

MINI-REVIEW

Involvement of EBV-encoded BART-miRNAs and Dysregulated Cellular miRNAs in Nasopharyngeal Carcinoma GenesisYuan-Jie Xie¹, Zhi-Feng Long², Xiu-Sheng He^{1*}**Abstract**

The definite molecular mechanisms underlying the genesis of nasopharyngeal carcinomas (NPCs) remain to be completely elucidated. miRNAs are small non-coding RNAs which are implicated in cell proliferation, apoptosis, and even carcinogenesis through negatively regulating gene expression post-transcriptionally. EBV was the first human virus found to express miRNAs. EBV-encoded BART-miRNAs and dysregulated cellular miRNAs are involved in carcinogenesis of NPC by interfering in the expression of viral and host cell genes related to immune responses and perturbing signal pathways of proliferation, apoptosis, invasion, metastasis and even radio-chemo-therapy sensitivity. Additional studies on the roles of EBV-encoded miRNAs and cellular miRNAs will provide new insights concerning the complicated gene regulated network and shed light on novel strategies for the diagnosis, therapy and prognosis of NPC.

Keywords: EBV-encoded BART-miRNAs - carcinogenesis - miRNAs - nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) is the most common head and neck cancer with high incidence in southern China, Southeast Asia, the Middle East and North Africa, especially in Cantonese populations of southern China (25–30 per 100,000 persons per year) (Li et al., 2012). Epstein-Barr virus (EBV) infection, genetic susceptibility, environmental factors and dietary habits are major etiological factors in the development of NPC (Cao et al., 2011; Polesel et al., 2013). However, the definite molecular mechanism underlying the carcinogenesis of NPC remains to be completely elucidated.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs with 20–23 nucleotides in length which negatively regulate gene expression post-transcriptionally through binding to complementary sequences in the 3'-untranslated region (3'-UTR) of target mRNAs, leading either to translation inhibition or mRNA cleavage which is depended on base-pairing conditions between miRNAs and their target mRNAs. Accumulating experimental studies indicated that miRNAs dysregulation was implicated in oncogenic cell processes, such as proliferation, apoptosis, metastasis and even therapy-resistance of carcinoma (Farazi et al., 2013). Recent researches had shown that EBV-encoded BART-miRNAs could interfere in the expression of viral and host cell genes in order to mask the viral-infected host cell from the immune response and facilitate NPC carcinogenesis (Cosmopoulos et al., 2009; Barth et al., 2011; He et al., 2012; Kim &

Lee, 2012; Marquitz & Raab-Traub, 2012), and the dysregulated cellular miRNAs can act as oncogenes or tumor suppressors perturbing signal pathways involved in carcinogenesis and aggressive tumour phenotype of NPC (Chen et al., 2009; Li et al., 2011; Liu et al., 2012; Luo et al., 2012). Here, besides summarizing the current findings on EBV-encoded BART-miRNAs and cellular miRNAs in NPC in briefly, we will discuss the mechanisms by which miRNAs dysregulation may play a role in carcinogenesis of NPC, and the possible use of miRNAs in the diagnosis, therapy and prognosis of NPC in future.

EBV-encoded BART-miRNAs and Genesis of NPC

Although the close association of NPC with EBV infection has been known for more than four decades, the exact role that EBV plays in the pathogenesis of NPC is still unclear. EBV is the first human virus found to express miRNAs. To date, a total of 25 EBV miRNA precursors, generating 48 mature miRNAs have been identified according to the Sanger database (<http://www.mirbase.org/index.shtml>, Release 19). EBV encoded miRNAs map to two regions of the viral genome (Figure 1). The BHRF1 transcript is located immediately upstream and downstream of the BHRF1 open reading frame and encodes three miRNA precursors with four mature miRNAs (miR-BHRF1-1, -2, -2* and -3). In the BART region, two clusters of miRNAs are generated and named miR-BART1 to 22 with the numbers corresponding to

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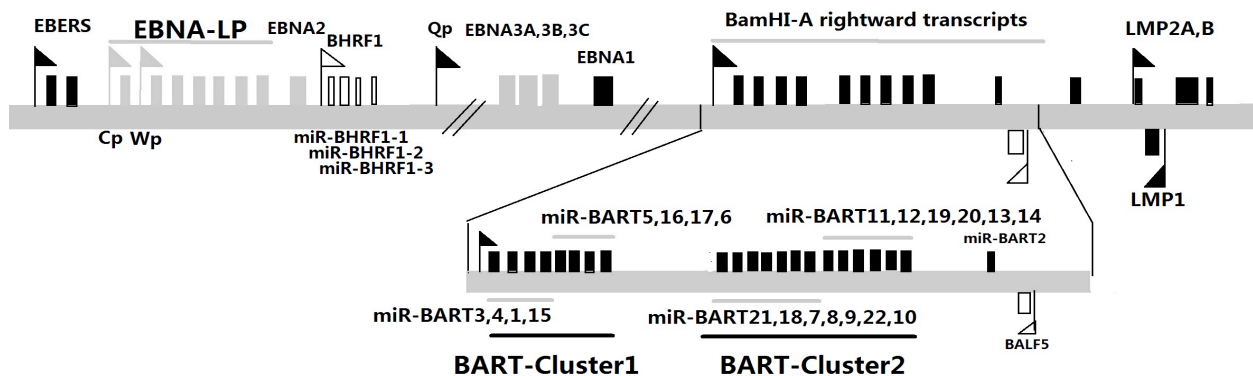


Figure 1. Schematic Representation of the Genomic Location of EBV miRNAs. The promoters and genes expressed in EBV-positive NPC tumours (latency II) are shown in black. The promoter and coding region of BHRF1 are indicated by the white pennant and box. Other promoters and genes are shown in grey. miR-BART2 runs in antisense orientation to the 3'UTR of the BALF5 DNA polymerase

