MicroRNAs: Biogenesis, Functions and Potential Biomarkers for Early Screening, Prognosis and Therapeutic Molecular Monitoring of Nasopharyngeal Carcinoma

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Abstract: According to reports published, the aberrant expression of microRNAs (miRNAs), a class of 19–25 nucleotide-long small non-coding RNAs, is responsible for human cancers, including nasopharyngeal cancer (NPC). The dysregulation of miRNAs that act either as a tumor suppressor or oncogene, leading to a wide range of NPC pathogenesis pathways, includes the proliferation, invasion, migration as well as the metastasis of NPC cells. This article reviews and highlights recent advances in the studies of miRNAs in NPC, with a specific demonstration of the functions of miRNA, especially circulating miRNAs, in the pathway of NPC pathogenesis. Additionally, the possible use of miRNAs as early screening and prognostic biomarkers and for therapeutic molecular monitoring has been extensively studied.

Keywords: microRNA; circulating microRNA; biomarker; nasopharyngeal carcinoma

1. Introduction

In 1993, Victor Ambros and colleagues discovered the first microRNA (miRNA), namely lin-4, in Caenorhabditis elegans. Up to date, with in-depth research on microRNA (miRNA), a new era of early diagnosis, prognosis of human cancers as well as cancer treatment has commenced [1,2]. miRNAs are the family of naturally occurring small non-coding RNAs within 19–25 nucleotides (nts) in length. They play multiple key roles in regulating a wide array of biological processes, including cell proliferation, differentiation, metabolism, stress response and apoptosis through the regulation of numerous target genes [3–5]. miRNAs regulate biological processes by binding to sequences in the 3′-untranslated region (3′-UTR) of their target mRNAs, eventually resulting in the degradation and/or repression of mRNA [3–5]. Additionally, miRNAs have been observed to interact with other regions of target genes, including 5′-UTR, as well as promoter and coding regions. Each miRNA is reported to be the mediator of multiple genes by acting at the post-transcriptional levels. Consequently, both the mutation of miRNAs and dysfunction of miRNA biogenesis may lead to various human diseases, including cancers [6]. Growing evidences have indicated that miRNAs are frequently dysregulated in various cancers. Therefore, understanding their profile in cancer may identify them as powerful post-transcriptional biomarkers as well as highlight their great biomarker potential for the early screening, prognosis and therapeutic targets of human cancers.

Cancers are a serious disease to human life and health and have become the second leading cause of human death globally, accounting for an estimated 9.6 million deaths in 2018. It was emphasized that new cases of cancer could reach 23.6 million in 2030 [7]. Among them, nasopharyngeal carcinoma (NPC), a prevalent nasopharyngeal malignant tumor with remarkable differences in distribution and...
which gravitates toward Southern Asia, is considered to be the most common head and neck cancer. Even though improvements in nasopharyngeal cancer treatment have been achieved, diagnosis at an advanced stage reduces the success rate of treatment as well as the survival of patients. Thus, early screening and diagnosis represent beneficial opportunities to increase the survival of patients as well as the effects of nasopharyngeal cancer treatment. However, the non-specific symptoms related to the early stage of NPC, as well as the deeply seated location of the nasopharynx, are major obstacles to an early screening of NPC [8]. Therefore, effective biomarkers are truly needed [9]. At present, much effort has been made to identify early biomarkers by focusing on the etiological factors that lead to nasopharyngeal tumorigenesis. In addition to the etiology of Epstein–Barr virus infection, recently, the aberrant expression of miRNAs has been demonstrated to play key roles in the pathogenesis of NPC. Hence, an adequate understanding of the roles of miRNAs in NPC pathogenesis will provide more opportunities as well as better strategies to identify them as effective biomarkers of NPC. Additionally, it will pave the way for a better application of their functions in clinics, i.e., developing them into a promising approach in NPC therapies. In this article, we will summarize the key roles of miRNAs in the pathogenesis of NPC. Additionally, updated miRNA-related research data will be presented. Finally, we will demonstrate the application of miRNAs in NPC early screening, prognosis as well as cancer treatment.

