

# miR-141 is up-regulated in biopsies from Vietnamese patients with nasopharyngeal carcinoma

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**Abstract:** Novel biomarkers for screening, diagnosis and monitoring the treatment of nasopharyngeal carcinoma (NPC), one of the most common cancers in Vietnam, are urgently required. Increasing evidence suggests that microRNA-141 (miR-141) is associated with NPC, owing to its ability to affect the expression of genes that modulate tumorigenesis. Unfortunately, research on miR-141 expression in Vietnamese patients is limited. Therefore, the objective of the current study was to evaluate miR-141 expression and assess whether miR-141 might be a potential biomarker for diagnosis of NPC in Vietnamese patients. Total RNA isolated from 40 NPC biopsy samples and 37 non-cancerous samples was analyzed by quantitative reverse-transcription PCR. The miR-141 expression levels were compared between NPC biopsy and non-cancerous samples. The frequency of miR-141 detection was 37.50% and 10.80% in the NPC and non-cancerous samples, respectively ( $p = 0.0143$ ). The miR-141 expression was 5.27 times higher in tumor samples than non-cancerous samples. Additionally, the RR (Relative risk) and OR (Odds ratio) were 1.83 (95%CI = 1.2576–2.6675,  $p = 0.0016$ ) and 4.95 (95%CI = 1.4625–16.7541,  $p = 0.01$ ), respectively. In conclusion, miR-141 was up-regulated in the biopsy samples and thus may be a potential biomarker for NPC in the Vietnamese population.

**Keywords:** Biomarkers; Carcinoma; microRNAs; Biopsy; Nasopharynx

## Introduction

Nasopharyngeal carcinoma (NPC), which has pronounced differences in distribution according to geography and ancestry, is the fifth most common cancer worldwide. NPC is present in 86,691 cases (age-standardized rate (ASR) = 1.2/100,000) and is responsible for 50,831 (ASR = 0.7/100,000) deaths annually in Vietnam.<sup>1,2,3</sup> Although patient diagnosis has improved in recent decades, most NPC patients are still diagnosed at an advanced stage of NPC, owing to ambiguous symptoms.<sup>4,5,6</sup> Therefore, improved identification of potential biomarkers associated with NPC progression is essential for the development of new diagnosis strategies and promising therapies.

The use of NPC biopsy samples is considered the “gold standard” in NPC diagnosis. Beside NPC biopsy samples, other types of samples, such as peripheral blood, nasopharyngeal swabs, throat swabs, plasma and



saliva have been used for detecting the etiological causes of NPC, including the presence of Epstein-Barr virus, methylation and microRNAs (miRNAs, denoted by the miR prefix) associated with NPC.<sup>7,8,9,10,11,12</sup> In our recent study, we found that among *EBNA-1*, *EBNA-2*, *LMP-1* and *LMP-2*, at least one EBV DNA was detectable in 57.9% of NPC samples, whereas a low frequency was found in non-cancerous swab samples.<sup>13</sup> Another recent study has also detected significantly elevated EBV DNA loads and miRNAs, such as *mir-bart-5p*, in nasopharyngeal swab samples from non-NPC patients.<sup>10,11,12</sup>

miRNAs are a small class of endogenous, functional non-RNAs that are highly conserved and are widely found in animals, plants, protists and viruses; miRNAs are involved in a wide range of biological processes, such as cell proliferation, differentiation, apoptosis and motility, through post-transcriptional repression of gene expression.<sup>6,14,15,16,17</sup> miRNA-141 (miR-141) is a member of the oncogenic miRNAs clustered with miR-200c, located at 12p13.31 (nt: 6964097-6964191, [+]). The dysregulation of miR-141 is associated with many human cancers, such as hepatocellular carcinoma, renal cell carcinoma, breast cancer, ovarian cancer and NPC.<sup>6,18,19,20,21</sup> However, only a few publications have described miR-141 profiles in NPC. Zhang et al.<sup>22</sup> have reported that miR-141 is up-regulated in NPC specimens compared with normal nasopharyngeal epithelium, and that it affects the cell cycle, apoptosis, cell growth, migration and invasion in NPC cells. Additionally, targets of miR-141, including *BRD3*, *PTEN* and *UBAP1*, have been found to be involved in a gene-miR-141 network contributing to NPC development.<sup>22</sup> Chen et al.<sup>23</sup> have reported that miR-141 is regulated by *SPLUNC1* (short palate, lung and nasal epithelium clone 1) and is related to the differentiation stages of NPC cell lines; notably, miR-141 expression is significantly elevated in NP-69 cells (a nasopharyngeal epithelial cell line) transfected with *LMP1*.<sup>23</sup> Another report has indicated that the expression of miR-141 is markedly increased in NPC tissues and is involved in *BRD7*-mediated cell progression and tumor formation through suppression of the *PTEN/AKT* axis in nasopharyngeal carcinoma, thus providing several promising targets