Table 1. Functional Roles of EBV-BART-miRNAs in Nasopharyngeal Carcinoma

BART miRNAs	Target gene	Functions of Target gene	Effects of BART miRNAs
miR-BART2-5p	<i>MICB</i>	Cellular ligand for NKG2D receptor in NK and T cells.	Interfere with host immune responses to protect infected cells from killing
miR-BART 3	<i>BALF5</i>	EBV lytic protein for viral replication	Suppresses lytic cycle to maintain EBV latency
	<i>IPO7</i>	Nuclear importer protein involved in immunity	
miR-BART6-5p	<i>Dicer</i>	RNase enzyme for miRNA maturation	Controls the biogenesis of miRNAs
	<i>EBNA2, Zta and Rta</i>	required for transition from type I, II latency to type III and lytic replication	
miR-BART22	<i>LMP2A</i>	involved in cellular transformation	Prevent viral infected cells from immune attack
	<i>MAP3K5</i>	protein phosphorylation of MAPK pathway downstream genes	
	<i>PUMA</i>	pro-apoptotic protein	
miR-BART1-3p,2-5p	<i>TP53INP1</i> and <i>MASPIN</i>	pro-apoptotic protein	Inhibit p53-induced apoptosis
BART cluster 1 and 2	<i>Bim</i>	pro-apoptotic protein	Inhibit Bax-dependent apoptosis
	<i>TOM22</i>	Mitochondrial receptor for Bax	
miR-BART16	<i>LMP1</i>	EBV latent protein with oncogenic potential	Inhibit Bax-dependent apoptosis
miR-BART1-5p, 16 and -17-5p	<i>PTEN</i>	a key tumor suppressor gene(TSG) related to PI3K/AKT pathway	Balances the proapoptotic effects of LMP1 from its oncogenic function
miR-BART9			Promote cell proliferation
miR-BART13, 14, 19-3p, 16 and -17-5p, 18-5p, and 10	<i>WIF1, NLK</i> and <i>APC</i>	Wnt and JAK-STAT pathway inhibitory genes respectively	Promote cell proliferation and carcinogenesis

the order in which they were original discovered. BART Cluster 1 and 2 encode 8 miRNA precursors (miR-BART1, 3-6 and 15-17) and 13 miRNA precursors (miR-BART7-14 and 18-22) respectively. The miR- BART2 is located lonely in antisense orientation to the 3' -UTR of the BALF5 DNA polymerase. In typical miRNA biogenesis, one strand of the miRNA hairpin is selected to be the mature miRNA, while the other strand quickly degraded. However, both strands of the original hairpin for BART-miRNAs persist and can, therefore, produce functionally relevant 44 mature miRNAs. To distinguish between the two potential miRNAs the suffixes -5p and -3p have been used to designate the 5' end of the hairpin and 3' end of the hairpin respectively. BHRF1-miRNAs, barely expressed in most NPC cells with EBV latent infection, are limited to cells displaying a type III latency and lytically infected cells (Zhu et al., 2009), however, BART-miRNAs, which are much lower in EBV-infected B cells, can be detected in all form of latency but are largely expressed in epithelial tumours of the nasopharynx and stomach (Kim et al., 2007; Chen et al., 2010). In fact, multiple reports have confirmed that all 44 BART-miRNAs were expressed in NPC. However, the expression of individual miRNAs appears to be highly variable. In general, the overall

expression levels of BART cluster 1 miRNAs appear slightly higher than those of cluster 2 miRNAs (Cai et al., 2006; Lo et al., 2012; Wong et al., 2012). The reason for the differences in abundance of the individual BART miRNA within EBV infected cell is not known. The differential splicing that occurs in the transcripts could possibly account for some of the differences (Edwards et al., 2008). Certainly, this also suggests that the different BART-miRNAs may provide distinctive contributions to carcinogenesis of EBV-associated NPC.

As far as is known, BART-miRNAs play key roles in maintaining EBV persistent latent infection by suppressing the host immune response and facilitating epithelial cell growth transformation by perturbing signal transduction pathways related to proliferation and apoptosis in the pathogenesis of NPC (Table 1). As shown in Table 1, miR-BART2-5p, 3, 6-5p and 22 can suppress themselves target genes and contribute to immune evasion of EBV-infected cells (Nachmani et al., 2009). miR-BART5 can suppress the expression of p53 up-regulated modulator of apoptosis (PUMA) which was found to be reduced in most NPC tissues and protect the cells from apoptosis, promoting host cell survival and facilitating cancer progression (Choy et al., 2008). miR-BART1-3p and 2-5p can target tumor

protein p53 inducible nuclear protein 1 (TP53INP1) and Maspin respectively, which are p53 downstream effectors, suggesting that p53-dependent tumor suppression may be inhibited despite accumulation of wild-type p53 in NPC (Cao et al., 2012). Bim, a pro-apoptotic protein, has also been identified as a target of BART cluster 1 and 2 miRNA, and functional assays proved that cells expressing either cluster 1 or 2 miRNAs were found to be more resistant to etoposide, a potent inducer of p53-dependent apoptosis (Marquitz et al., 2011). miR-BART16 can inhibit the apoptotic process by targeting TOM22, a mitochondrial receptor for Bax (Dolken et al., 2010). In vitro expression of miR-BART7 could enhance proliferation, migration, and invasion of NPC cells. Furthermore, NPC cells expressing miR-BART7 were more resistant to cisplatin. High-throughput gene expression analysis showed that miR-BART7 could affect multiple cancer-related pathways (Chan et al., 2012). As an important oncogenic protein produced by EBV, LMP1 engages in a number of signal pathways (such as NF- κ B, JNK-p38MAPK, PI3K-Akt and JAK-STAT), which modulate expression of a variety of downstream molecules to promote cell growth, suppress apoptosis, enhance cell invasion and even angiogenesis (Shair et al., 2008; You et al., 2011; Dawson et al., 2012). miR-BART1-5p, 16 and 17-5p can target the 3'-UTR of the LMP1 gene and negatively regulate the proper expression of LMP1 protein. These miRNAs also modulate LMP1-induced NF- κ B signal transduction pathway and alleviate the cisplatin sensitivity of LMP1-expressing NPC cells (Lo et al., 2007). Additionally, EBV miRNAs can work collaboratively to activate Wnt pathway by down-regulating Wnt inhibitory genes such as WIF1 (target of miR-BART19-3p, 13), NLK (target of miR-BART14, 19-3p, 18-5p, 10), and APC (miR-BART 19-3p, 17-5p, 10) (Webb et al., 2008; Wong et al., 2012), and miR-BART9 can inhibit the expression of phosphatase and tensin homologue (PTEN) related to AKT/PI3K pathway, a key tumor suppressor gene (TSG) in NPC (Xu et al., 2004; Wong et al., 2012).