2. Brief Understanding of miRNAs Biogenesis

Mature miRNAs are generated through a two-step processing of primary miRNAs in both the nuclear and cytoplasmic regions. miRNAs can be divided into intronic or intergenic miRNAs (Figure 1) [5,10,11]. Almost half of miRNA genes are located in the intergenic region (between two genes) and may exist either as a cluster of miRNA genes or a single miRNA gene under the control of its own promoter [5,11].

![Figure 1. Location of the microRNA (miRNAs) gene in the genome: (A) intergenic miRNAs: miRNAs located between two genes; (B) intronic miRNA: miRNAs located in the region of intron in a gene.](image)

The biogenesis of miRNAs is classified into two categories: the canonical pathway and non-canonical pathway. In the canonical pathway, the primary miRNAs (pri-miRNAs) are transcribed from their genes and cleaved into pre-miRNAs by two main enzymes, Drosha and Dicer [5]. In this dominant pathway, the biogenesis of miRNAs, as well as the mechanism of regulation of their target genes’ expression, is summarized in the steps shown in Figure 2. The mature miRNA originates from a long primary miRNA (pri-miRNA) with the structure of 5’-7 methyl-guanosine capped and 3’ polyadenylated, and it is transcribed by RNA-polymerase II (RNA-polyII) from its own promoter or the promoter of the host genes in which it is contained; it is controlled by RNA-polyII-associated transcription factors and epigenetic regulators [12–14]. Pri-miRNA contains a hair-spin structure which varies from hundreds to thousands of base pairs in length [10,15]. Pri-miRNA is cleaved at the stem of the hair-spin structure, leading to the release of a ~60–70 nucleotide hair-spin structure, which is termed as the precursor miRNA (pre-miRNA). This process is catalyzed by the microprocessor, which contains the nuclear RNase III-type protein Drosha and its cofactor, the DiGeorge syndrome critical region eight (DGCR8) protein found in humans, and takes place in the nucleus [16]. The pre-miRNA with a stem-loop structure is exported from the nucleus into the cytoplasm for further processing by exportin-5 (EXP 5), originally known as a minor export factor for tRNAs, and the Ras-related nuclear protein guanosine triphosphate (RAN-GTP) [11,15,17]. Then, the terminal loop of the pre-miRNA is removed by RNA III Dicer and its cofactor transactivation-responsive RNA-binding protein (TRBP), thereby releasing a
~20–22 nucleotide miRNA-duplex, which contains two 5’ phosphorylated sequence strands with 3’ overhangs, named the mature miRNA guide strand (miRNA) and complementary passenger strand (miRNA*) [17,18]. The miRNA-duplex is loaded into an Argonaute protein (Ago protein) to generate a RNA-induced silencing complex (RISC), and the miRNA passenger is degraded. The mature miRNA is incorporated into the miRNA-induced silencing complex (miRISC), which participates in regulating the gene expression via the interaction between a region called the “seed” (2–8 nt), a site located at the 5’ part of miRNA (positions 2–7) and the target mRNAs 3’-UTR by a Watson–Crick complementary sequence, leading to the degradation of the mRNA (in case of perfect pairing between the miRNAs/mRNA target) or the blocking of mRNA translation (in case of imperfect pairing (Figure 3)) [3,11,19]. Up to date, some miRNAs are generated by different non-canonical pathways, including: (1) miTrons (pri-miRNAs are encoded in the introns of coding genes); (2) Dicer-independent miRNAs; (3) tRNA-derived miRNAs [5]. These non-canonical pathways use many different combinations of proteins which are involved in another pathway—the canonical pathway, including Dicer, exportin-5 andAGO2. It is noted that the Dicer protein is necessary in both canonical and non-canonical pathways, while Drosa and DGCR8 are only functioned to canonical miRNAs. In other words, the non-canonical miRNAs could be generated in the case of a Drosha and DGCR8 absence [5].

Figure 2. The biogenesis of miRNAs: the canonical pathway. pri-miRNA: primary miRNA; pre-miRNA: precursor miRNA; DGCR8: DiGeorge syndrome critical region 8; RAN-GTP: Ras-related nuclear protein guanosine triphosphate; TRBP: transactivation-responsive RNA-binding protein; Ago: Argonaute protein; RISC: RNA-induced silencing complex; miRISC: miRNA-induced silencing complex.

