



Upregulation of miRNA-155 in Nasopharyngeal Carcinoma Patients

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Abstract

Background: The upregulation of miRNA-155 (miR-155) has been associated with oncogenesis of many human tumors, including nasopharyngeal carcinoma (NPC). However, the profile of miR-155 in Vietnamese NPC patients has not been investigated. The current study aimed to evaluate the miR-155 expression and assess whether miR-155 is a potential biomarker for diagnosis of NPC in Vietnamese patients.

Methods: In current case-control study, total of RNA was isolated from 60 biopsy NPC samples and 60 non-cancerous swab samples were analyzed by Reverse-transcription PCR, qualitative Real-time PCR.

Results: The frequency of miR-155 detection were 78.33%, 15.0% in NPC and non-cancerous samples ($P < 0.05$), respectively. The miR-155 expression level was 4.92 times higher in tumor samples than non-cancerous sample.

Conclusion: Taken together, miR-155 in NPC was upregulated. It may serve as a potential biomarker for NPC in the Vietnamese population.

Keywords: Mirn155 microRNA; Nasopharyngeal carcinoma; Vietnamese

Introduction

microRNAs (miRs, miRNAs), originally discovered in *Caenorhabditis elegans* by Lee et al, typically represent ~20 nucleotides in length, are the abundant class of evolutionarily conserved, small non-coding RNAs (1). miRNAs play important roles in regulation of gene expression by binding to sequences in 3'-untranslated region (3'-UTR) of their target mRNAs, resulting block their translation (2,3). Their target regulation activities are involved in numerous cellular processes including cell proliferation, differentiation, apoptosis and metabolism (2). Recent years, growing evidences

indicated the regulation of miRNAs have been reported as both oncogenes and tumor suppressor genes that the abnormal expression of miRNAs contributes to many human tumor pathogenesis, including nasopharyngeal carcinoma (NPC) and other cancers (4-11). Therefore, miRNAs can be clinically applied, it is necessary to identify miRNAs play major functions as whether oncogenic miRNAs or tumor suppressor role in the pathogenesis of human tumorigenesis.



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The understanding of miRNAs' role will be able to develop the molecular target therapy by suppression or reactivation of them. miR-155, an evolutionarily conserved miRNA, is encoded by the MIR155 host gene (also named MIR155HG). The dysregulation of miR-155 are involved in different biological processes including hematopoiesis inflammation, immunity, as well as various human cancers, such as NPC, breast cancer, etc. (5, 6, 12-15). Unlike the other miRNAs, miR-155 has been widely studied in immune-biology, determined as the regulator of immune system (12, 16, 17). There are only few publications carried on the detection of miR-155 expression in NPC. miR-155 was up-regulated in NPC tissue and partly driven by LMP1 and LMP2 as well as Epstein-Barr virus negative NPC derived cell lines. The potential of miR-155 as the target-therapeutic treatment for NPC should be further investigated (7). Additionally, the oncogenic role of miR-155 has been studied and evaluated in NPC cell proliferation, migration as well as invasion. The oncogenic role of miR-155 was reported in that the miR-155 expression was up regulated in NPC cell line and EBV-positive NPC tissue samples collected from Chinese NPC patients. However, this study is carried on the limit amount of samples (15).

Vietnam, located in Southern Asia, is well known as the high incidence and mortality rate of nasopharyngeal carcinoma within 86,691 cases (Age-standardized rate –ASR=1.2/100,000) and 50,831 (ASR=0.7/100,000) deaths (18, 19). However, to our knowledge, the detection and evaluation of miR-155 expression in Vietnamese NPC patients have not been reported yet. Therefore, compared

to previous studies, the current study was the first case-control study, which focused on the Vietnamese local clinical NPC samples within the expanded sample size.

In the light of this, the aim of the study was to explore the characteristics of the expression of miR-155 in Vietnamese NPC samples; we determined the expression of miR-155 in NPC tissues collected from local patients in the comparison to non-cancerous samples to reveal potential therapeutic biomarkers for NPC in near future.