EBV-regulated miRNAs and NPC Genesis

Besides encoding BART-miRNAs, researches also showed that EBV severely dysregulated the miRNAs profiles of the host cell such as miR-146a, miR-155, miR-10b and miR-203 which were regulated by LMP1 and involved in the development of NPC (Kok et al., 2010). LMP1 induced the expression of miR-146a predominantly through two NF- κ B binding sites in the miR-146a promoter. The up-regulated miR-146a played a role in the induction or maintenance of EBV latency by modulating innate immune responses to the virus-infected host cell (Cameron et al., 2008). However, miR-146a expression was down-regulated in many solid tumors and NK/T cell lymphoma, and there was consistent evidence that miR-146a may act as a tumor suppressor by targeting some mRNAs, such as TRAF6, Notch1 and EGFR (Mei et al., 2011; Paik et al., 2011). There are few reports about the functions and mechanism of miR-146a contributing to NPC directly or indirectly. A recent study found that single nucleotide polymorphism

(SNP) in miR-146a rs2910164C>G was associated with the predisposition of NPC in Chinese population (Lung et al., 2012). LMP1 and LMP2A encoded by EBV could further enhance the expression of miR-155 in NPC CNE1 and TW03 cells. Downregulation of JMJD1A by miR-155 was significantly correlated with N stage in TNM classification, a lower five-year survival rate, and a lower five-year disease-free survival rate of NPC patients (Du et al., 2011). miR-10b over-expression induced by LMP1 promoted the metastasis of NPC cells and accelerated the death of tumor-bearing nude mice (Li et al., 2010). miR-203 was low expression in NPC tissues that were latently infected with EBV. LMP1 can downregulate cellular miR-203 and promote cell proliferation through JNK and NF- κ B pathways. E2F3 and CCNG1 were identified as target genes of miR-203. Ectopic expression of miR-203 inhibited EBV-induced S-phase entry and transformation *in vivo* (Yu et al., 2012).

So the BART-miRNAs and cellular miRNAs dysregulated by productions of EBV especially LMP1, which are major contribution factors of EBV to oncogenesis of NPC, can perturb host immune response and signal transduction pathways related to proliferation and apoptosis of NPC cell. However, the overall biologic effects of these miRNAs related to EBV infection on NPC progression have not been fully explored. It will be urgent to clarify whether any of these BART miRNAs contribute to EBV oncogenesis and these miRNAs how to work in concert to facilitate carcinogenesis of NPC *in vivo*.

Dysregulated Cellular miRNAs Function as Oncogenes in NPC

Besides EBV-related miRNAs, miRNA expression profiling of NPC tissues had been studied by different labs and some aberrantly-expressed miRNAs screened in NPC tissue, which could promote an aggressive phenotype by changing the expression of their mRNA targets involved in related signal pathways (Sengupta et al., 2008; Chen et al., 2010; Li et al., 2011). Obviously, some discrepancies of outcomes were found in these studies and very little overlap of miRNA expression data could be seen, which maybe arise from sample selection or preparation, experimental design, assays and/or data analysis. However, some aberrant miRNAs had been verified by experiments which function as either oncogenes or tumor suppressors, interfering with invasion and metastasis, and even modulating the radio-chemotherapy sensitivity of NPC.

miRNAs function as oncogenes, which are often up-regulated in tumors and commonly termed oncomirs, can negatively regulate the expression of tumor suppressor gene, and/or genes responsible for apoptosis, proliferation and differentiation. Researches have showed that miR-141, miR-18a, miR-144, miR-18b, miR-214, miR-421 and miR-663 were up-regulated in NPC specimens and cell lines. miR-141 contributed to NPC development through targeting BRD3, UBAP1 and PTEN and regulating the Rb/E2F, JNK2 and AKT pathways to affect cell cycle, apoptosis, cell growth, migration and invasion of NPC cells (Zhang et al., 2010). miR-18a, a member of the

oncogenic miR-17-92 cluster, promoted the growth, migration and invasion of NPC cells by regulating Dicer1 expression, which caused the global downregulation of miRNA expression levels including miR-200 family and miR-143. Clinical parameter studies showed that higher levels of miR-18a correlated with NPC advanced stage, lymph node metastasis, EBV infection and a higher death rate from NPC, indicating oncogenic roles in NPC development (Luo et al., 2013). miR-144 and miR-18b, functioned as an onco-miRNA in NPC by targeting and suppressing the expression of PTEN and connective tissue growth factor (CTGF) respectively (Yu et al., 2013; Zhang et al., 2013). miR-214 not only can promote NPC cell proliferation and invasion abilities *in vitro*, but also can accelerate tumor formation and lung metastasis in a mouse xenograft model by targeting Lactoferrin (LTF) (Deng et al., 2013). miR-421 inhibited forkhead box protein O4 (FOXO4) by directly targeting FOXO4 following downregulation of p21, p27, Bim and FASL expression and induced NPC cell growth and apoptosis resistance (Chen et al., 2013). miR-663 was previously reported to be decreased and identified as a tumor suppressor in gastric cancer (Pan et al., 2010). However, miR-663 was found to be up-regulated in NPC cells and tissue samples (Yi et al., 2012). Indeed, inhibition of miR-663 impaired the proliferation of NPC cells *in vitro* and the NPC tumor growth of xenografts in nude mice. Mechanistically, miR-663 directly targeted p21 (WAF1/CIP1) to promote the cellular G1/S transition and acted as an oncogene in NPC.

