Review Article

Study of the correlationship link between microRNAs and nasopharyngeal carcinoma

Xin-Hua Xu, FRCOG, MM, *Yang Liu,* FRCOG, MM.

ABSTRACT

سرطان البلعوم الأنفي(NPC) هو مرض عديد الجينات تتصل بمجموعة متنوعة من العوامل. مع تطور تقنيةmicroRNAs (miRNAs) ،وجد الباحثون وجود صلة وثيقة بين miRNAs وNPC. يوجد لmiRNAs تعبيرات مختلفة في NPC وأنسجة البلعوم العادي؛ العلاج الإشعاعي والعلاج الكيميائي يؤثر أيضاً على تعبير لل miRNAs في أنسجة NPC. وأظهرت الدراسات أيضاً أن خلل الانقسام والتعبير المنتبذ لل miRNAs شاركوا في حدوث وتطور NPC عن طريق التأثير في التعبير عن الجينات المسرطنة أو الجينات الكابتة للأورام ،أو تعمل على نقل مسارات الإشارات ذات الصلة،فلها أهمية كبيرة للتحقيق من miRNAs وحدوث وتطور NPC للوقاية والعلاج من NPC.

Nasopharyngeal carcinoma (NPC) is a polygenic disease related to a variety of factors. With the development of the microRNAs (miRNAs) technique, researchers found a close link between miRNAs and NPC. Differentially expressed miRNAs exist in NPC and normal nasopharyngeal tissues; radiotherapy and chemotherapy also affect the expression of miRNAs in NPC tissues. Further studies showed that dysregulation and ectopic expression of miRNAs were involved in the occurrence and development of NPC by affecting the expression of oncogenes or tumor suppressor genes, or acting on relevant signal transduction pathways. It is of great significance to investigate the miRNAs related to the occurrence and development of NPC for the prevention and treatment of NPC.

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From the Oncology Department, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yiling Road, Yichang, China.

Address correspondence and reprint request to: Prof. Xin-Hua Xu, Oncology Department, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, 183 Yiling Road, Yichang 443003, China. Tel. +86 (717) 6487360. Fax. +86 (717) 6481291. E-mail: xuxinhua@medmail.com.cn

Nasopharyngeal carcinoma (NPC) is a multi-gene genetic disease, which occurs mainly in southern China, Southeast Asia, and North Africa. Genetic susceptibility, Epstein-Barr virus (EBV) infection, environmental, dietary, such as intake of pickled foods and other factors are associated with the tumorigenesis and development of NPC. Its development is also a multistage process, depending on the regulation of gene expression in time and space. Due to its uncertain early symptoms and late diagnosis, NPC has caused a serious global health problem. Therefore, it is necessary to clarify the cellular and molecular mechanisms of dynamic development of NPC, to determine its biomarker in process and detect its high-risk factors. MicroRNAs (miRNAs), as a negative post-transcriptional regulator, have been a hotspot in research for their dysregulation, ectopic expression, and involvement in biological processes of tumor.[1,2] Here, we summarized the miRNAs that were involved in the occurrence and development of NPC, providing new ideas for monitoring and treatment of NPC.

The structure and biological characteristics of microRNAs. MicroRNAs are a class of high abundance, evolutionarily conserved, endogenous non-coding small single-stranded RNAs molecules, consisting of 18 to 25 nucleotides (nt), widely present in plants and animals. They are transcribed by RNA polymerase II, to generate primary miRNA transcripts (pri-miRNAs), which are processed into approximately 70-nt precursor-miRNA (pre-miRNAs) with a hairpin structure by enzyme Drosha, a RNaseIII endonuclease located in the nucleus. After being transported to the cytoplasm by Exportin 5, pre-miRNAs are further processed by another RNase III endonuclease Dicer, which interacts with a group