for NPC diagnosis. Furthermore, inhibitors specific to miR-141 may provide a promising strategy for NPC therapy.<sup>6</sup>

To our knowledge, no studies of the types of miR-141 expression in Vietnamese NPC patients have been reported to date. Therefore, the current study is the first case-control study on Vietnamese clinical NPC samples. The aim of the study was to access the differential expression of miR-141 in biopsy samples from NPC patients and non-cancerous nasopharyngeal swab samples to reveal potential biomarkers for NPC.

## Methodology

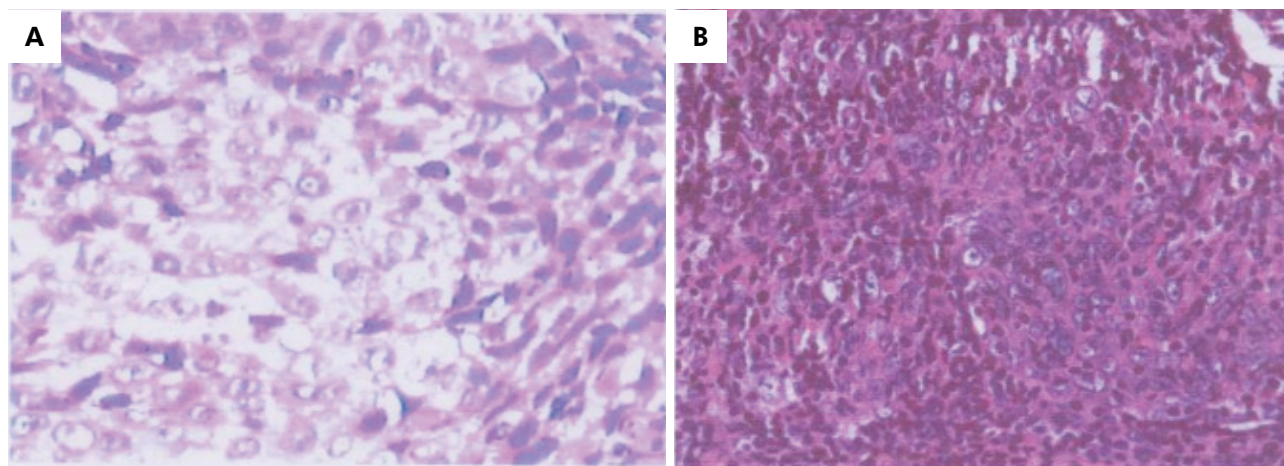
### Ethics statement

Institutional Ethics Board approval was obtained from the Medical Ethics Committee of the Cho Ray Hospital, Ho Chi Minh City, Vietnam (decision number 516/BVCR-HDDD). Collection of all samples used in this study was approved by the Cho Ray Hospital, and samples were obtained from all participants in the current study. The patients were required to provide signed informed consent forms.

### Sample collection

A case-control study was carried out. A total of 40 NPC biopsy samples were archived from the Cho Ray Hospital, Vietnam. All samples were submitted to the histopathological department, and NPC was confirmed through immunohistochemistry. The mean age of the NPC patients was 49.6 (range from 15 to 72). The subjects included 31 males (accounting for 77.5%) and nine females (accounting for 22.5%). The NPC biopsy samples were confirmed as non-keratinizing squamous cell carcinoma and undifferentiated carcinoma via hematoxylin and eosin staining in histological examination (Figure 1). For non-cancerous controls, 37 nasopharyngeal swab samples that were negative for nasopharyngeal carcinoma were collected from non-NPC patients. All samples were placed in 1.5-ml tubes containing PBS buffer and stored at -20°C for further experiments.