Figure 3. The interaction types of miRNA–mRNA (target mRNA). Site matching between the seed region of miRNA (at positions 2–8) and the 3’-end of the target mRNA. The character of N represents the single Watson–Crick paring nucleotide. Pol(A): Poly-A tail; A: Adenine.
3. The Roles of miRNAs in NPC Tumorigenesis: Potential Biomarkers for NPC Early Screening, Prognosis, and Therapy

The first study to reveal the roles of miRNA in nasopharyngeal tumorigenesis was published in 2008 by Sengupta and colleagues [20]. In their report, by using microarray-based approaches, they identified eight miRNAs with significantly different expressions in laser-capture micro-dissected NPC samples compared with normal nasopharyngeal epithelial samples. Among them, six miRNAs, including hsa-miR-29c, hsa-miR-34b/c, hsa-miR-212, hsa-miR-216, and hsa-miR-217, exhibited a lower expression in tumor cells, and two miRNAs, hsa-miR-151 and hsa-miR-192, showed a higher expression in NPC tumor cells. Particularly, the target genes were reported to be associated with the lower expression of hsa-miR-29c, and its 15 target genes’ levels, including FLJ12505, COL4A1, COL4A2, COL5A2, COL3A1, COL1A2, FBN1, SPARC, COL15A1, FUSIP1, COL1A1, TFEC, IFNG, LAMC1, LAMC1, showed a two- and six-fold increase in NPC tumors compared with normal nasopharyngeal epithelium. Their abnormal expression was reported to be associated with tumor cell invasiveness, metastasis and metastatic potential, which are prominent characteristics of NPC. Hence, since the first report, growing evidences on miRNA regulatory networks in NPC progression have emerged. Over the years, numerous studies have indicated that a high proportion of miRNAs play a significant role in NPC pathogenesis. The dysregulation of miRNAs that act either as tumor suppressors (down-regulation) or oncogenes (up-regulation) leads to the abnormal regulation of a range of NPC-associated biological pathways, such as cell proliferation, apoptosis, survival and metastasis, which play a crucial role in nasopharyngeal carcinogenesis (Figure 4) [21,22].

In terms of the mechanism, miRNAs act as oncogenic and tumor suppressor miRNAs in tumorigenic events. In the function of tumor suppressor miRNAs, miRNAs are down-regulated,
resulting in a potentially enhanced expression of oncogenes, promoting cell proliferation, migration, angiogenesis, invasion, and metastasis, whereas oncogenic miRNAs are over-expressed in nasopharyngeal tumorigenesis, leading to the inhibition of tumor suppressor genes, and, as a result, they increase the proliferation, migration and invasion of NPC cells as well as the loss of cell cycle regulation and they inhibit cell apoptosis.

3.1. miRNAs as Tumor Suppressor Genes

Generally, tumor suppressor miRNAs can down-regulate different oncogenes, which are revealed to promote cell proliferation, migration, angiogenesis, invasion, and metastasis [23]. miRNAs that act as tumor suppressors are reported to be often down-regulated in NPC pathogenesis [22,24,25]. The down-regulation of tumor repressor miRNAs, which are negative regulators of protein-coding genes, leads to an up-regulation of their target genes and the subsequent alterations of the associated cellular pathways in NPC [24]. Many miRNAs (including hsa-Let-7, hsa-miR-9, hsa-miR-26, hsa-miR-29, hsa-miR-30, hsa-miR-34, hsa-miR-201, hsa-miR-218, hsa-miR-223, hsa-miR-331, hsa-miR-429, etc.) are reported to be tumor suppressor genes that play vital roles in nasopharyngeal carcinoma [26–55]. Bioinformatic prediction tools in combination with experimental analysis, including NPC culture cell analysis, case-control studies, etc., have demonstrated that miRNAs could influence multiple steps of tumorigenesis by interacting with target genes (Table 1). Understanding the role of tumor suppressor genes in NPC has the potential to establish the prognostic biomarkers and therapeutic targets of NPC.