Dysregulated Cellular miRNAs Function as Tumor-suppressors in NPC

In contrast to oncomirs, miRNAs with tumor suppressive activity by promoting cellular differentiation and/or apoptosis, are regarded as a tumor suppressors, which are often down-regulated in cancer cells compared to normal cells. To date, several miRNAs including miR-218, miR-216b, miR-26a, miR-101, miR-98, let-7, miR-138, miR-200 have been shown to be reduced significantly in NPC tissues and cell lines. Direct interaction between miR-218 and the 3'-UTR of mRNAs encoding ROBO1, survivin and connexin43 were validated in NPC, cervical, and breast cell lines, and suppressive effects of miR-218 on NPC survival and migration were rescued by enforced expression of survivin and ROBO1, respectively (Alajez et al., 2011). miR-216b was related to advanced clinical stage and lymph node metastasis of NPC. Both *in vitro* and *in vivo* miR-216b attenuated NPC cell proliferation, invasion and tumor growth in nude mice and exerted its tumor suppressor function through targeting KRAS and inhibition of the KRAS-related AKT and ERK pathways (Deng et al., 2011). Enhancer of Zeste homolog 2 (EZH2), as a member of the polycomb group (PcG) gene family, was associated with a higher risk of relapse in NPC patients. Using a luciferase-based assay, miR-98, miR-26a, and miR-101 were validated as bona fide regulators of EZH2 expression (Alajez et al., 2010; Yu et al., 2013). miR-98 was under-expressed in relapsed NPC patient samples, strongly suggesting miR-98 functioned as a tumor-suppressing modulator in NPC biology. miR-26a

functioned as a growth-suppressive miRNA in NPC by repressing the expression of c-myc, Cyclin D3 and E2, CDK4 and CDK6 while enhancing CDK inhibitors p14ARF and p21CIP1 in an EZH2-dependent manner (Lu et al., 2011). The let-7 miRNA family (-a, -b, -d, -e, -g, and -i) was involved in the proliferation, apoptosis and invasion of cancer cells and downregulated in numerous types of cancer, including lung cancer, gastric tumors, colon cancer (Akao et al., 2006; Ohshima et al., 2010; Xia et al., 2010). Ectopic expression of the let-7 family in nasopharyngeal carcinoma cells resulted in inhibition of cell proliferation through downregulation of c-Myc expression (Wong et al., 2011), and miR-138 can lead to cell cycle arrest and apoptosis and dramatically suppress NPC cell lines growth, proliferation and colony formation *in vitro* and inhibit tumorigenesis *in vivo* by targeting and down-regulating expression of CCND1 which was widely up-regulated in NPC tissues (Liu et al., 2012). A series of gain-of-function and loss-of-function studies showed that over-expression of miR-200a inhibits C666-1 cell growth, migration and invasion, whereas its knock-down stimulates these processes in CNE-1 cells (Xia et al., 2010). ZEB2 and CTNNB1 were the functional downstream targets of miR-200a. Interestingly, knock-down of ZEB2 solely impeded NPC cell migration and invasion, whereas CTNNB1 suppression only inhibited NPC cell growth, suggesting that the inhibitory effects of miR-200a on NPC cell growth, migration and invasion are mediated by distinct targets and pathways (Xia et al., 2010).

Dysregulated Cellular miRNAs Related to Invasion and Metastasis of NPC

Invasion and metastasis are prominent characteristics of NPC and are major causes for NPC deaths (Yoshizaki et al., 2012). Although evidences were mounting that miRNAs had direct relevance in metastasis, such information has only begun to surface, leaving many areas in this field yet to be explored. Recent findings had demonstrated that some miRNAs including miR-10b, which was induced by EBV-encoded LMP1 and mentioned above, miR-149, miR-29c, miR-143, miR-26a and miR-375 were involved in invasion and metastasis of NPC, in which miR-149 acted as the activator by downregulating the expression of E-cadherin, regulating epithelial-mesenchymal transition (EMT) and promoting the proliferation, mobility and invasion of NPC cell lines, whereas miR-29c, miR-143, miR-26a and miR-375 were suppressor in invasion and metastasis of NPC. In NPC tumors, the lower miR-29c levels were correlated with higher levels of multiple mRNAs such as collagen 3A1, 4A1, 15A1, laminin γ 1, and thymine-DNA glycosylase (TDG), whose 3'-UTRs can bind miR-29c at target sequences conserved across many vertebrates and most of which were associated with tumor cell invasiveness and metastatic potential (Sengupta et al., 2008). miR-143 played a role in regulating the invasiveness and metastasis of NPC, and overexpression of miR-143 caused a significant reduction of the adhesion ability of the highly metastatic NPC cell line (Zhong et al., 2013). miR-26a inhibited invasiveness and metastasis

of NPC cells through the repression of EZH2 (Yu et al., 2013). miR-375 may be the suppressor of metastasis by targeting metadherin (MTDH), which was significantly increased in NPC samples. Ectopic expression of miR-375 and knock-down MTDH by siRNA both decreased cell viability and clonogenic survival, cell migration, as well as *in vivo* tumor formation. NPC patients with high levels of MTDH expressed would experience significantly lower survival and, in particular, higher distant relapse rates (Hui et al., 2011).

Dysregulated Cellular miRNAs Modulate Radio-chemotherapy Sensitivity of NPC

Radiotherapy, especially intensity-modulated radiotherapy (IMR) with adjuvant chemotherapy were the primary treatment for NPC, but radio-chemotherapy resistance severely reduces the cure effect of NPC (Ng et al., 2011). Albeit the causes of radio-chemotherapy resistance remained to be unclear, recent researches showed that some miRNAs involved in the radio-chemotherapy sensitivity in NPC and some drugs such as Statins can induce the expression of tumor suppressor Let-7a and suppressing miR-21 and miR-155 in NPC cell (Wang et al., 2011). Although reports on miRNAs associated with response to radio-chemotherapy are little to date, the emerging data gives hope that panels of miRNAs will be identified to predict radio-chemotherapy sensitivity in NPC. Ectopic restoration of miR-29c could enhance the sensitivity of NPC cells to IR and cisplatin treatment by promoting apoptosis through repressing expression of anti-apoptotic factors, Mcl-1 and Bcl-2 in NPC tissues and cell lines (Zhang et al., 2013). With the same effects of prothymosin alpha (PTMA) siRNA, miR-1 transfection can accelerate the apoptotic process by targeting PTMA gene in a number of cell lines including NPC-TW06 treated with apoptosis inducers such as actinomycin D, camptothecin and etoposide which are also the chemotherapeutic drugs in clinical cancer therapy (Wu et al., 2011). Therefore miR-1 transfection and PTMA siRNA may have potential applications as an adjuvant in cancer chemotherapy. Although miR-205 was significantly reduced in breast tumors in which miR-205 act as the tumor suppressor through directly targeting of oncogenes such as ErbB3 and Zeb1 (Wu & Mo, 2009; Wijnhoven et al., 2010), miR-205 has been shown to be more elevated in radio-resistant NPC cell line (CNE-2R) with suppressed PTEN protein expression than that in its parental cell line CNE-2. Knock-down miR-205 in CNE-2R cells compromised the inhibition of PTEN and increased cell apoptosis. Significantly, PTEN was downregulated at late stages of NPC, and miR-205 was significantly elevated followed the radiotherapy (Qu et al., 2012). On the other hand, miRNA-324-3p was significantly decreased in CNE-2R cells and its downstream target WNT2B was up-regulation. miRNA-324-3p contributes to the radioresistance of NPC by regulating the WNT2B signalling pathway (Li et al., 2013). These suggested that both miR-205 and miRNA-324-3p may be potential predictive biomarkers for radiosensitivity of NPC and serve as targets for achieving successful radiotherapy in