Methods

Ethics statement

Institutional Ethics Board approval was obtained from the Medical Ethics Committee of the Cho Ray Hospital, Ho Chi Minh City, Vietnam. (The decision number of the permission from Ethical committee: 516/BVCR-HDDD, Cho Ray Hospital, Ho Chi Minh City, Vietnam). All the samples used in this study were agreed by Cho Ray Hospital and obtained from all participants in current study. The patients were required to be agreed and to sign on the consent forms.

In current study, 60 NPC biopsy tissues were archived and admitted from the Cho Ray Hospital, Vietnam. All of those samples were submitted to the Histopathological Department. They were subsequently proved histologically to have NPC by Immunohistochemistry (IHC) confirmed. The NPC biopsy samples were positively confirmed as NPC by hematoxylin and eosin for histological examination (Fig. 1).

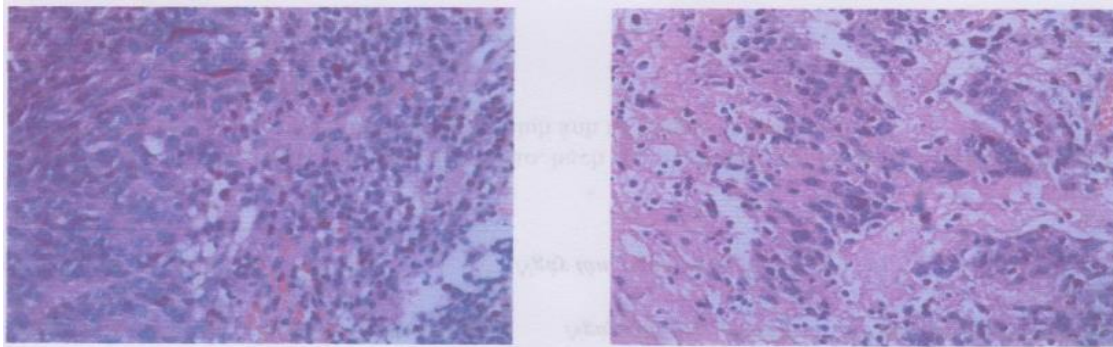


Fig. 1: Histological examination of undifferentiated nasopharyngeal carcinoma

For non-cancerous control, 60 nasopharyngeal swab samples, which were negative for nasopharyngeal carcinoma, were collected from non-NPC patients. All the samples were placed in 1.5-ml tubes containing PBS buffer and stored at -20°C for further experiments.

Total RNA isolation, Reverse transcriptase PCR assay and detection of miR-155

The isolation of RNA, including miRNAs were isolated from NPC biopsy samples and non-cancerous samples by applying mirVana™ miRNA Isolation Kit (Ambion, Life Technology) according to the manufacturer's instructions. cDNA was reverse transcribed from approximately 5 ng of Total RNA by using TaqMan® Advanced miRNA cDNA Synthesis Kit. The detection of miR-155 was determined by qualitative Real-time PCR (qRT-PCR) assay with Taqman™ Advanced miRNA assays kit (ThermoFisher Scientific). About 5 μl of RT reaction product was used to each reaction for detection of miR-155. The UniSp6 rRNA was used as the internal control candidate to normalize the Ct values because of the non-differential expression level in tumor and health adjacent samples.

Statistical analysis

Data were analyzed using Medcalc® Version 12.7.0.0. All *P*-values were two-side, and values less than 0.05 were considered significant. All values were reported as mean \pm SD. The relative expression of miR-155 as determined using q-PCR was analyzed using the $2^{-\Delta\Delta\text{Ct}}$ method. Finding was greater and less than 1 was determined to classify up-regulation and down-regulation, respectively. Chi-test was used to determine the association between the expression of miR-155 and NPC status. Moreover, the association between expression of miR-155 and risk of NPC was estimated by computing OR, RR and 95% confidence intervals (CI).

