APPLIED BIOINFORMATICS TOOLS FOR ANALYSIS OF MICRORNA-214 EXPRESSION AND PREDICTION OF ITS POTENTIAL TARGETS GENES IN NASOPHARYNGEAL CARCINOMA

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ABSTRACT

miRNA (microRNA) are short RNA molecules in length from 20 to 24 nucleotides that have been shown to play an important role in regulating gene expression in many different types of human cancer. Meanwhile, miRNA-214 is one of the known miRNAs involved in the formation of nasopharyngeal carcinoma (NPC) through overexpression that promotes proliferation and development of cancer cells. However, in Vietnam, the study of miR-214 related to NPC has not been conducted yet. With the aims to develop the further studies of miR-214 on NPC in Vietnamese patients, in this initial study, we conducted the analysis of miR-214 expression in previous publications, as well as the prediction of miR-214 potential target genes, which involved in many cellular pathways. Here we applied bioinformatics tools to predict miRNAs and their targets, and discuss the role of miR-214 in the context of human cancers. As the results, miR-214 acted as the oncogenic roles in NPC, relevanted to many pathways, such as cell proliferation, apoptosis, metastasis and invasion through the its target genes *LTF*, *Bim, Bax*, *LINC0086*, etc. In conclusion, the use of computional approaches facilitate the further experimental validation of miRNAs in general, particularly miR-214, in Vietnamese NPC patients.

Keywords: MicroRNA; MiRNA-214; Nasopharyngeal carcinoma.

1. Introduction

Nasopharyngeal carcinoma (NPC), a malignant tumor arising from the nasopharyngeal epithelium, has pronounced differences in distribution according to geography and ancestry, is the fifth most common cancer worldwide (Khoo & Pua, 2013). Growing evidences indicated that miRNAs have been implicated as both oncogenes and tumor suppressors that the abnormal expression (positive up-regulation or down-regulation) of miRNAs contributes to various human tumor pathogenesis through interacting with its target genes. Many previous studies reported that the abnormal expression of miRNAs could be served as the potential biomarkers for the diagnosis and therapy of human cancer (Lan et al., 2015;

Yuan et al., 2018). Among them, miR-214 has been reported to be aberrant expressed in many human cancers, including NPC. However, numerous studies have shown the conflicting results regarding to whether the role of miRNA is an oncogenic miRNA or tumor suppressor miRNA in NPC. On the other hand, the prediction of its target gene, for which the target gene acts as an oncogene or a tumor suppressor gene, combined to miR-214, give a hand for development of the novel molecular target diagnosis and treatments.

In Vietnam, the study of miRNAs, in particularly miR-214 on NPC has not been studied yet. In order to build a scientific basis for experimental early detection and prognosis of NPC based on the identification of miRNAs expression, specifically miR-214, we conducted: the systematic revision of miR-214, based on the previous publications, in NPC, to determine the miR-214 roles in NPC. Moreover, the prediction of its potential target genes, which relevant to NPC tumorigenesis pathways, was performed by the application of bioinformatics tools, such as TargetScan 7.2, DAVID 2008 Functional Annotation Bioinformatics Microarray Analysis Tools, etc. This initial study will play a role in building the foundation for the development of the following research direction related to miRNAs in general and effect of miR-214 to the formation and development of NPC in the Vietnamese population.

2. Material and method

2.1. Data collection

Several keywords, such as "microRNA-214", "nasopharyngeal carcinoma", etc., were applied to retrieve the information of miR-214 by collected the previous publications on many databases, included PubMed, Embase, Web of Science databases, Embase Database, etc. The studies fulfilling the following criteria were included in the systematic review. Inclusion criteria: [1] Case-control study or cohort study that published the miRNA-214 expression profile, and clinicopathological feature. [2] Studies that investigated expression of miRNAs in NPC. [3] Study that mention detection, analysis method of miRNA-214 expression.

2.2. MicroRNA targets prediction bioinformatic

The miRBase database (http://www.mirbase.org/) was used for finding out the basic information of miR-214. Possible target genes and signal pathways were validated by utilizing Pictar (https://pictar.mdc-berlin.de/), TargetScan 7.2 (http://www.targetscan.org/vert_72/), and miRDB (http://mirdb.org/), which identifies binding sites targeted by single miRNA.DAVID 2008 Functional Annotation Bioinformatics Microarray Analysis Tools (http://niaid.abcc.ncifcrf.gov/) was used to classify the functions of the target genes, which were predicted from Pictar, TargetScan 7.2 and miRDB online softwares.