NPC.

As demonstrated above, many dysregulated cellular miRNAs involved in carcinogenesis of NPC by targeting gene and disturbing signal transduction pathway related to proliferation, apoptosis, migration, invasion and radio-chemotherapy sensitivity. Notably, despite much progress have been made in miRNAs implicated in carcinogenesis of NPC, the comprehensive profiles of aberrant miRNAs in the progression of NPC and their functions need to be further elucidated, and identifying the coordinated regulatory networks of miRNA-mRNA and transcription factor-miRNA maybe more meaningful to clarify important roles of miRNAs in the development of NPC.

Clinical-applied Perspectives of miRNAs in Diagnosis, Prognosis and Therapy of NPC

At present, the prognosis of most patients with NPC is poor, because NPC is usually diagnosed at the advanced stages due to its deep anatomical position, without special symptoms and lack of effective diagnostic biomarkers, so search for easily available biomarkers in serum or plasma for early diagnosis of NPC is urgent (Cho, 2007). Some proteins such as fibronectin, Mac-2 binding protein and cell-free EBV-DNA had previously been described as biomarkers (Tsu et al., 2007), but they cannot be considered as ideal biomarkers for all NPC patients because lack of sensitivity and specificity. Being able to regulate the most cellular processes related to carcinogenesis, it is logical to hypothesize that cell free miRNAs would be potential biomarkers of early clinical diagnosis/prognosis and even further developed into therapeutic agents (Qu et al., 2011). Recent studies have provided evidence that a combination of two differentially expressed serum miRNAs miR-15b and miR-27b have the potential to be sensitive, cost-effective biomarkers for early detection of non small cell lung cancer (NSCLC) (Hennessey et al., 2012). More importantly, the discovery of BART-miRNAs in plasma, existing in exosomes from both NPC xenografts and cell lines which contain stable miRNAs and protect from endogenous RNase activity, enforced the possibility that BART-miRNAs act as early diagnosis and prognosis biomarkers of NPC (Jr Meckes et al., 2010).

Recently, Liu et al established a signature of 5 miRNAs (miR-142-3p, miR-29c, miR-26a, miR-30e and miR-93) from the 41 differentially expressed miRNAs between nasopharyngeal carcinoma and non-cancer nasopharyngitis tissues as independent prognostic factors in NPC patients (Liu et al., 2012). Similarly, 4 miRNAs including miR-17, miR-20a, miR-29c, and miR-223 were found to be expressed differentially in the serum of NPC compared with that of non-cancerous control. Based on this, a diagnosis equation with Ct difference method has been established to distinguish NPC cases and non-cancerous controls and validated with high sensitivity and specificity (Zeng et al., 2012). The miR-BART7 was detectable in all the patient plasma samples and was independent of the EBV DNA level. The plasma ebv-miR-BART7 could be a potential biomarker for undifferentiated NPC and used in NPC screening, especially in cases where

EBV DNA is not detectable (Wong et al., 2012) .

In addition, the low expression of Dicer and Drosha, which were important microRNA-processing enzymes, was significantly correlated with shorter progression-free survival (PFS) and overall survival (OS) of NPC patients (Guo et al., 2012). A prognostic score model combining the Dicer1 expression and TNM stage had a better prognostic value than the TNM stage alone model or Dicer1 expression alone model. miR-218 down-regulated and ROBO1 overexpression were significantly associated with worse overall and nodal relapse-free survival (Alajez et al., 2011). The SNP rs4919510C>G in miRNA-608 may be a biomarker to predict locoregional recurrence (LRR) in radiotherapy-treated NPC patients (Zhou et al., 2013). These evidences from the studies outlined above indicated the potential of some miRNAs and proteins related to miRNAs as noninvasive biomarkers in NPC. However, further investigations are indispensable to validate their functions and targets of more microRNAs during multistage carcinogenesis of NPC, thus ultimately making miRNAs biomarker more meaningful and improving diagnosis and prognosis of patients sufficiently.

Besides the potential as predictive biomarkers, the ability of a miRNA targeting many genes would be hopeful about the therapeutic potential of miRNAs in human carcinoma. Antagomirs (AMOs) or locked-nucleic-acid antisense oligonucleotides (LNA) can pair-up with miRNAs and prevent them from acting on their target mRNAs, so silencing of miRNAs to normalize the function of oncomirs is theoretically feasible. Alternatively, to increase the expression of a miRNA with tumor suppressing function, pri-miRNA and miRNA mimics delivered by viral or liposomal vector are showing promise in achieving miRNA introduction (Oh et al., 2009; Garzon et al., 2010). However, researches about the therapeutic potential of miRNAs and regulatory mechanism related to miRNA expression itself are still in its infancy. The proliferation and invasion ability of 5-8F cells were significantly inhibited by transfecting recombinant lentivirus vector for shRNA targeting EZH2 (Liang et al., 2012). The ectopic expression of miRNAs such as miR-138, miR-216a can dramatically suppress cell proliferation and colony formation in vitro and inhibit tumorigenesis *in vivo* (Deng et al., 2011; Liu et al., 2012). Although additional investigations are necessary to fully exploit the use of miRNAs in NPC patients, it suggested that some miRNAs have potential to act as therapeutic targets or replacement therapies. With the deepening of the research, transfecting tumour-suppressor miRNAs or antagonizing oncogenic miRNAs, combination with radiotherapy and chemotherapy for the treatment of patients with NPC will be conceivable in the future.