### Table 1. List of miRNAs that act as tumor suppressor genes in nasopharyngeal carcinoma (NPC).

| miRNAs       | Functions and Target Genes                                                                                                                                 |
|--------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Let-7 family | Promoting the cell proliferation and apoptosis, the epithelial mesenchymal transition by directly regulating the MYC [25], high-mobility group A2 (HMGA2) [26,27], and enhancer of zeste homolog 2 (EZH2) [28]. |
| hsa-miR-9    | Inhibiting the nasopharyngeal tumor cell proliferation, migratory and invasion by targeting the 3′ untranslated region of chemokine (C-X-C motif) receptor 4 (CXCR4) through activation of the Mitogen-activated protein kinase (MAPK) pathway [29,30]; modulating the immune response through targeting numerous interferon-regulated genes: IFIH1, IFI27, PSB9_HUMAN, PSMB8, IFI5, PSMB16, IFIT2, TRAIL, IFIT1, PSB8_HUMAN, IRF1, B2M and GPB1, MHC class I molecules and interleukin (IL)-related genes: IL20RB, GALT, IL7, IL11, IL11R, IL1A, IL6 and IL17R [31]. |
| hsa-miR-16   | Inhibiting the NPC cell proliferation, migration, invasion, metastatic colonization by targeting fibroblast growth factor 2 (FGF2) via phosphoinositide-3-kinase/akt (PI3K/AKT) and mitogen-activated protein kinase (MAPK) signaling pathways [32]. Additionally, Knocking-down CDK4 leads to the induction of miR-16, resulting in inhibiting the cell growth [33]. |
| hsa-miR-26   | Suppressing the growth of NPC cell as well as the formation of colony by inducing G1 cell-cycle arrest. Additionally, hsa-miR-26 targets the CDK inhibitors p14 (ARF), p21 (CIP1), zeste homolog 2 (EZH2). T cell lymphoma invasion and metastasis 1 (TIAM1), leading to decrease oncogenic properties of migration, invasion, and cell survival [34,35]. |
| hsa-miR-29c  | Suppressing the NPC cells migration and invasion through the hsa-miR-29c/TIAM1 pathway to inhibit the translation of T cell lymphoma invasion and metastasis (TIAM1) [36,37], as well as targeting the multiple miRNAs, which encoded extracellular matrix proteins, includes seven collagens, laminin γ1, fibrillin, and secreted protein, acidic, cysteine-rich (SPARC) [20]. Additionally, miR-29c can involve to numerous pathways to suppress the proliferation, survival, and motility of NPC cells [21]. |
| hsa-miR-30a  | Regulating the invasion and metastasis of nasopharyngeal cancer through epithelial-mesenchymal transition by inhibiting the E-cadherin via targeting 3′-untranslated region of E-cadherin [38]. |
| hsa-miR-34b, hsa-miR-449a | Inhibiting the nasopharyngeal malignancy progression through targeting lactate dehydrogenase A (LDHA) [39]. |
| hsa-miR-34c  | Suppressing the growth and metastasis of NPC tumor by targeting MET proto-oncogene (MET) through the pathway of hsa-miR-34c/MET pathway [40]. |
| hsa-miR-98, hsa-miR-101 | Inhibiting the cellular processes, including cell differentiation, development as well as apoptosis through targeting the expression EZH2 [34]. |
| hsa-miR-124 | Inhibited cell growth, migration and invasion by repressing Foxq1 expression [41]. |
Table 1. Cont.