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of proteins including Argonaute2 (Ago2) to mediate the process of pre-miRNAs, and the assembly of the miRNA-containing RNA-induced silencing complex (miRISC), to generate the approximately 22-nt mature miRNAs, and exert their effects through the miRISC.[3] The mutation/genetic polymorphisms of the promoter region in pri-miRNAs, pre-miRNAs, or miRNAs can affect the shear, processing, maturation and expression of miRNAs. Studies have shown that more than 50% of miRNAs have been mapped to tumor-associated genomic regions or fragile sites, and differential expression of miRNAs could be caused by the chromosome anomalous amplification, deletion, or translocation in these regions.[4] Similar to protein-coding genes, the transcription of miRNAs is also regulated by transcription factors (TFs), and the aberrant regulation of miRNAs by TFs can lead to phenotypic variations, even diseases. The TFs that play an important regulatory role in the miRNAs expression include ETS2, MYB, SP1, KLF6, NFE2, PCBP1, and TMEM54.[5] Closing down chromatin structure by excessive DNA methylation/decreased histone modification levels also inhibited the miRNAs expression.[6] Regarding the functionary mechanism of miRNAs, compared with the exogenous RNA interference (RNAi) effector molecules, the significant difference is that miRNA targets its mRNA by complete or incomplete complementary pairing, then induces the degradation of mRNAs through the classic RNAi pathway or inhibiting protein translation, or guiding its targeted mRNA to deadenylate rapidly, resulting in fast decay of mRNA or depression of mRNA level.[1] The MiRNAs have been found to regulate genes involved in a series of diverse biological processes, including development, differentiation, stress response, angiogenesis, adhesion, proliferation, and apoptosis and so on. The dysregulation of miRNAs may play a crucial role in tumor pathogenesis.[2]

MicroRNAs and nasopharyngeal carcinoma. Research has shown that miRNAs are expressed differentially in NPC tissue/cell lines with various degrees of differentiation and normal nasopharyngeal epithelial (NPE). Chemoradiotherapy can affect their expression in cell lines, with different sensitivity to chemotherapy and radiotherapy and the direction of regulation. This is not only related to the dysregulation of miRNAs, but also EBV, which makes a great contribution to NPC, and has the function of encoding miRNAs. The MiRNAs expressed differentially is involved in the development of NPC by acting on the special genes or signaling pathway, and is closely associated with their expression level.

MicroRNAs differentially expressed in NPC at various degrees of differentiation and normal nasopharyngeal epithelium. Li et al[7] detected 8 cases of NPC tissues, and 4 cases of normal nasopharyngeal tissues with a total of 735 human miRNAs in each sample using an Illumina microarray platform. Thirty-four miRNAs in all were identified to be differentially expressed. Of these, compared with the normal samples, MiR-18a, which belongs to miR-17-92 cluster was the only overexpressed gene, with 33 underexpressed miRNAs including the miR-34b/miR-34c cluster, the miR-195/miR-497 cluster, and most members of the let-7 family. The MiR-34b and miR-34c were the top 2 underexpressed miRNAs in NPC among these miRNAs. The MiRNA microarray assay was performed on biopsies from different clinical stages of NPC by Luo et al,[3] and their studies showed that miR-29a/c, miR-34b, miR-34c-3p, miR-34c-5p, miR-429, miR-203, miR-222, miR-1/206, miR-141, miR-18a/b, miR-544, miR-205, and miR-149 may play important roles in the development of NPC. Liu et al[8] determined 5 miRNAs that were significantly associated with disease-free survival (DFS), distant metastasis-free survival (DMFS), and overall survival (OS) of NPC patients. Of the 5 miRNA of NPC patients, expression levels of 4 miRNAs (miR-142-3p, miR-29c, miR-26a, and miR-30e) were positively associated with DFS, DMFS, and OS in comparison to non-cancer nasopharyngitis tissues, whereas the expression level of the other one (miR-93) was inversely associated with DFS, DMFS, and OS. When combined with TNM Classification of Malignant Tumors stage, more accurate prognostic assessment value can be obtained. Xia et al[9] found that the endogenous miR-200a expression level increased with the degree of differentiation in a panel of NPC cell lines, namely, undifferentiated C666-1, low-differentiated CNE-2 and HNE1, and high-differentiated CNE-1 cells. In addition, through a series of gains and the absence of functional studies, they detected that over expression of miR-200a inhibited C666-1 cell growth, invasion, and migration, while its knock-down stimulated these processes in CNE-1 cells. Therefore, it is hypothesized that miR-200a may be related to the differentiation of NPC.