Conclusions

EBV-encoded miRNAs and host cellular miRNAs play important roles in the process of carcinogenesis of NPC. EBV-encoded miRNAs can interfere in the expression of viral and host cell genes to prevent the viral-infected host cell from the immune response, promote proliferation and suppress apoptosis which all facilitate carcinogenesis

and aggressive tumour phenotype of NPC. Aberrantly-expressed cellular miRNAs in NPC can act as oncogenes or tumor suppressors by changing the expression of their mRNA targets, and some miRNAs are related to invasion, metastasis and radio-chemotherapy sensitivity of NPC. The association of miRNA dysregulation with carcinogenesis of NPC and the functional analysis of specific miRNAs suggested the feasibility of using miRNAs as biomarkers and targets of therapeutic intervention in the future, although additional investigations need to be done.

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References

- Akao Y, Nakagawa Y, Naoe T (2006). let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. *Biol Pharm Bull*, **29**, 903-6.
- Alajez NM, Lenarduzzi M, Ito E, et al (2011). MiR-218 suppresses nasopharyngeal cancer progression through downregulation of survivin and the SLIT2-ROBO1 pathway. *Cancer Res*, **71**, 2381-91.
- Alajez NM, Shi W, Hui AB, et al (2010). Enhancer of Zeste homolog 2 (EZH2) is overexpressed in recurrent nasopharyngeal carcinoma and is regulated by miR-26a, miR-101, and miR-98. *Cell Death Dis*, **1**, e85.
- Barth S, Meister G, Grasser FA (2011). EBV-encoded miRNAs. *Biochim Biophys Acta*, **1809**, 631-40.
- Cai X, Schafer A, Lu S, et al (2006). Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathog*, **2**, e23.
- Cameron JE, Yin Q, Fewell C, et al (2008). Epstein-Barr virus latent membrane protein 1 induces cellular MicroRNA miR-146a, a modulator of lymphocyte signaling pathways. *J Virol*, **82**, 1946-58.
- Cao JY, Mansouri S, Frappier L (2012). Changes in the nasopharyngeal carcinoma nuclear proteome induced by the EBNA1 protein of Epstein-Barr virus reveal potential roles for EBNA1 in metastasis and oxidative stress responses. *J Virol*, **86**, 382-94.
- Cao SM, Simons MJ, Qian CN (2011). The prevalence and prevention of nasopharyngeal carcinoma in China. *Chin J Cancer*, **30**, 114-9.
- Chan JY, Gao W, Ho WK, Wei WI, Wong TS (2012). Overexpression of Epstein-Barr virus-encoded microRNA-BART7 in undifferentiated nasopharyngeal carcinoma. *Anticancer Res*, **32**, 3201-10.
- Chen HC, Chen GH, Chen YH, et al (2009). MicroRNA deregulation and pathway alterations in nasopharyngeal carcinoma. *Br J Cancer*, **100**, 1002-11.
- Chen L, Tang Y, Wang J, Yan Z, Xu R (2013). miR-421 induces cell proliferation and apoptosis resistance in human nasopharyngeal carcinoma via downregulation of FOXO4. *Biochem Biophys Res Commun*, **435**, 745-50.
- Chen SJ, Chen GH, Chen YH, et al (2010). Characterization of Epstein-Barr virus miRNAome in nasopharyngeal carcinoma by deep sequencing. *PLoS One*, **5**, doi:pii: e12745. 10.1371/journal.pone.0012745.
- Cho WC (2007). Nasopharyngeal carcinoma: molecular