| miRNAs   | Functions and Target Genes                                                                                                                                                                                                 |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hsa-miR-138 | Suppressing cell proliferation, colony formation and nasopharyngeal tumorigenesis by knocking-down the expression of Cyclin D1 (CCND1) [42].                                                                                       |
| hsa-miR-142 | Suppressing NPC cell proliferation, invasion and metastasis by directly binding to 3′-UTR region of suppressor of cytokine signaling 6 (SOCS6) [43]. Additionally, the hypermethylation of DNMT1 leading to the silence of hsa-miR-142 also promotes the metastasis through targeting zinc finger E-box binding homeobox 2 (ZEB2) [44]. |
| hsa-miR-200b | Inhibiting the NPC cell growth, migration, and invasion. EBV-encoded EB nuclear antigen 1 (EBNA-1) suppresses the expression of hsa-miR-200b, results in upregulating zinc finger E-box binding homeobox 1 and 2 (ZEB1, ZEB2) [46]. Additionally, Notch1 was identified as the direct target gene of hsa-miR-200b [45]. |
| hsa-miR-204 | Inhibiting the invasion of NPC. Latent membrane protein 1 (LMP-1) of Epstein-Barr Virus suppresses the expression of miR-204 through activating Stat-3 and enhances cell division cycle 42 (CDC42) to enhance the NPC invasion [46]. |
| hsa-miR-200b | Inhibiting the NPC cell proliferation, invasion and cell growth by targeting KRAS through the inhibition of the KRAS-related AKT and ERK pathways [47], as well as binding to the 3′-untranslated region (UTR) of PKCα [48]. |
| hsa-miR-216b | Targeting to enhancer of zeste homolog 2 (EZH2) to inhibit the differentiation, development, and apoptosis [34]. Additionally, causing the significant toxicity in NPC leads to the inhibition of NPC cell growth via the SLIT-ROBO pathway [49]. |
| hsa-miR-218 | Promoting the nasopharyngeal cell transformation via the positively control protein synthesis by stimulating ribosomal protein mRNA translation and ribosome biogenesis [57]. |
| hsa-miR-223 | Promoting the nasopharyngeal carcinoma cells migration and invasion, which related genes, including E-cadherin, Vimentin, and MMP-9, were identified [58]. Additionally, over-expression of hsa-miR-223 directly suppresses the expression of PTEN, which is a key regulator of AKT phosphorylation, resulting in enhancing the radio-resistance of NPC via the PTEN/AKT pathway [61]. |
| hsa-miR-21 | Promoting the radio-resistance by targeting Rab27B [64]. |

3.2. miRNAs as Oncogenes

Several miRNAs are reported as oncogenes, known as oncogenic miRNAs (onco-miRNAs), which are over-expressed in nasopharyngeal tumorigenesis. Their activities have been found to increase the proliferation, migration and invasion of NPC cells, the loss of cell cycle regulation and inhibition the cell apoptosis [22,24,56]. Many onco-miRNAs (including hsa-miR-10a/b, hsa-miR-17, hsa-miR-18, hsa-miR-21, hsa-miR-93, hsa-miR-141, hsa-miR-144, hsa-miR-155, hsa-miR-205, hsa-miR-214, hsa-miR-378, hsa-miR-421, hsa-miR-663, hsa-miR-3135, etc.) are reported to be associated with nasopharyngeal tumorigenesis (Table 2) [57–92].

Table 2. List of miRNAs as oncogenes in NPC.

| miRNAs   | Functions and Target Genes                                                                                                                                                                                                 |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hsa-miR-10a | Fascinating the ability of nasopharyngeal cell transformation via the positively control protein synthesis by stimulating ribosomal protein mRNA translation and ribosome biogenesis [57]. |
| hsa-miR-10b | Promoting the nasopharyngeal carcinoma cells migration and invasion, which related genes, including E-cadherin, Vimentin, and MMP-9, were identified [58]. Additionally, over-expression of hsa-miR-10b also correlated with LMP-1 and Twist1 through the regulation of LMP1/Twist1 pathway in NPC malignancy [59]. |
| hsa-miR-17 | Promoting the proliferation of nasopharyngeal cell by targeting p21 [60]. Additionally, the over-expression of hsa-miR-17 directly suppresses the expression of PTEN, which is a key regulator of AKT phosphorylation, resulting in enhancing the radio-resistance of NPC via the PTEN/AKT pathway [61]. |
| hsa-miR-18a | Promoting the nasopharyngeal malignant progression by widespread downregulation of the miRNome and regulating Dicer1 expression [62]. Moreover, hsa-miR-18a promotes the progression of NPC through miR-18a/SMG1/mTOR pathway [63]. |
| hsa-miR-20a | Promoting the radio-resistance by targeting Rab27B [64]. |
| hsa-miR-21 | Promoting migration and proliferation by inhibiting the B cell CLL/lymphoma 2 (BCL2) expression [65]. Additionally, the expression of STAT3 activates the hsa-miR-21, resulting in inducing the nasopharyngeal carcinoma cell proliferation and suppressing the cell apoptosis through targeting PTEN gene (PTEN/AKT pathway) [66]. |
was identified by Chim et al. in 2008 [99]. They discovered the existence of placental miRNAs, (ECmiRNA) [94]. Two major populations of c-miRNAs (including non-vesicle-associated c-miRNAs and progression, tumor colony formation, invasion, metastasis as well as radio/vesicle-associated c-miRNAs, which reflect the mechanism of c-miRNAs release) have been identified. Processes 2020