Effects of chemoradiotherapy on nasopharyngeal microRNAs expression. Zhang et al[10] investigated the impact of 5-Fluorouracil (5-FU) in combination with cisplatin on the miRNAs expression profile of NPC cell line CNE, and high-throughput miRNAs array technology and the stacking-hybridized universal tag (SHUT) assay were performed. The study indicated that

5-FU in combination with cisplatin could significantly alter the global expression profile of miRNAs in CNE cells. After 48 hours of treatment with a low-dose (10% inhibitory concentration [IC10]) of 5-FU and/or cisplatin, the majority of key miRNAs were shown to be regulated. When compared to 431 miRNAs detected in the control cells, 184 miRNAs were significantly expressed in the 5-FU-treated cells, whereas 336 miRNAs were expressed in the cisplatin-treated cells, and 13 miRNAs in the cells treated with the combination of 2 drugs. Importantly, most of these key miRNAs that were shown to be regulated miRNAs are associated with tumor development, progression, and metastasis. When examining whether miR-7 expression differed significantly between CNE-1 and CNE-2 cells, Chen et al[11] used low radiosensitive NPC cells CNE-1, and high radiosensitive NPC cells CNE-2 exposed to 0 Gy (false irradiation), 2 Gy (low-dose irradiation), and 8 Gy (high-dose irradiation) x-ray. The total RNAs of the cell lines were extracted 10 hours after radiation for reverse transcription of miR-7. They found that the expression of miR-7 upregulated after x-ray in the low radiosensitive cells, while this downregulated in high radiosensitive cells, with a large amplitude adjustment after low-dose irradiation, and a minor amplitude adjustment after high-dose irradiation. Among them, CNE-1 cells with a 2 Gy exposure had the highest expression level of miR-7, whereas the non-irradiated CNE-1 cells had the lowest expression. While CNE-2 cells exposed to 2 Gy x-ray had the lowest expression level of miR-7, and the non-irradiated CNE-2 cells had the highest expression level. Therefore, they speculated that miR-7 may play an important role in the radioresistance of NPC cells to x-ray, and the suppression of miR-7 expression may elevate the radiosensitivity of NPC cells. The above findings clarified the potential mechanisms of NPC chemoradiotherapy, and provided new clues for the treatment of NPC.

Related research regarding miRNAs and the pathogenesis of nasopharyngeal carcinoma. Numerous studies have shown the anomalous expression of miRNA processing enzymes, single nucleotide polymorphisms (SNPs), and the interaction of miRNAs with latent membrane protein 1 (LMP1) encoded by EBV, human telomerase reverse transcriptase enzyme (hTERT), COX-2, VEGF, and signal transduction pathways involved in the development, invasion, and metastasis of NPC.

MiRNA processing enzymes and nasopharyngeal carcinoma. The MiRNA processing enzymes Drosha and Dicer play a pivotal role in the maturation of miRNAs. It is reported[12] that the mean protein expression levels of Dicer and Drosha were significantly downregulated in NPC cell lines and tissue specimens in comparison with NPE. Moreover, Dicer and Drosha protein expression levels were not correlated with age, gender, or lymph node metastasis, but were significantly lower in stage III + IV than in stage I + II. The Kaplan-Meier survival analysis showed that NPC patients with low expression levels of the Drosha and Dicer proteins had a significantly shorter PFS and OS than those with high levels of Drosha and Dicer. Therefore, the expression levels of Dicer and Drosha enzymes can be used as potential prognostic biomarkers for NPC. Luo et al[13] found that after miR-18a transduction into NPC tissues, miR-18a negatively regulated Dicer1 by binding to the 3' untranslated regions (3'UTR) of Dicer1, causing the global down regulation of miRNA expression levels including the miR-200 family and miR-143, which directly induced aberrant expression of the epithelial mesenchymal transition marker E-cadherin and the oncogene K-Ras, to promote the growth, invasion, and migration of NPC cells. The clinical parameter also showed that higher levels of miR-18a were correlated with NPC advanced stage, lymph node metastasis, EBV-infection, and a higher NPC mortality. Therefore, the expression levels of the miRNAs-processing enzymes are associated with the occurrence and development of NPC, and it also becomes a potential regulatory target of NPC.