- biomarker discovery and progress. *Mol Cancer*, **6**, doi: 10.1186/1476-4598-6-1
- Choy EY, Siu KL, Kok KH, et al (2008). An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. *J Exp Med*, **205**, 2551-60.
- Cosmopoulos K, Pegtel M, Hawkins J, et al (2009). Comprehensive profiling of Epstein-Barr virus microRNAs in nasopharyngeal carcinoma. *J Virol*, **83**, 2357-67.
- Dawson CW, Port RJ, Young LS (2012). The role of the EBV-encoded latent membrane proteins LMP1 and LMP2 in the pathogenesis of nasopharyngeal carcinoma (NPC). *Semin Cancer Biol*, **22**, 144-53.
- Deng M, Tang H, Zhou Y, et al (2011). miR-216b suppresses tumor growth and invasion by targeting KRAS in nasopharyngeal carcinoma. *J Cell Sci*, **124**, 2997-3005.
- Deng M, Ye Q, Qin Z, et al (2013). miR-214 promotes tumorigenesis by targeting lactotransferrin in nasopharyngeal carcinoma. *Tumour Biol*, **34**, 1793-800.
- Dolken L, Malterer G, Erhard F, et al (2010). Systematic analysis of viral and cellular microRNA targets in cells latently infected with human gamma-herpesviruses by RISC immunoprecipitation assay. *Cell Host Microbe*, **7**, 324-34.
- Du ZM, Hu LF, Wang HY, et al (2011). Upregulation of MiR-155 in nasopharyngeal carcinoma is partly driven by LMP1 and LMP2A and downregulates a negative prognostic marker JMJD1A. *PLoS One*, **6**, e19137.
- Edwards RH, Marquitz AR, Raab-Traub N (2008). Epstein-Barr virus BART microRNAs are produced from a large intron prior to splicing. *J Virol*, **82**, 9094-106.
- Farazi TA, Hoell JI, Morozov P, Tuschl T (2013). MicroRNAs in human cancer. *Adv Exp Med Biol*, **774**, 1-20.
- Garzon R, Marcucci G, Croce CM (2010). Targeting microRNAs in cancer: rationale, strategies and challenges. *Nat Rev Drug Discov*, **9**, 775-89.
- Guo X, Liao Q, Chen P, et al (2012). The microRNA-processing enzymes: Drosha and Dicer can predict prognosis of nasopharyngeal carcinoma. *J Cancer Res Clin Oncol*, **138**, 49-56.
- He ML, Luo MX, Lin MC, Kung HF (2012). MicroRNAs: potential diagnostic markers and therapeutic targets for EBV-associated nasopharyngeal carcinoma. *Biochim Biophys Acta*, **1825**, 1-10.
- Hennessey PT, Sanford T, Choudhary A, et al (2012). Serum microRNA biomarkers for detection of non-small cell lung cancer. *PLoS One*, **7**, e32307.
- Hui AB, Bruce JP, Alajez NM, et al (2011). Significance of dysregulated metadherin and microRNA-375 in head and neck cancer. *Clin Cancer Res*, **17**, 7539-50.
- Jr Meckes DG, Shair KH, Marquitz AR, et al (2010). Human tumor virus utilizes exosomes for intercellular communication. *Proc Natl Acad Sci U S A*, **107**, 20370-5.
- Kim DN, Chae HS, Oh ST, et al (2007). Expression of viral microRNAs in Epstein-Barr virus-associated gastric carcinoma. *J Virol*, **81**, 1033-6.
- Kim DN, Lee SK (2012). Biogenesis of Epstein-Barr virus microRNAs. *Mol Cell Biochem*, **365**, 203-10.
- Kok KH, Lei T, Jin DY (2010). Identification and validation of the cellular targets of virus-encoded microRNAs. *Methods Mol Biol*, **667**, 319-26.
- Li G, Liu Y, Su Z, et al (2013). MicroRNA-324-3p regulates nasopharyngeal carcinoma radioresistance by directly targeting WNT2B. *Eur J Cancer*, doi: 10.1016/j.ejca.2013.03.001.
- Li G, Wu Z, Peng Y, Liu X, et al (2010). MicroRNA-10b induced by Epstein-Barr virus-encoded latent membrane protein-1 promotes the metastasis of human nasopharyngeal carcinoma cells. *Cancer Lett*, **299**, 29-36.
- Li JX, Lu TX, Huang Y, Han F (2012). Clinical characteristics of recurrent nasopharyngeal carcinoma in high-incidence area. *Sci World J*, doi: 10.1100/2012/719754
- Li T, Chen JX, Fu XP, et al (2011). microRNA expression profiling of nasopharyngeal carcinoma. *Oncol Rep*, **25**, 1353-63.
- LIANG BJ, LI XP, LU J, et al (2012). [Effects of enhancer of zeste homolog (EZH2) downregulation on the proliferation and invasion of nasopharyngeal carcinoma cell and the possible mechanisms]. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*, **47**, 298-304.
- Liu N, Chen NY, Cui RX, et al (2012). Prognostic value of a microRNA signature in nasopharyngeal carcinoma: a microRNA expression analysis. *Lancet Oncol*, **13**, 633-41.
- Liu X, Lv XB, Wang XP, et al (2012). MiR-138 suppressed nasopharyngeal carcinoma growth and tumorigenesis by targeting the CCND1 oncogene. *Cell Cycle*, **11**, 2495-506.
- Lo AK, Dawson CW, Jin DY, Lo KW (2012). The pathological roles of BART miRNAs in nasopharyngeal carcinoma. *J Pathol*, **227**, 392-403.
- Lo AK, To KF, Lo KW, et al (2007). Modulation of LMP1 protein expression by EBV-encoded microRNAs. *Proc Natl Acad Sci U S A*, **104**, 16164-9.
- Lu J, He ML, Wang L, et al (2011). MiR-26a inhibits cell growth and tumorigenesis of nasopharyngeal carcinoma through repression of EZH2. *Cancer Res*, **71**, 225-33.
- Lung RW, Wang X, Tong JH, et al (2012). A single nucleotide polymorphism in microRNA-146a is associated with the risk for nasopharyngeal carcinoma. *Mol Carcinog*, doi: 10.1002/mc.21937.
- Luo Z, Dai Y, Zhang L, et al (2013). miR-18a promotes malignant progression by impairing microRNA biogenesis in nasopharyngeal carcinoma. *Carcinogenesis*, **34**, 415-25.
- Luo Z, Zhang L, Li Z, et al (2012). An in silico analysis of dynamic changes in microRNA expression profiles in stepwise development of nasopharyngeal carcinoma. *BMC Med Genomics*, doi: 10.1186/1755-8794-5-3.
- Marquitz AR, Mathur A, Nam CS, Raab-Traub N (2011). The Epstein-Barr Virus BART microRNAs target the pro-apoptotic protein Bim. *Virology*, **412**, 392-400.
- Marquitz AR, Raab-Traub N (2012). The role of miRNAs and EBV BARTs in NPC. *Semin Cancer Biol*, **22**, 166-72.
- Mei J, Bachoo R, Zhang CL (2011). MicroRNA-146a inhibits glioma development by targeting Notch1. *Mol Cell Biol*, **31**, 3584-92.
- Nachmani D, Stern-Ginossar N, Sarid R, Mandelboim O (2009). Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB to escape recognition by natural killer cells. *Cell Host Microbe*, **5**, 376-85.
- Ng WT, Lee MC, Hung WM, et al (2011). Clinical outcomes and patterns of failure after intensity-modulated radiotherapy for nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys*, **79**, 420-8.
- Oh SY, Ju Y, Park H (2009). A highly effective and long-lasting inhibition of miRNAs with PNA-based antisense oligonucleotides. *Mol Cells*, **28**, 341-5.
- Ohshima K, Inoue K, Fujiwara A, et al (2010). Let-7 microRNA family is selectively secreted into the extracellular environment via exosomes in a metastatic gastric cancer cell line. *PLoS One*, **5**, e13247.
- Paik JH, Jang JY, Jeon YK, et al (2011). MicroRNA-146a downregulates NFkappaB activity via targeting TRAF6 and functions as a tumor suppressor having strong prognostic implications in NK/T cell lymphoma. *Clin Cancer Res*, **17**, 4761-71.
- Pan J, Hu H, Zhou Z, et al (2010). Tumor-suppressive mir-663 gene induces mitotic catastrophe growth arrest in human