expression of miR-21 was associated with relapse-free survival [100]. Hence, the study highlighted in the serum from di

levels of tumor-related miRNAs, including miR-155, miR-210, and miR-21, were significantly higher reverse-transcription real-time PCR [99]. Two months later, Lawrie et al. in 2008 reported that the including miR-141, miR-149, miR-299-5p, and miR-135b, in maternal plasma using quantitative However, the mechanism of c-miRNAs release is still unclear [98]. The first profile of c-miRNAs c-miRNAs Functions and Target Genes

| miRNAs     | Functions and Target Genes                                                                                                                                                                                                 |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hsa-miR-30a| Increasing the nasopharyngeal carcinoma cell invasion and metastasis through epithelial-mesenchymal transition by targeting E-cadherin gene via GF-IR-Src-MicroRNA-30a-E-Cadherin Pathway [67,68]. |
| hsa-miR-93 | Promoting cell proliferation, invasion and metastasis via the inhibition of transforming growth factor-β receptor II (TGFβRII) through the attenuation of Smad-dependent TGF-β signaling and the activation of PI3K/Akt pathway [69] and disabled homolog-2 (DAEB2) [70]. Additionally, hsa-miR-93 enhances the cell proliferation by directly targeting expression of CDKNA gene [71]. |
| hsa-miR-141| Increasing the cell growth, migration, invasion as well as the regulation of cell cycle, and reducing the cell apoptosis through inhibiting BMI1 via hsa-miR-141/BMI1 signaling axis [72], and increasing the tumor formation by targeting phosphatase and tensin homolog (PTEN) via BRD7/hsa-miR-141/PTEN/AKT pathway [73,74]. |
| hsa-miR-144| Promoting the cell proliferation, migration, invasion through the repression of PTEN to active PI3K/Akt pathway [75,76]. Additionally, downregulation of hsa-miR-144 by triptolide enhanced the formation of p85α-PTEN complex, resulting in causing the S phase arrest of NPC cells [77]. |
| hsa-miR-149| Promoting the epithelial-mesenchymal transition, nasopharyngeal cell mobility and invasion was facilitated by LncRNA-LINC00460 through sponging hsa-miR-149-5p [78,79]. |
| hsa-miR-155| Promoting the nasopharyngeal carcinoma cells’ proliferation, migration and invasion through regulating the PTEN and AKT signaling. Additionally, over-expression of hsa-miR-205 resulted in the down-regulation of E-cadherin and up-regulation of Snail proteins, led to the NPC cell proliferation, invasion [83]. The repression of apoptosis and stimulation of cell proliferation were also through targeting tumor protein p53-inducible nuclear protein 1 [84]. |
| hsa-miR-205| Promoting the nasopharyngeal carcinoma cells’ proliferation, invasion and metastasis through the inhibition of p53 and p53-dependent pathways. Moreover, hsa-miR-205 up-regulated the expression of E-cadherin, which is a negative regulator of cell proliferation and invasion [85]. |
| hsa-miR-214| Promoting the cell proliferation and repressing the cell apoptosis by targeting Bim (Bcl-2-interacting mediator of cell death) [85]. Additionally, the targeting of hsa-miR-214 is responsible for downregulating LTF in the NPC specimens [86]. |
| hsa-miR-378| Promoting the nasopharyngeal cancer cell proliferation, colony formation, and invasion by downregulating the Transducer of ERBB2 (TOB2) expression [67]. |
| hsa-miR-421| Promoting the cell proliferation and apoptosis via downregulating the expression of FOXO4 [88]. |
| hsa-miR-504| Promoting the nasopharyngeal malignant progression through the targeting EIF4A2 and HSPG2 [90]. |
| hsa-miR-774| Promoting cell proliferation, invasion and metastasis via the inhibition of TGF-βRI kinase activity. Additionally, hsa-miR-774 inhibited the expression of Snail proteins, leading to the NPC cell proliferation, invasion [83]. |
| hsa-miR-3182| Promoting the cell proliferation and inhibition of cell apoptosis through targeted expression of E-cadherin and up-regulation of Snail proteins, leading to the NPC cell proliferation, invasion [83]. |
| hsa-miR-30a| Increasing the nasopharyngeal carcinoma cell invasion and metastasis through epithelial-mesenchymal transition by targeting E-cadherin gene via GF-IR-Src-MicroRNA-30a-E-Cadherin Pathway [67,68]. |
| hsa-miR-141| Promoting cell proliferation, invasion and metastasis via the inhibition of transforming growth factor-β receptor II (TGFβRII) through the attenuation of Smad-dependent TGF-β signaling and the activation of PI3K/Akt pathway [69] and disabled homolog-2 (DAEB2) [70]. Additionally, hsa-miR-93 enhances the cell proliferation by directly targeting expression of CDKNA gene [71]. |
| hsa-miR-149| Promoting the epithelial-mesenchymal transition, nasopharyngeal cell mobility and invasion was facilitated by LncRNA-LINC00460 through sponging hsa-miR-149-5p [78,79]. |
| hsa-miR-155| Promoting the nasopharyngeal carcinoma cells’ proliferation, migration and invasion through regulating the PTEN and AKT signaling. Additionally, over-expression of hsa-miR-205 resulted in the down-regulation of E-cadherin and up-regulation of Snail proteins, led to the NPC cell proliferation, invasion [83]. The repression of apoptosis and stimulation of cell proliferation were also through targeting tumor protein p53-inducible nuclear protein 1 [84]. |
| hsa-miR-205| Promoting the nasopharyngeal carcinoma cells’ proliferation, invasion and metastasis through the inhibition of p53 and p53-dependent pathways. Moreover, hsa-miR-205 up-regulated the expression of E-cadherin, which is a negative regulator of cell proliferation and invasion [85]. |
| hsa-miR-214| Promoting the cell proliferation and repressing the cell apoptosis by targeting Bim (Bcl-2-interacting mediator of cell death) [85]. Additionally, the targeting of hsa-miR-214 is responsible for downregulating LTF in the NPC specimens [86]. |
| hsa-miR-378| Promoting the nasopharyngeal cancer cell proliferation, colony formation, and invasion by downregulating the Transducer of ERBB2 (TOB2) expression [67]. |
| hsa-miR-421| Promoting the cell proliferation and apoptosis via downregulating the expression of FOXO4 [88]. |
| hsa-miR-504| Promoting the nasopharyngeal malignant progression through the targeting EIF4A2 and HSPG2 [90]. |
| hsa-miR-774| Promoting cell proliferation, invasion and metastasis via the inhibition of TGF-βRI kinase activity. Additionally, hsa-miR-774 inhibited the expression of Snail proteins, leading to the NPC cell proliferation, invasion [83]. |
| hsa-miR-3182| Promoting the cell proliferation and inhibition of cell apoptosis through targeted expression of E-cadherin and up-regulation of Snail proteins, leading to the NPC cell proliferation, invasion [83]. |