MicroRNAs involved in the process of EBV-induced nasopharyngeal carcinoma. It is known that EBV contributes to the occurrence of NPC. It is shown that EBV encodes miRNAs, including ebv-miR-BHRF1-1 ~ 3 located in the viral genome BHRF1 region, and ebv-miR-BART1~22 located in the BART region.[14] In the latest studies, Wong et al[15] detected EBV-miRNA in NPC. They found that compared with NPE tissues, 29 EBV-miRNA was significantly up regulated in NPC. Most EBV microRNAs were generally higher than human microRNAs apart from hsa-miR144 and hsa-miR451, and were predominantly from the BART region, with BART6-5p changed most significantly. The expression abundance of all the differentially expressed EBV microRNAs was higher universally in tumors than that in non-tumors, with a range of 38 (BART11-3p) to 357 (BART1-3p) in nontumors, and 81 (BART11-3p) to 2600(BART7) in tumor biopsies. The EBV-miRNA copy numbers in NPC patients serum were positively correlated with that in the cell lines and tissues. In addition, the expression levels of the 12 EBV miRNAs significantly changed

were compared between EBV-positive NPC cell line (C666) and EBV-positive immortalized nasopharynx epithelium (NP460hTERTþEBV) by Q-PCR. The results indicated that the 12 EBV MiRNAs were all differentially expressed at higher levels in C666 (with copy numbers ranging from 67 to nearly 107) than in NP460hTERTþEBV, even though EBV latent infection presented in both epithelial cell lines. Plasma ebv-miR-BART7 levels from undifferentiated NPC patients were measured by Chan et al[16] using real-time quantitative polymerase chain reaction (RT-PCR). The results showed that the plasma ebv-miR-BART7 level was significantly higher in NPC patients when compared with that from healthy individuals. Furthermore, the ebv-miR-BART7 was detectable in all plasma samples of patients. More significantly, the plasma ebv-miR-BART7 level was independent of the level of EBV DNA. High-throughput gene expression analysis suggested that ebv-miR-BART7 enhanced proliferation, invasion, and migration of NPC cells by affecting multiple tumor-related pathways in vitro. Moreover, NPC cells expressing ebv-miR-BART7 were more resistant to cisplatin. Therefore, plasma EBV-miR-BART7 can be used for NPC screening, especially when EBV DNA cannot be detected, and EBV-miR-BART7 can be suggested as a potential biomarker for undifferentiated NPC.

In addition, LMP1 is an important type of tumorigenic virus protein. It is reported that the level of miR-203 was downregulated substantially in NPC tissues that were latently infected with EBV. When the latent EBV genome or suppressed LMP1 is removed, the miR-203 expression can be restorated.[17] While ectopic expression of miR-203 suppressed EBV-induced S-phase entry and transformation in vivo, overexpression of its targets E2F3 and CCNG1 could overcome the effects of miR-203 mimics on the cell cycle. Jun N-terminal protein kinase (JNK) and NF-B inhibitors blocked miR-203 down regulation was also observed. Du et al[18] assessed the function of miR-155 in NPC. They found that LMP1 and LMP2A encoded by EBV could enhance the expression of miR-155 in NPC CNE1 and TW03 cells. Moreover, MiR-155 could downregulate the expression of putative targets of miR-155 JMJD1A and BACH1, whereas, miR-155 inhibitors could upregulate JMJD1A expression in NPC cell lines. Most importantly, JMJD1A is associated with the N stage of TNM staging and cacoethic prognosis of NPC patients. Therefore, it is expected that miR-155 and JMJD1A can be used as potential therapeutic targets in NPC. That LMP1 could induce the expression of human telomerase reverse transcriptase (hTERT) and promote cell immortalization has also been reported.[19] Therefore, miRNAs may increase the understanding of host-virus interaction, and serum miRNAs level can be used as a biomarkers of high-risk populations for NPC screening.

MicroRNAs and single nucleotide polymorphisms. The SNPs are a type of DNA sequence polymorphism caused by variations of a single nucleotide at the level of the genome. Genetic research has identified several SNPs associated with the risk effect of NPC, such as HLA, cytochrome P4502E1 (CYP2E1), glutathione-S-transferase M1 (GSTM1), and integrin laminin-α9(ITGA9).[20] Lung et al[21] recently found that 3 functionally mature miRNAs, namely, miR-146a, miR-146a*C, and miR-146a*G, were all upregulated in NPC specimens. More importantly, miR-146a*C is preferentially expressed in the CC genotype, which were further found to elevate the risk of NPC susceptibility.