3. Results

3.1. Location, sequence of miRNA-214

miR-214 is located at 1q.24.3 (nt: 172138798 - 172138907, [-]), is the same location with the miRNA-3120. However, the length of miRNA-3120 is shorter than miR-214, therefore, miR-3120 is located inside the gene of miRNA-214. According to miRNA Database, the precusor-miRNA-214, which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the two sequences, terms hsa-miR-214-5p (hsa-miR-214*) and hsa-miR-214-3p (hsa-miR-214) (Fig. 1).

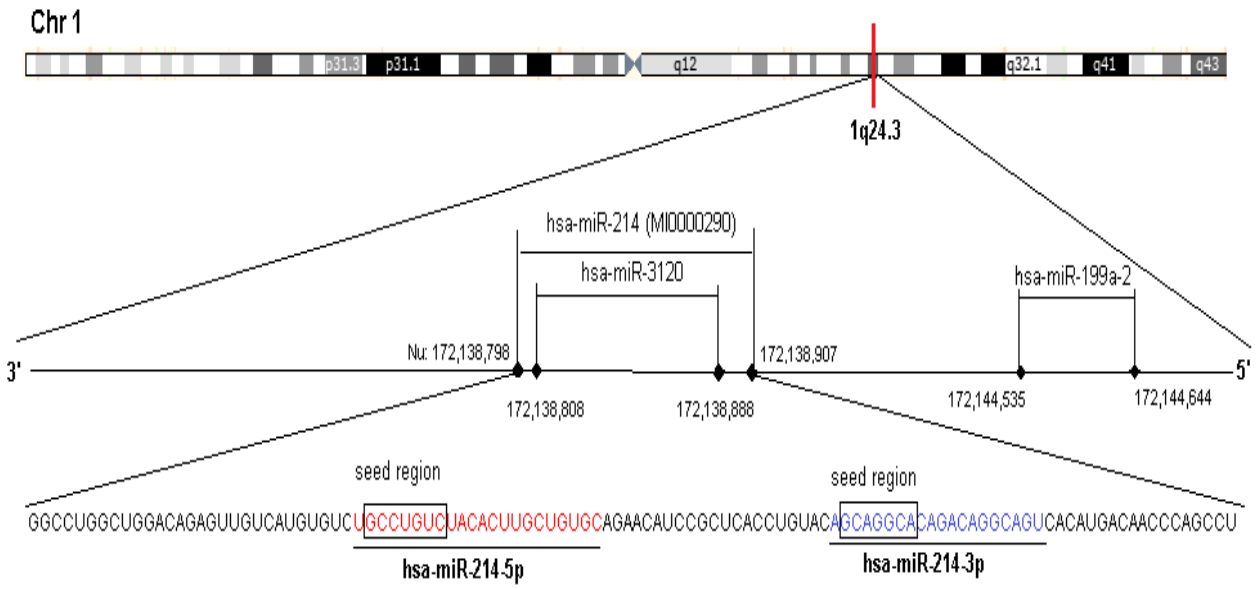

Figure 1. Location of miR-214. Mature-hsa-miRNA-214-5p is red sequence, mature-hsa-miRNA-214-3p is blue sequence.

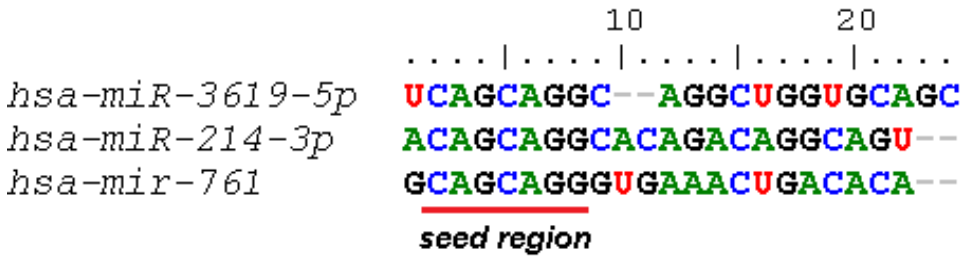

Figure 2. Sequences of the three member of miRNA-214/761/3619-5p family (miR-3619-5p, miR-214-3p, miR-761).

