetic miR-34a against human multiple myeloma was shown by repression of targets BCL-2 and CDK6.	86
Melanoma	MiR-34a targeting c-Met is partially responsible for decrease in cell growth and migration.	88
Leukemia	Introduction of miR-34a in K562 cells inhibits cell proliferation, induces cell-cycle arrest and promotes megakaryocyte differentiation.	89
	PMA induces miR-34a expression, which may explain PMA-induced megakaryocytic differentiation of the human chronic myelocytic leukemia cell line K562.	90
Pancreatic cancer	Restoration of miR-34a in pancreatic CSCs significantly reduced *in vitro* migration, invasion and anchorage-independent growth.	91
Osteosarcoma	MiR-34a inhibits growth and metastasis of osteosarcoma cells both *in vitro* and *in vivo* through targeting c-Met.	37
Hepatocellular carcinoma	MiR-34a inhibits migration and invasion of human hepatocellular carcinoma cells.	92
Ewing's sarcoma	MiR-34a is one of five identified microRNAs suggested to be predictors of outcome in Ewing's sarcoma.	93
Ovarian cancer	Levels of miR-34a are downregulated in ovarian cancers patients. Introduction of miR-34a in ovarian cancer cells resulted in reduced proliferation, motility and invasion.	94

Abbreviations: CSC, cancer stem cell; PMA, phorbol 12-myristate 13-acetate

companies finalizing preclinical research phases and proceeding to clinical trials. A phase I, open-label, multicenter study (NCT01829971) in liver cancer using miR MRX34, which restores the level of miR34a, is underway. The study evaluating the safety, pharmacokinetics and pharmacodynamics of MRX34 will be completed in 2014, and future research should be extended to other types of cancer. Currently, there is no report regarding the *in vivo* pharmacokinetics of miRs. Also, the effective delivery of synthetic therapeutic miRs to the desired target tissues will be a challenge. These challenges are a fundamental part of current miR research, and an exhaustive study of *miR-34a* can lead the way.

Conflict of Interest

The authors declare no conflict of interest.

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