- gastric cancer cells. *Oncol Rep*, **24**, 105-12.
- Polesel J, Serraino D, Negri E, et al (2013). Consumption of fruit, vegetables, and other food groups and the risk of nasopharyngeal carcinoma. *Cancer Causes Control*, **24**, 1157-65.
- Qu C, Liang Z, Huang J, et al (2012). MiR-205 determines the radioresistance of human nasopharyngeal carcinoma by directly targeting PTEN. *Cell Cycle*, **11**, 785-96.
- Qu H, Xu W, Huang Y, Yang S (2011). Circulating miRNAs: promising biomarkers of human cancer. *Asian Pac J Cancer Prev*, **12**, 1117-25.
- Sengupta S, den Boon JA, Chen IH, et al (2008). MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. *Proc Natl Acad Sci U S A*, **105**, 5874-8.
- Shair KH, Schnegg CI, Raab-Traub N (2008). EBV latent membrane protein 1 effects on plakoglobin, cell growth, and migration. *Cancer Res*, **68**, 6997-7005.
- Twu CW, Wang WY, Liang WM, et al (2007). Comparison of the prognostic impact of serum anti-EBV antibody and plasma EBV DNA assays in nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys*, **67**, 130-7.
- Wang W, Le W, Cho DY, Hwang PH, Upadhyay D (2011). Novel effects of statins in enhancing efficacy of chemotherapy in vitro in nasopharyngeal carcinoma. *Int Forum Allergy Rhinol*, **1**, 284-9.
- Webb N, Connolly G, Tellam J, Yap AS, Khanna R (2008). Epstein-Barr virus associated modulation of Wnt pathway is not dependent on latent membrane protein-1. *PLoS One*, **3**, e3254.
- Wijnhoven BP, Hussey DJ, Watson DI, et al (2010). MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. *Br J Surg*, **97**, 853-61.
- Wong AM, Kong KL, Tsang JW, Kwong DL, Guan XY (2012). Profiling of Epstein-Barr virus-encoded microRNAs in nasopharyngeal carcinoma reveals potential biomarkers and oncomirs. *Cancer*, **118**, 698-710.
- Wong TS, Man OY, Tsang CM, et al (2011). MicroRNA let-7 suppresses nasopharyngeal carcinoma cells proliferation through downregulating c-Myc expression. *J Cancer Res Clin Oncol*, **137**, 415-22.
- Wu CD, Kuo YS, Wu HC, Lin CT (2011). MicroRNA-1 induces apoptosis by targeting prothymosin alpha in nasopharyngeal carcinoma cells. *J Biomed Sci*, doi: 10.1186/1423-0127-18-80.
- Wu H, Mo YY (2009). Targeting miR-205 in breast cancer. *Expert Opin Ther Targets*, **13**, 1439-48.
- Xia H, Cheung WK, Sze J, et al (2010). miR-200a regulates epithelial-mesenchymal to stem-like transition via ZEB2 and beta-catenin signaling. *J Biol Chem*, **285**, 36995-7004.
- Xia H, Ng SS, Jiang S, et al (2010). miR-200a-mediated downregulation of ZEB2 and CTNNB1 differentially inhibits nasopharyngeal carcinoma cell growth, migration and invasion. *Biochem Biophys Res Commun*, **391**, 535-41.
- Xia XM, Jin WY, Shi RZ, Zhang YF, Chen J (2010). Clinical significance and the correlation of expression between Let-7 and K-ras in non-small cell lung cancer. *Oncol Lett*, **1**, 1045-1047.
- Xu X, Yang H, Huo X (2004). [Expression and significance of PTEN in nasopharyngeal carcinoma]. *Lin Chuang Er Bi Yan Hou Ke Za Zhi*, **18**, 658-9.
- Yi C, Wang Q, Wang L, et al (2012). MiR-663, a microRNA targeting p21 (WAF1/CIP1), promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma. *Oncogene*, **31**, 4421-33.
- Yoshizaki T, Ito M, Muroto S, et al (2012). Current understanding and management of nasopharyngeal carcinoma. *Auris Nasus Larynx*, **39**, 137-44.
- You S, Zhang F, Meng F, et al (2011). EBV-encoded LMP1 increases nuclear beta-catenin accumulation and its transcriptional activity in nasopharyngeal carcinoma. *Tumour Biol*, **32**, 623-30.
- Yu H, Lu J, Zuo L, et al (2012). Epstein-Barr virus downregulates microRNA 203 through the oncoprotein latent membrane protein 1: a contribution to increased tumor incidence in epithelial cells. *J Virol*, **86**, 3088-99.
- Yu L, Lu J, Zhang B, et al (2013). miR-26a inhibits invasion and metastasis of nasopharyngeal cancer by targeting EZH2. *Oncol Lett*, **5**, 1223-8.
- Yu X, Zhen Y, Yang H, et al (2013). Loss of connective tissue growth factor as an unfavorable prognosis factor activates miR-18b by PI3K/AKT/C-Jun and C-Myc and promotes cell growth in nasopharyngeal carcinoma. *Cell Death Dis*, **4**, e634.
- Zeng X, Xiang J, Wu M, et al (2012). Circulating miR-17, miR-20a, miR-29c, and miR-223 combined as non-invasive biomarkers in nasopharyngeal carcinoma. *PLoS One*, **7**, e46367.
- Zhang JX, Qian D, Wang FW, et al (2013). MicroRNA-29c enhances the sensitivities of human nasopharyngeal carcinoma to cisplatin-based chemotherapy and radiotherapy. *Cancer Lett*, **329**, 91-8.
- Zhang L, Deng T, Li X, et al (2010). microRNA-141 is involved in a nasopharyngeal carcinoma-related genes network. *Carcinogenesis*, **31**, 559-66.
- Zhang LY, Ho-Fun LV, Wong AM, et al (2013). MicroRNA-144 promotes cell proliferation, migration and invasion in nasopharyngeal carcinoma through repression of PTEN. *Carcinogenesis*, **34**, 454-63.
- Zhong W, He B, Quan T, et al (2013). [Expression of miR-143 in nasopharyngeal carcinoma cell lines and its effect on cell adhesion ability]. *Nan Fang Yi Ke Da Xue Xue Bao*, **33**, 582-5.
- Zhou Y, Zheng J, Deng J, et al (2013). A sequence polymorphism in miRNA-608 predicts recurrence after radiotherapy of nasopharyngeal carcinoma. *Cancer Res*, **73**, 5151-62.
- Zhu JY, Pfuhl T, Motsch N, et al (2009). Identification of novel Epstein-Barr virus microRNA genes from nasopharyngeal carcinomas. *J Virol*, **83**, 3333-41.