MiRNA-214 is belonged to the miRNA-214/761/3619-5p family. The miRNA-214/761/3619-5p family includes three members (miRNA-214-3p, miRNA-761 and miRNA-3619-5p), which classified based on the sequence homology in their seed regions "CAGCAGG" (Fig. 2). The seed region of mature miRNAs bound to 3'UTRs on target mRNA with complete or incomplete matching. The binding between miRNA and its target mRNA through the seed region leads to the degradation of mRNA or the blocking of mRNA translation (Filipowicz et al., 2008).

When comparing the human pre-miRNA-214 with pre-miRNA-214 of 22 different species showed highly conserved in the sequences. As the result, both mature-miRNA-214-5p (miRNA-214*) and mature-miRNA-214-3p (miRNA-214) are conserved in many species, have conserved sequence: GCCUGUCU/UCA CUUGCUGUGC and ACAGCAGGCACAGA CAGGCAG, respectively (Fig. 3).

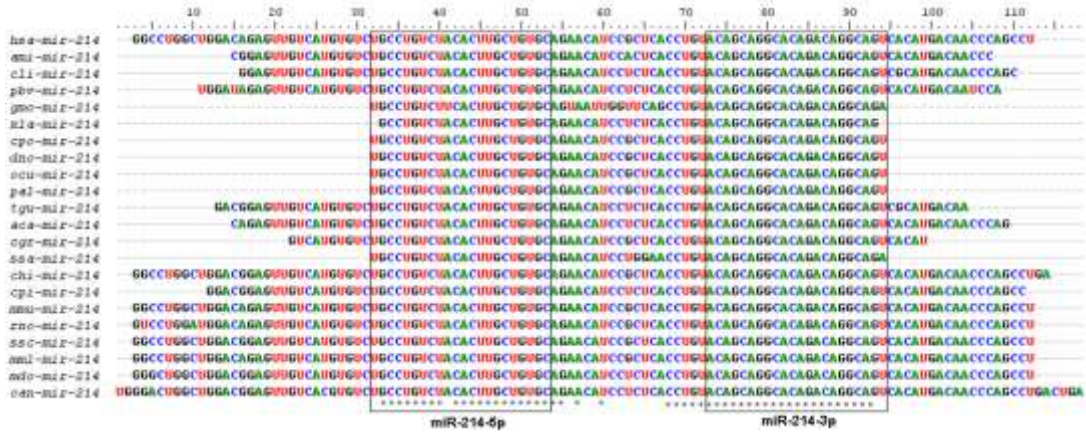

Figure 3. The highly conserved nucleotides of miR-214 are is observed in many species. MiR-214 precusor sequences of 22 species were downloaded and aligned by BioEdit software. The star (*) represents nucleotieds are similar in all species (hsa: *Homo sapiens*; ami: *Alligator mississippiensis*; cli: *Columba livia*; pbv: *Python bivittatus*; gmo: *Gadus morhua*; xla: *Xenopus laevis*; cpo: *Cavia porcellus*; dno: *Dasypus novemcinctus*; ocu: *Oryctolagus cuniculus*; pal: *Pteropus alecto*; tgu: *Taeniopygia guttata*; aca: *Anolis carolinensis*; cgr: *Cricetulus griseus*; ssa: *Salmo salar*; chi: *Capra hircus*; cpi: *Chrysemys picta*; mmu: *Mus musculus*; rno: *Rattus norvegicus*; ssc: *Sus scrofa*; mml: *Macaca mulatta*; mdo: *Monodelphis domestica*; oan: *Ornithorhynchus anatinus*).

3.2. The expression of miRNA-214 in NPC

Up to April, 2019, the total of seven publications, included Case – Control study and Cohort study, related to the expression of miR-214 in NPC was shown in Table 1. The results of those studies showed that miR-214 affected different target genes involved in several signal pathways: signaling pathways in cell, such as cell proliferation, apoptosis, etc.

Table 1

A concise overview of previous studies related to miR-214 in NPC

Study	Year	Country	Assay	Type of study/ Sample	Expression / Target of miR-214
Deng et al.	2013	China4	qRT-PCR	**Case - control** Case: 27 NPC biopsy. Control: 10 normal nasopharyngeal epithelial tissues.	Up-expression in NPC patient → Oncogenic miR-214. miR-214 regulated signal pathway LTF/AKT.
Plieskatt et al.	2014	United Stage	qRT-PCR	**Case – control** Case: 4 FFPE tissue and 54 serum from NPC patients Control: 12 non-cancerous FFPE tissue and 26 non-cancerous serum	Down-expression in NPC patient → Tumor suppressor miR-214.