Results

cDNA UniSp6 rRNA (abbreviated as U6) were detected in both the case and control group by Real-time PCR, resulting the Ct value of cDNA U6 were 29.05 ± 0.38 and 29.53 ± 0.31 in the case and control group, respectively. The *p* value ($P=0.3253$) indicated the expression of U6 was no difference between those two groups (Fig. 2A).

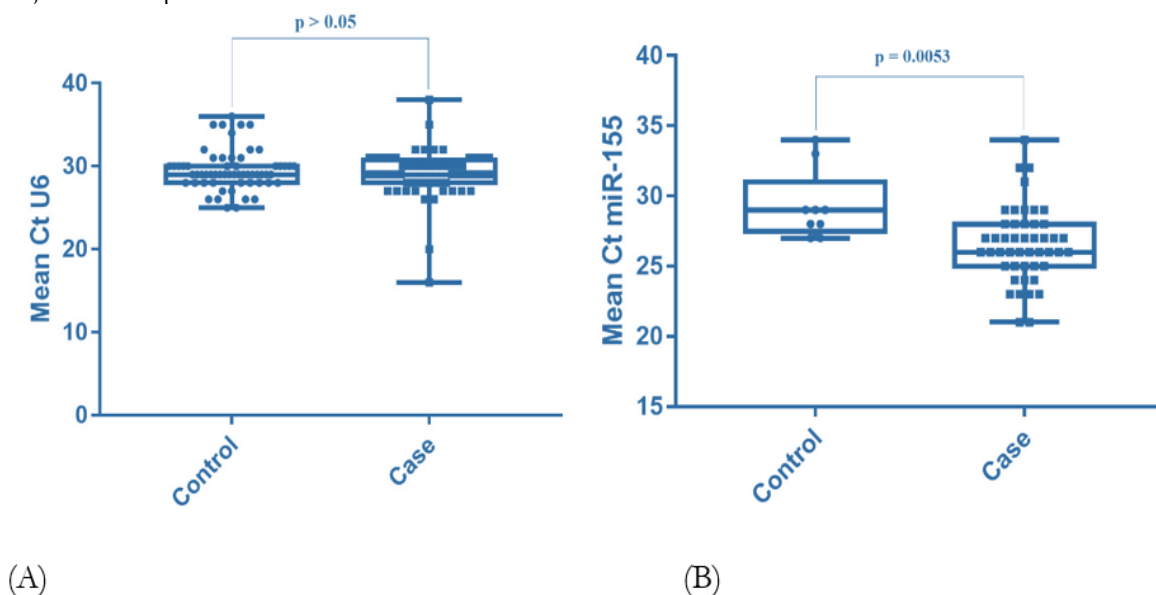


Fig.2: The mean of Ct value (mean \pm SE) of (A) UniSp6 rRNA; (B) miR-155 in the case group and control group. Each black dot was indicated the Ct value of each sample

Therefore, U6 was used as a reference gene (internal control) to normalize miR-155 expression in the comparison between the NPC group (case group) and non-cancerous group (control group). In current study, the expression of miR-155 in both NPC biopsy samples and non-cancerous samples were detected by qRT-PCR. The proportion of positive and negative case in NPC samples were 78.33% (47 of 60 cases) and 21.67% (13 of 60 case), respectively. In the case of non-cancerous group, the positive and negative rate were 15.0% (9 of 60 cases) and 85.0% (51 of 60 cases), respectively. A *P*-value ($P=0.0053$) showed the significantly higher correlation between the miR-155 and NPC (Fig. 2B).

Based on the proportion of miR-155 detection, the odds ratio value was computed between the expression of miR-155 and NPC. Odds ratio was 20.49 (95%CI=8.02–52.33, $P<0.0001$). Additionally, the mean of Ct values of miR-155 in the case group and control group were 26.55 ± 0.39 and 28.33 ± 0.83 , respectively. The relative quantification of miR-155 expression between the case group and control group was analyzed by the $2^{-\Delta\Delta C_t}$ method, as the result, the expression of miR-155 levels was 4.92 times higher in tumor samples in comparison with the non-cancerous samples ($P=0.022$) (Fig. 3).