MicroRNAs inhibit the expression of cell cycle-related proteins. Cyclin is a class of protein family closely related to the functional state of the cell cycle, and its expression level changes with the cell cycle. It plays a regulatory role in different stages of the cell cycle by binding with a specific protein kinase and activating its activity. The studies found that MiRNAs may mediate the occurrence and development of NPC through the inhibition of cell cycle-related protein expression. It has been reported that miR-34 downregulated significantly in NPC, and it is a direct target of the tumor suppressor gene p53. The latest study by Cha et al[22] found that miR-34 targeted a set of highly conserved sites in the UTR of Wnt and EMT genes, specifically WNT1, WNT3, LRP6, AXIN2, β-catenin, LEF1 and Snail, leading to inhibition of TCF/LEF transcriptional activity and the EMT procedures. The loss of p53 function increased Wnt activities and promoted the Snail-dependent EMT procedures. In clinical specimens, the transcription of TCF/LEF was closely associated with functionality of p53 and miR-34. This will not only indicate the physiological relevance of p53-miR-34-Wnt signaling network, also show the potential and pervasive influence of miR-34 absence on the oncogenic pathway in human tumors.

Another study reported that miR-663 plays a role as a proto-oncogene in NPC.[23] The MiR-663 directly targeted P21 (WAF1/CIP1) to promote the conversion of cells in the G1/S-phase, and the silencing of p21 could restore the inhibitory effects of miR-663 on the G1/S transition. The MiR-138 generally downregulated in NPC specimens and NPC cell lines was negatively correlated with gene expression of its novel target cell cycle proteins D1 (CCND1). The ectopic expression of miR-138 dramatically inhibited cell proliferation and

colony formation in vitro and suppressed tumorigenesis in vivo. Therefore, it is suggested that miR-138 may exert as a tumor suppressor gene by inhibiting CCND1 gene expression in NPC.[24] The let-7 family[25] have been demonstrated as negative regulators of the Ras gene to induce cell cycle arrest and inhibit the cell proliferation and metastasis. The miR-144[26] could enhance PAKT and cyclin D1 expression by inhibiting PTEN (tensin homologue) expression to activate the PI3K/Akt pathway, promoting G(1)-phase transition and reducing E-cadherin, and to promote cell proliferation, invasion, and migration. The studies above provide a satisfactory reference for diagnostic markers and treatment in the process of NPC.

MicroRNAs regulate apoptosis. Tumorigenesis is closely related to apoptosis suppression. The gene expression microarray analysis of NPC cells transfected with miR-1 and luciferase reporter vector assays used by Wu et al[27] showed that miRNA-1 could directly target the PTMA (thymosin alpha) gene and induce apoptosis of NPC cells. Therefore, the PTMA siRNA may have potential auxiliary application value in cancer chemotherapy. It is also reported that low expression of miR-29c was positively associated with therapeutic resistance of NPC patients. The ectopic restoration of miR-29c could promote apoptosis by inhibiting the expression of anti-apoptotic factor Mcl-1 and Bcl-2, and substantially enhance the sensitivity of NPC cells to ionizing radiation (IR) and cisplatin treatment.[28] Therefore, it is expected that miR-29c serve as a potential therapeutic sensitizer in NPC treatment.

MicroRNAs involvement in tumor angiogenesis. Tumor angiogenesis is essential for tumor development. Studies showed that MiRNAs participate in tumor angiogenesis, and promoted the progress of NPC. It has been found that miR-218 is frequently downregulated in NPC and could inhibit NPC progression via downregulating survivin (BIRC5) and acting on the SLIT2-ROBO1 pathway involved in tumor angiogenesis.[29] Therefore, it is expected that restoring miR-218 expression in NPC might be widely used in clinical treatment. Li et al[30] constructed a type of shRNA, which is safer and more effective than traditional shRNA, based on miRNA structure. Then they further established a miR-155-based pLVTHM/shRNAmir lentiviral vector, and successfully silenced COX-2 that were involved in both tumor angiogenesis, invasion, and metastasis of NPC cells. Thus, miR-155 is also a potential target for the prevention and treatment of NPC. It is also reported that miRNA suppressed NPC cell growth by downregulating VEGF.[31] Overall, miRNAs may mediate the evolution of NPC via involvement in tumor angiogenesis.