Study	Year	Country	Assay	Type of study/ Sample	Expression / Target of miR-214
Zhang et al.	2014	China	qRT-PCR and FISH	**Case - control** Case: 5 NPC biopsy. Control: 5 adjacent tumor from NPC patients.	Up-expression in NPC patient → Oncogenic miR-214. miR-214 regulated signal pathway Bim/ cell enhancement and apoptosis.
Lu et al.	2014	China	qRT-PCR	**Case - control** 20 blood of NPC patients. 10 healthy control subjects. **Cohort study** 40 blood of NPC patients. 15 healthy control subjects.	Up-expression in NPC patient → Oncogenic miR-214.
He et al.	2015	China	qRT-PCR	**Case - control** 16 NPC biopsy. 16 adjacent tumor from NPC patients.	Up-expression in NPC patient → Oncogenic miR-214. miR-214 regulated signal pathway Bax/ cell enhancement and apoptosis.
Li et al.	2016	China	qRT-PCR	**Case – control** 8 NPC tissue samples 2 non-cancerous tissues	Down-expression in NPC patient → Tumor suppressor miR-214.
Guo et al.	2017	China	qRT-PCR	**Case - control** 20 NPC biopsy. 20 non-cancerous serum. **Cohort study** 112 NPC biopsy. 56 adjacent tumor from NPC patients.	Up-expression in NPC patient → Oncogenic miR-214. miR-214 regulated signal pathway LINC0086/ cell enhancement and apoptosis.

qRT-PCR: Quantitative Reverse transcription - PCR; FISH: fluorescence in situ hybridization.

In general, the expression of miRNA-214 is unclear. According to Zhang et al. (2014), Lu et al. (2014), Guo et al. (2017), He et al., (2015), miRNA-214 was up-expressed in cancerous samples. In contrast, according to Plieskatt *et al.* (2014), Li *et al*. (2016), the expression of miRNA-214 was decreased in cancerous samples. According to research by Deng et al. (2013), miRNA-214 increasing expression in metastatic tissue and cell lines, but decreased expression in nasopharyngeal tumor samples. Therefore, the expression of miRNA-214 in NPC is still controversial and unclear. Therefore, it is necessary to conduct

the research relevant to the expression of miR-214 in Vietnamese NPC patients based on the information of current systematic revision.

3.3. Bioinformatics analysis of potential targets of miR-214

TargetScan 7.2, Pictar and miRDB were used to predict the targets that were possibly regulated by miR-214. Using TargetScan 7.2, miR-214 was predicted to regulate 3361 targets, Pictar software has predicted that 525 transcripts were regulated by miR-214, whereas miRDB predicts that 790 genes are targets of miR-214. As the results, 48 targets gene were predicted by both TargetScan 7.2, Pictar and miRDB. To evaluate the possible regulation mechanisms of miR-214, as well as their relationships in the process of cell, we next performed a functional analysis on 48 targets of the miR-214 based network using DAVID 2008 Functional Annotation Bioinformatics Microarray Analysis Tools. The results were shown in Fig. 4 and Table 2.