RNA, including miRNAs, was isolated from NPC biopsy samples and non-cancerous samples



**Figure 1.** Histological examination of (A) undifferentiated carcinoma (WHO type 3) and (B) non-keratinizing squamous cell carcinoma (WHO type 2).

with a *mirVana*<sup>TM</sup> miRNA Isolation Kit (Ambion, Life Technology) according to the manufacturer's instructions. cDNA was reverse transcribed from approximately 5 ng of Total RNA with a SensiFAST<sup>TM</sup> cDNA synthesis Kit (Bioline). The detection of miR-141 was performed with a quantitative real-time PCR (qRT PCR) assay with Taqman<sup>TM</sup> Advanced Advance miRNA kits (Thermo Fisher Scientific). Five microliters of the RT reaction product was used in each reaction for detection of miR-141. The UniSp6 rRNA was used as the internal control to normalize Ct values, because of the non-differential expression in tumor and adjacent healthy samples.

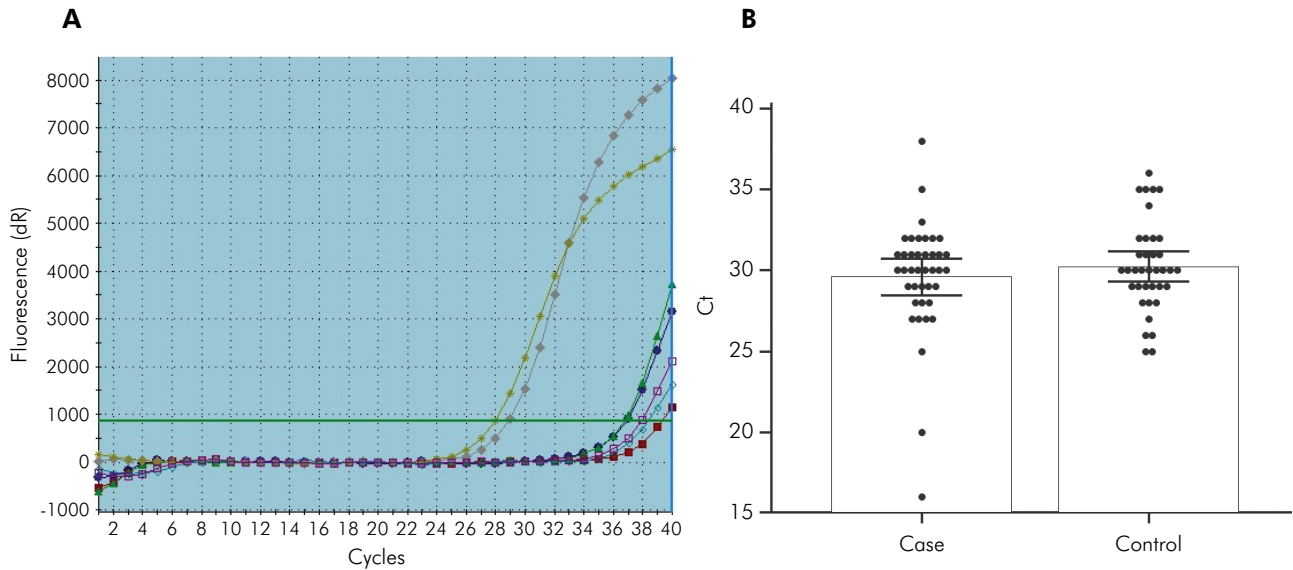
### Statistical analysis

Data were analyzed in Medcalc® Version 12.7.0.0. All *p* values were two-sided, and values less than 0.05 were considered significant. All values are reported as mean ± SD. The relative expression of miR-141, as determined with q-PCR, was analyzed with the  $2^{-\Delta\Delta Ct}$  method. Values greater and less than one were used to indicate up-regulation and down-regulation, respectively. Chi square tests were used to determine the association between miR-141 expression and NPC status. Moreover, the association between the expression of miR-141 and NPC risk was estimated by computing the OR, RR and 95% confidence intervals (CI).