MicroRNAs and other related pathways. Wong et al[32] reported that the let-7 family might inhibit NPC cell proliferation by downregulating c-Myc gene expression. The demethylation drugs (5-azacitidine and zebularine) caused activation of let-7 expression in poorly differentiated NPC cells. Therefore, DNA methylation is considered to be a potential regulatory pathway. However, the extent of DNA hypermethylation/hypomethylation in regulating let-7 expression still requires further clarification. In addition, in NPC samples, MiR-375 expression was significantly decreased, while MTDH was significantly increased. The MTDH proved to be a target of miR-375. Reexpression of miR-375 and silencing of MTDH by siRNA both reduced cell viability, clonogenic survival, and tumor formation in vivo. The NPC patients with high levels of MTDH experienced significantly lower survival, particularly, higher distant recurrence rates.[33] Accordingly, it seems that MiRNAs may be involved in the occurrence and development of NPC via interaction with multiple genes/pathways.

From the above-mentioned studies, we can conclude that miRNAs play an important role in the development of NPC. Part of this role is positively correlated with the occurrence and development of NPC, while part is negatively correlated. It is important that existing research provides new ideas that the prevention and treatment of NPC may benefit from regulating the dysregulated miRNAs. The miRNAs that are involved in the occurrence and development of NPC are summarized in **Table 1**.

Perspectives. With continual development of miRNA technology, more and more miRNAs that are involved in the process of NPC and their regulatory mechanism were investigated and found. Furthermore, it has been found that partial serum miRNAs levels were significantly correlated with miRNAs in tissues, which provides a reference and strategy for NPC screening, diagnosis, staging, treatment, and prognosis. Moreover, researchers combined the classic siRNA with miRNA techniques, thereby increasing the sensitivity and accuracy of detection. However, NPC is a polygenic disease; one miRNA targets multiple genes and is also regulated by multiple genes, thus forming complex gene regulatory networks, which brings a certain degree of difficulty to research of the exact mechanism. Therefore, there is still a long way to go before complete elucidation of the function of miRNAs, their related targets, and regulatory networks. However, this will enable broad application prospects for miRNAs to be used in the clinical diagnosis and treatment of tumors.

Table 1 - MicroRNAs (miRNA) that were abnormally expressed in nasopharyngeal carcinoma cell lines.

MicroRNAs	Biological functions	Expression
miR-18a	Involved in cell growth, invasion and metastasis	↑
miR-34b cluster	Involved in regulating signaling pathways and cell cycle	↓
miR-34c cluster	Involved in regulating signaling pathways and cell cycle	↓
miR-195 cluster	Involved in regulating signaling pathways, cell cycle and apoptosis	↓
miR-497 cluster	Involved in regulating signaling pathways, cell cycle and apoptosis	↓
let-7 cluster	Involved in regulating cell cycle cell proliferation, metastasis	↓
miR-200a	Involved in cell growth, invasion and metastasis	↑
miR-29a/c	Involved in regulating signaling pathways, cell cycle and apoptosis	↓
miR-26a	Involved in mitosis and cell cycle	↑
miR-30e	Undecided	↑
miR-93	Undecided	↓
miR-1/206/29c	Involved in inducing apoptosis, inhibiting invasion and metastasis	↓
miR-141	Involved in inducing cell cycle, apoptosis, growth, migration, invasion	↑
miR-149	Undecided	↑
miR-18a/b	Undecided	↑
miR-205	Undecided	↑
miR-544	Undecided	↑
miR-99a/b	Undecided	↑
miR-203	Involved in regulating signaling pathways, cell cycle and immortalized	↓
miR-222	Undecided	↓
miR-429	Undecided	↓
miR-7	Inhibiting apoptosis	↑
ebv-miR-BHRF1-1~3	Involved in cell proliferation, invasion, metastasis, immortalized, inhibiting apoptosis	↑
ebv-miR-BART1~22	Involved in cell proliferation, invasion, metastasis, immortalized, inhibiting apoptosis	↑
miR-146a, miR-146a*C	Involved in genetic polymorphisms of genes	↑
miR-146a*G	Involved in genetic polymorphisms of genes	↑
miR-138	Involved in regulating cell cycle	↓
miR-663	Involved in regulating cell cycle	↑
miR-144	Involved in regulating signaling pathways, cell cycle, invasion and metastasis	↑
miR-218	Down regulating survivin (BIRC5), involved in tumor angiogenesis	↓
miR-155	Involved in tumor angiogenesis, invasion, and metastasis	↑

ebv - Epstein-Barr virus, BHRF - BamHI H rightward fragment, BART - BamHI A rightward transcript, ↑- upregulated, ↓- downregulated