4. Circulating miRNA: Potential Values in Nasopharyngeal Carcinoma Early Screening, Prognosis and Cancer Treatment

As described above, the over-expression of oncogenic miRNAs or down-expression of tumor suppressor miRNAs are reported to be strongly associated with nasopharyngeal tumorigenesis, progression, tumor colony formation, invasion, metastasis as well as radio/chemical resistance. Notably, recent growing evidence indicates that several miRNAs could be detected in biological fluids, including blood serum/plasma, tears, urine, etc., which reflect the pathophysiological condition of cancer [93–97]. These miRNAs are termed circulating miRNAs (c-miRNAs) or extracellular miRNA (ECmiRNA) [94]. Two major populations of c-miRNAs (including non-vesicle-associated c-miRNAs and vesicle-associated c-miRNAs, which reflect the mechanism of c-miRNAs release) have been identified. However, the mechanism of c-miRNAs release is still unclear [98]. The first profile of c-miRNAs was identified by Chim et al. in 2008 [99]. They discovered the existence of placental miRNAs, including miR-141, miR-149, miR-299-5p, and miR-135b, in maternal plasma using quantitative reverse-transcription real-time PCR [99]. Two months later, Lawrie et al. in 2008 reported that the levels of tumor-related miRNAs, including miR-155, miR-210, and miR-21, were significantly higher in the serum from diffuse large B-cell lymphoma compared with the control sera. Notably, the high expression of miR-21 was associated with relapse-free survival [100]. Hence, the study highlighted
c-miRNAs as non-invasive powerful diagnostic markers for human cancers. Since then, the new class of miRNA, c-miRNAs, has opened up new molecular markers for human cancer monitoring.