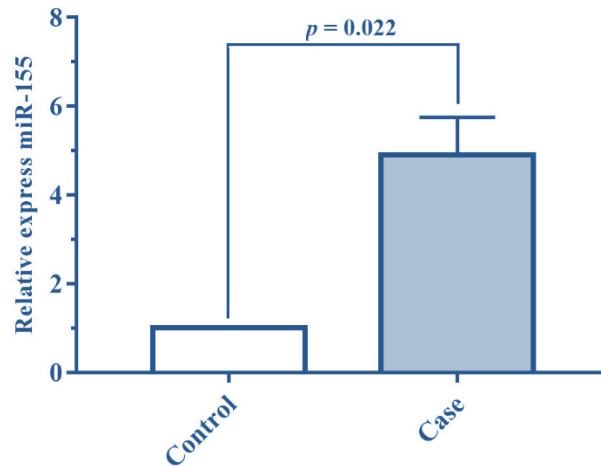


Fig. 3: qRT-PCR showed the level expression of miR-155 was up-regulated in NPC biopsy samples compared with the non-cancerous samples

Discussion

In current initial case-control study, we aimed to find out the association between the miR-155 expression and NPC. Up to date, there are many researches of abnormal regulation of miR-155 associated the pathogenesis of various human tumors, including NPC (5, 6, 12-15).

In our study, the positive rate of miR-155 expression in NPC biopsy sample was 78.33% (meant Sensitivity = 78.33%), indicated that 78.33% of the NPC biopsy samples will be positive for miR-155 expression. The strong correlation between

NPC tumorigenesis and miR-155 positive was detected ($P<0.05$). It indicated the upregulation of miR-155 in NPC. Additionally, the overexpression of miRNA levels was once confirmed by 4.92 times higher in tumor samples in comparison with the non-cancerous sample. Compared to previous studies, the over-expression of miR-155 was also observed in NPC cell line and NPC tissue samples. The up-regulated miR-155 was detected in EBV-positive NPC tissue samples and was correlated with plasma LMP1 DNA copies (15). Another study also indicated the potential of miR-155 as the therapeutic targets for NPC in further studies

based on the observation of upregulation of miR-155 in NPC samples (7). The main difference between this study and previous publications is that we focused on the Vietnamese population, the country with the highest mortality.

The upregulation of miR-155 was strongly correlated with NPC risk with the significant statistic through the calculating the OR ($P < 0.0001$). The odds for a positive expression of miR-155 in NPC was 20.49 times higher than in the case of cancer without expression of miR-155 (OR=20.49, 95%CI=8.02–52.33, $P < 0.0001$). Therefore, in the light of this, even though the limit amount of samples enrolled in current study, we investigated in this study the differential expression of potential miR-155 in NPC biopsy samples and healthy nasopharyngeal swabs.

Finally, we concluded that the same concept of miR-155 role with previous studies is that: miR-55 is up-regulated, and acts as an oncogenic role in NPC development, not excepting Vietnamese population, and could be proposed as potential markers for NPC in Vietnamese NPC patients. For further studies, identification of association between miR-155 expression and EBV-positive NPC case, including EBNA5, LMPs, as well as the non-invasive specimens, such as throat swabs, nasopharyngeal swabs, as the biomarkers for the screening and early diagnosis of NPC in Vietnamese population, will be continuously studied. Additionally, the potential of miR-155 will be further studied as the target therapeutic treatment for NPC.

Conclusion

The oncogenic role of miR-155 was observed in NPC biopsy samples, which collected from Vietnamese patients. This observation was concluded based on the calculation of the miR-155 expression in NPC samples compared to non-cancerous samples. The percentage of miR-155 detection in cancerous and non-cancerous samples were 78.33%, and 15.0%, ($P < 0.05$), respectively. miR-155 was up regulated 4.92 times in NPC samples

compared with the non-cancerous samples in the Vietnamese population.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declared that they have no competing interests.

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