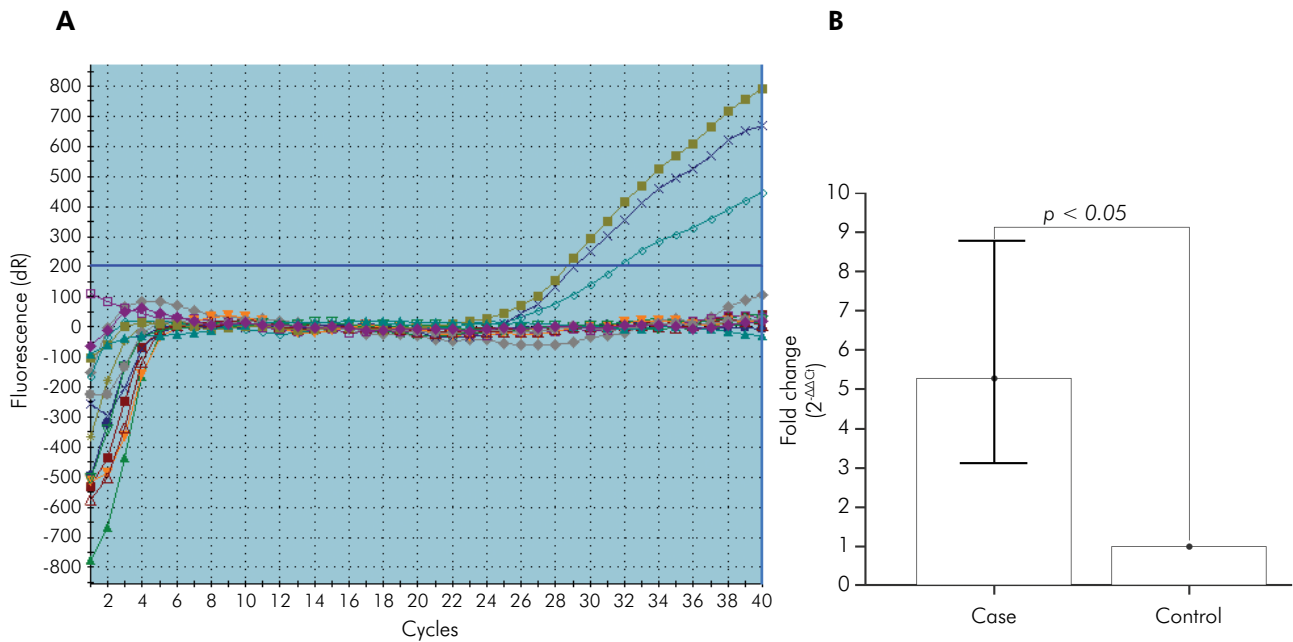
## Results

First, UniSp6 rRNA was detected in both case and control samples through real-time PCR. Both the NPC biopsies and non-cancerous controls were positive for UniSp6 rRNA (Figure 2). The mean Ct values for UniSp6 rRNA in the case and control groups were  $29.60 \pm 0.56$  and  $30.24 \pm 0.46$ , respectively. Therefore, UniSp6 was suitable as a reference gene (internal control) to normalize miR-141 expression between the case and control groups.

In the current case-control study, miR-141 expression in the NPC biopsy samples was positive in 37.50% (15 of 40 cases) and negative in 62.50% (25 of 40 cases). In the non-cancerous samples, miR-141 expression was positive in 10.80% (4 of 37 cases) and negative in 89.20% (33 of 37 cases). The expression of miR-141 in NPC biopsy samples was significantly higher than that in non-cancerous samples ( $p = 0.0143$ ). The mean Ct values of miR-141 in the case and control groups were  $26.27 \pm 0.95$  and  $29.50 \pm 1.56$ , respectively. Relative quantification of miR-141 expression between the case and control groups was determined with the  $2^{-\Delta\Delta Ct}$  method. The miR-141 level was 5.28 times higher in tumor samples than in non-cancerous samples ( $2^{-\Delta\Delta Ct} = 5.28$  (3.16–8.82)) (Figure 3). Additionally, the OR and RR values computed between the expression of miR-141 and NPC were 4.95 (95%CI = 1.4625–16.7541,  $p = 0.01$ ) and 1.83 (95%CI = 1.2576–2.6675,  $p = 0.0016$ ), respectively.



**Figure 2.** (A) UniSp6 rRNA expression detected in a representative healthy control. (B) The mean Ct values (mean  $\pm$  SE) of UniSp6 rRNA in the case and control groups. Each black dot indicates the Ct value of each sample.



**Figure 3.** (A) miR-141 expression detected/undetected in representative NPC biopsy samples. (B) qRT-PCR showing up-regulation of miR-141 in NPC biopsy samples compared with non-cancerous samples.