Due to their many essential characteristics, c-miRNAs have been considered as valuable biomarkers for the early screening and diagnosis of human cancers, including NPC. c-miRNAs are stable in circulation and resistant to RNase activities in the extracellular environment [93,94]. Additionally, c-miRNAs remain stable with a considerable level of expression even when they are subjected to harsh conditions, including an extreme pH level (high or low pH), extended storage, boiling, and multiple freeze-thaw cycles [93,94,101]. However, the mechanisms by which c-miRNAs maintain their remarkable stability in a RNase-rich extracellular environment as well as in harsh conditions are still not well understood. The levels of c-miRNAs could easily be evaluated by various methods, such as qRT-PCR and oligonucleotide microarray [102,103]. Therefore, c-miRNAs are identified as suitable molecular targets for human cancer early screening, prognosis as well as treatment.

In several reports, interestingly, the potential uses of the c-miRNA expression profile are reported as biomarkers for early screening, and the prognosis and diagnosis of NPC [96,104]. In the study conducted by Zeng et al. in 2012, they performed the evaluation of serum miRNA profiling on the sera of twenty NPC patients and twenty non-cancerous controls. Four serum c-miRNAs, c-miR-223, c-miR-29c, c-miR-20a and c-miR-17, were significantly expressed in NPC patients’ sera compared with the expression in the non-cancerous controls. Thus, serum-based biomarkers, especially the four serum c-miRNAs, are potential biomarkers for NPC diagnosis [105]. Yi et al. in 2019 reported that the low expression of c-miR-31-5p was significantly associated with the NPC tumor-node-metastasis (TNM) stage: Stage I + II vs. III + IV, T1 vs. T2 + T3 + T4 and N1 + N3 vs. N0. Moreover, c-miR-31-5p showed a moderate diagnostic performance. Therefore, c-miR-31-5p was concluded as the non-invasive valuable, and as a novel biomarker for the early diagnosis of NPC. Especially, it was reported to be an attractive therapeutic molecular target for NPC treatment [106]. Exosome-associated miRNAs, a type of vesicle-associated c-miRNA, are derived from cancer and could interact with endothelial cells, thereby enhancing proliferation, migration as well as angiogenesis [107]. Exosomal miR-24-3p was reported to be involved in nasopharyngeal pathogenesis by mediating T-cell suppression via the repression of fibroblast growth factor 11 (FGF11), and it may serve as a potential biomarker in NPC [108]. Up to date, exosome-associated miRNAs are still of interest in finding potential biomarkers for NPC. Other exosome-associated miRNAs, such as metastasis-associated miR-23a [109], exosomal miR-9 [107], exosomal miR-34c [110], etc., have been reported. Their data suggested that exosomal miRNAs reflect the pathogenesis of NPC and that there is the possibility of exosome-based therapies for NPC in the future.

5. miRNAs as Novel Molecular Targets for NPC Therapies

As described, strikingly different miRNA expressions have been observed in NPC; therefore, the activation of tumor suppressor miRNAs and/or the inhibition of oncogenic miRNAs may open up a novel approach for the development of therapeutics/treatments for NPC. Up to date, an increasing number of studies have focused on the possibility of miRNAs serving as molecular targets for NPC therapies. In the study conducted by Kang et al. in 2016, they investigated the effects of miR-24 on the radio-sensitivity of NPC cells. They found that the miR-24/SP1 pathway contributed to the reduction in radio-resistance in human NPC [111]. Therefore, the finding of the miR-24/SP1 pathway may help our understanding of radio-sensitivity in NPC and represents a promising therapeutic molecular target. Thus, based on the roles of miRNAs, more and more studies are applying miRNAs to NPC therapy.

6. Conclusions

Since the first discovery of miRNAs, numerous studies have focused on their roles in human tumorigenesis, including NPC. As described in this article, the abnormal expression of miRNAs, especially c-miRNAs, have been shown to play important roles in the hallmarks of NPC by targeting many pathways, including cell proliferation, invasion and migration. Especially, such detectable
changes in non/less invasive samples (plasma, serum, etc.) make them promising non-invasive biomarkers for the early screening, prognosis, and therapeutic molecular target monitoring of NPC.

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