References

1. Olson P, Lu J, Zhang H, Shai A, Chun MG, Wang Y, et al. MicroRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer. ***Genes Dev*** 2009; 23: 2152-2165.
2. Sini RA, Trink B, Nissan A. The role of microRNA in tumorigenesis: key players or innocent bystanders. ***J Surg Oncol*** 2009; 99: 135-136.
3. Luo Z, Zhang L, Li Z, Li X, Li G, Yu H, et al. An in silico analysis of dynamic changes in microRNA expression profiles in stepwise development of nasopharyngeal carcinoma. ***BMC Med Genomics*** 2012; 5: 3.
4. Choudhry H, Catto JW. Epigenetic regulation of microRNA expression in cancer. ***Methods Mol Biol*** 2011; 676: 165-184.
5. Drakaki A, Iliopoulos D. MicroRNA Gene Networks in Oncogenesis. ***Curr Genomics*** 2009; 10: 35-41.
6. Farazi TA, Spitzer JI, Morozov P, Tuschl T. miRNAs in human cancer. ***J Pathol*** 2011; 223: 102-115.
7. Li T, Chen JX, Fu XP, Yang S, Zhang Z, Chen KhH, et al. microRNA expression profiling of nasopharyngeal carcinoma. ***Oncol Rep*** 2011; 25: 1353-1363.
8. Liu N, Chen NY, Cui RX, Li WF, Li Y, Wei RR, et al. Prognostic value of a microRNA signature in nasopharyngeal carcinoma: a microRNA expression analysis. ***Lancet Oncol*** 2012; 13: 633-641.
9. Xia H, Ng SS, Jiang S, Cheung WK, Sze J, Bian XW, et al. miR-200a-mediated downregulation of ZEB2 and CTNNB1 differentially inhibits nasopharyngeal carcinoma cell growth, migration and invasion. ***Biochem Biophys Res Commun*** 2010; 391: 535-541.
10. Zhang X, Li W. 5-Fluorouracil in combination with cisplatin alters the microRNA expression profile in the CNE nasopharyngeal carcinoma cell line. ***Mol Med Rep*** 2012; 6: 303-308.
11. Chen ZX, Sun AM, Chen Y, Liu Y, Zhan JF, Chen LH, et al. [Effects of radiosensitivity and X-ray dose on miR-7 expression in nasopharyngeal carcinoma]. ***Nan Fang Yi Ke Da Xue Xue Bao*** 2010; 30: 1810-1812, 1816. Chinese
12. Guo X, Liao Q, Chen P, Li X, Xiong W, MA J, et al. The microRNA-processing enzymes: Drosha and Dicer can predict prognosis of nasopharyngeal carcinoma. ***J Cancer Res Clin Oncol*** 2012; 138: 49-56.