## Discussion

The amplification of nucleic acids from nasopharyngeal swabs and throat swabs has been

found to be suitable for all types of testing, owing to stability at room temperature and in storage at 4°C.<sup>10</sup> Additionally, according to the WHO, many types of swab specimens, including nasopharyngeal swabs, are standard protocols used to obtain samples for

clinical and quarantine station diagnosis. The use of nasopharyngeal swabs has many advantages in studying the expression of potential miRNA biomarkers in throat swabs of healthy control and NPC patients.<sup>11</sup> In the current study, we investigated patients who underwent nasopharyngeal biopsy with imaging and biopsy results, confirmed the samples for NPC and performed an initial evaluation of miR-141 expression in Vietnamese NPC patients. Many studies have demonstrated that dysregulation of miRNAs is associated with the pathogenesis of various human tumors. Recently, a distinct miRNA signature has been proposed as a potential biomarker for the diagnosis of various types of human cancer.<sup>6</sup> Previous studies have demonstrated that the use of miRNAs, a class of non-protein encoding RNA molecules that function in epigenetic regulation, are a novel class of therapeutic targets for the treatment of cancer.<sup>24,25</sup> One of them, miR-141, has been well documented to have both oncogenic and tumor suppressive roles in human malignancies. The oncogenic role has been observed in ovarian cancer and NPC.<sup>6,22,23,26,28</sup> In the current study, we detected positive miR-141 expression in 37.50% of NPC biopsy samples (sensitivity = 37,50%), thus suggesting that 37.50% of NPC samples can be expected to be positive for miR-141 expression. Moreover, the miR-141 expression in the NPC samples was higher than that in non-cancerous samples ( $p = 0.0143$ ), thus indicating that the expression of miR-141 is strongly associated with NPC. Additionally, the miRNA expression was confirmed to be 5.27 times higher in tumor samples than non-cancerous samples ( $2^{-\Delta\Delta Ct} = 5.27$ ). Up-regulated expression of miR-141 was observed in NPC samples, as has also been observed in other populations and NPC cell lines. Up-regulated expression of miR-141 has been reported by Zhang et al.,<sup>22</sup> Chen et al.<sup>23</sup> and Liu et al.<sup>6</sup> in NPC tissue samples collected from The Second Xiangya Hospital of Central South University (Changsha, China) and The Second Xiangya Hospital and Hunan Province Tumor Hospital, and in NPC cell lines, as compared with non-cancerous samples. However, in Vietnam, no studies on miR-141 in NPC has been reported. This is a follow-up study based on previous findings regarding the use of biopsies to assess miRNA properties. The main difference between this study

and previous publications is that we focused on the Vietnamese population, the country with the high mortality from NPC worldwide. The up-regulation of miR-141 was strongly correlated with NPC risk, and the results were significant on the basis of the RR and OR ( $p < 0.05$ ). miR-141 highly associated with higher odds, counting for 4.95 times, of NPC cancer (OR = 4.95, 95%CI = 1.4625–16.7541,  $p = 0.01$ ). Moreover, the expression of miR-141 was 1.83 times higher than no miR-141 expression in NPC (RR = 1.83, 95%CI = 1.2576–2.6675,  $p = 0.0016$ ). Therefore, even though limited samples were analyzed in the current study, we investigated the potential differential expression of miR-141 in NPC biopsy samples and healthy nasopharyngeal swabs. Finally, we suggest that, in agreement with findings from previous studies, miR-141 is up-regulated and has an oncogenic role in NPC development, including in the Vietnamese population; thus, it is a potential marker for NPC in Vietnamese NPC patients. In further studies, miR-141 obtained from non-invasive specimens, such as throat swabs or nasopharyngeal swabs, will be investigated as a biomarker for the screening and early diagnosis of NPC in the Vietnamese population.

## Conclusion

In the present study, the potential differential expression of miR-141 in NPC biopsy samples and nasopharyngeal swabs from healthy individuals was investigated. The miRNA was detected in 37.50% of cancerous and 10.80% of non-cancerous samples ( $p = 0.0143$ ). The present study demonstrated that miR-141 is up-regulated 5.25 fold in NPC samples compared with non-cancerous samples in the Vietnamese population. Therefore, miR-141 may be a potential diagnostic biomarker for NPC in Vietnamese patients. To develop a non-invasive method for clinical applications in screening and early diagnosis of NPC, further investigations should be performed in larger groups of samples as well as non-invasive samples.

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