13. Luo Z, Dai Y, Zhang L, Jiang C, Li Z, Yang J, et al. miR-18a promotes malignant progression by impairing microRNA biogenesis in nasopharyngeal carcinoma. ***Carcinogenesis*** 2013; 34: 415-425.
14. Gourzones C, Gelin A, Bombik I, Klibi J, Verillaud B, Guigay J, et al. Extra-cellular release and blood diffusion of BART viral micro-RNAs produced by EBV-infected nasopharyngeal carcinoma cells. ***Virol J*** 2010; 7: 271.
15. Wong AM, Kong KL, Tsang JW, Kwong DL, Guan XY. Profiling of Epstein-Barr virus-encoded microRNAs in nasopharyngeal carcinoma reveals potential biomarkers and oncomirs. ***Cancer*** 2012; 118: 698-710.
16. Chan JY, Gao W, Ho WK, Wei WI, Wong TS. Overexpression of Epstein-Barr virus-encoded microRNA-BART7 in undifferentiated nasopharyngeal carcinoma. ***Anticancer Res*** 2012; 32: 3201-3210.
17. Yu H, Lu J, Zuo L, Yan Q, Yu Z, Li X, et al. Epstein-Barr virus downregulates microRNA 203 through the oncoprotein latent membrane protein 1: a contribution to increased tumor incidence in epithelial cells. ***J Virol*** 2012; 86: 3088-3099.
18. Du ZM, Hu LF, Wang HY, Yan LX, Zeng YX, Shao JY, et al. Upregulation of MiR-155 in nasopharyngeal carcinoma is partly driven by LMP1 and LMP2A and downregulates a negative prognostic marker JMJD1A. ***PLoS One*** 2011; 6: e19137.
19. Yip YL, Tsang CM, Deng W, Cheung PY, Jin Y, Cheung AL, et al. Expression of Epstein-Barr virus-encoded LMP1 and hTERT extends the life span and immortalizes primary cultures of nasopharyngeal epithelial cells. ***J Med Virol*** 2010; 82: 1711-1723.
20. Lo KW, Chung GT, To KF. Deciphering the molecular genetic basis of NPC through molecular, cytogenetic, and epigenetic approaches. ***Semin Cancer Biol*** 2012; 22: 79-86.
21. Lung RW, Wang X, Tong JH, Chau SL, Lau KM, Cheng SH, et al. A single nucleotide polymorphism in microRNA-146a is associated with the risk for nasopharyngeal carcinoma. ***Mol Carcinog*** 2013; 52 Suppl 1: S28-S38.
22. Cha YH, Kim NH, Park C, Lee I, Kim HS, Yook JI. MiRNA-34 intrinsically links p53 tumor suppressor and Wnt signaling. ***Cell Cycle*** 2012; 11: 1273-1281.
23. Yi C, Wang Q, Wang L, Huang Y, Li L, Liu L, et al. MiR-663, a microRNA targeting p21(WAF1/CIP1), promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma. ***Oncogene*** 2012; 31: 4421-4433.
24. Liu X, Lv XB, Wang XP, Sang Y, Xu S, Hu K, et al. MiR-138 suppressed nasopharyngeal carcinoma growth and tumorigenesis by targeting the CCND1 oncogene. ***Cell Cycle*** 2012; 11: 2495-2506.
25. Wang QZ, Lv YH, Gong YH, Li ZF, Xu W, Diao Y, et al. Double-stranded Let-7 mimics, potential candidates for cancer gene therapy. ***J Physiol Biochem*** 2012; 68: 107-119.
26. Zhang LY, Ho-Fun Lee V, Wong AM, Kwong DL, Zhu YH, Dong SS, et al. MicroRNA-144 promotes cell proliferation, migration and invasion in nasopharyngeal carcinoma through repression of PTEN. ***Carcinogenesis*** 2013; 34: 454-463.
27. Wu CD, Kuo YS, Wu HC, Lin CT. MicroRNA-1 induces apoptosis by targeting prothymosin alpha in nasopharyngeal carcinoma cells. ***J Biomed Sci*** 2011; 18: 80.
28. Zhang JX, Qian D, Wang FW, Liao DZ, Wei JH, Tong ZT, et al. MicroRNA-29c enhances the sensitivities of human nasopharyngeal carcinoma to cisplatin-based chemotherapy and radiotherapy. ***Cancer Lett*** 2013; 329: 91-98.
29. Alajez NM, Lenarduzzi M, Ito E, Hui AB, Shi W, Bruce J, et al. MiR-218 suppresses nasopharyngeal cancer progression through downregulation of survivin and the SLIT2-ROBO1 pathway. ***Cancer Res*** 2011; 71: 2381-2391.
30. Li G, Li XP, Jiang L, Lu J, Liu X, Chen SJ. [Silencing of COX-2 in nasopharyngeal carcinoma cells with a shRNAmir lentivirus vector]. ***Nan Fang Yi Ke Da Xue Xue Bao*** 2009; 29: 1111-1114. Chinese
31. Cai X, Wei W, Zhao S, Tang Y, He C, Wang C. [Inhibition of the expression of VEGF gene in nasopharyngeal carcinoma cells by microRNA]. ***Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*** 2010; 24: 703-707. Chinese
32. Wong TS, Man OY, Tsang CM, Tsao SW, Tsang RK, Chan JY, et al. MicroRNA let-7 suppresses nasopharyngeal carcinoma cells proliferation through downregulating c-Myc expression. ***J Cancer Res Clin Oncol*** 2011; 137: 415-422.
33. Hui AB, Bruce JP, Alajez NM, Shi W, Yue S, Perez-Ordonez B, et al. Significance of dysregulated metadherin and microRNA-375 in head and neck cancer. ***Clin Cancer Res*** 2011; 17: 7539-7550.

Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.