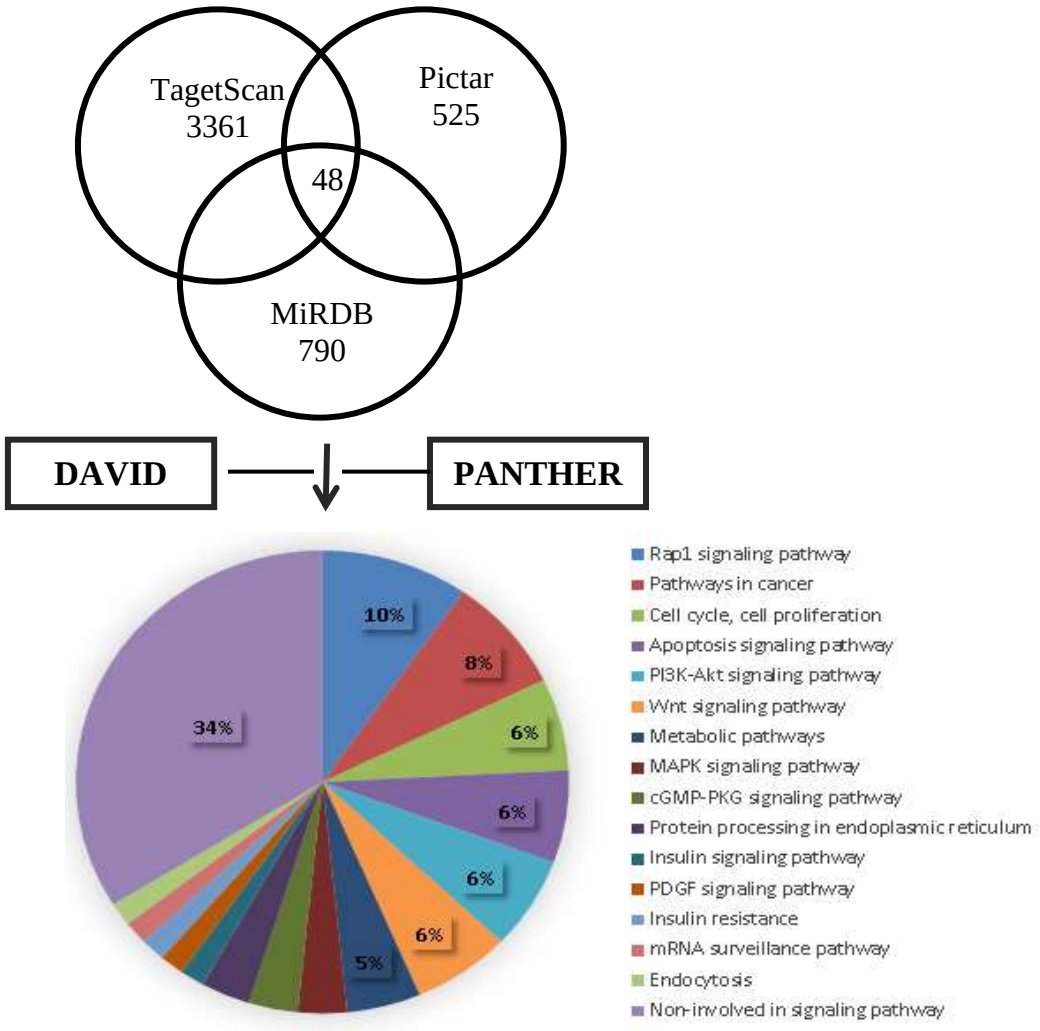

Figure 4. Functions of predicted miR-214 target genes

Table 2

Targets of miRNA-214 and their signal pathway

Pathways	**Target Genes**
Rap1 signaling pathway	*ADORA2A, GRIN1, RASSF5, CSF1, PLCB4, PGF*
Pathways in cancer	*CRKL, RASSF5, PLCB4, PGF, Bax*
Cell cycle, cell proliferation	*CLASP1, DUSP15, PGF, YWHAZ*
Apoptosis signaling pathway	*BCL2L11, Bax, LTF, LINC0086*
PI3K-Akt signaling pathway	*CSF1, PGF, YWHAZ, BCL2L11*
Wnt signaling pathway	*SMARCC1, PLCB4, PCDH20, PSEN1*
Metabolic pathways	*HSD17B8, CDIPT, PLCB4*
MAPK signaling pathway	*CRKL, PPM1A*
cGMP-PKG signaling pathway	*PDE5A, PLCB4*
Protein processing in endoplasmic reticulum	*SEC24C, Bax*
Insulin signaling pathway	*TRIP10*
PDGF signaling pathway	*SRGAP3*
Insulin resistance	*PPARGC1B*
mRNA surveillance pathway	*CPSF4*
Endocytosis	*SNX12*

The results showed that the genes were largely involved in many signaling pathways, which are considered to be important roles in tumorigenesis, such as: cell cycle, tight junction, Wnt signaling pathway, MAPK signaling pathway, Jak-STAT signaling pathway, etc. Some genes are noted for participating in many important signaling pathways, such as: *YWHAZ*, *BCL2L11*, *Bax*, *PGF*, *PLCB4*, and *PPP2CB*. For example, the *YWHAZ* gene involved in many signaling pathways, such as: cell cycle, cell proliferation, PI3K-Akt signaling pathway, PI3K-Akt signaling pathway; especially, *YWHAZ* effect on infection of EBV (one of main causes in NPC).

Among of predicted target genes, some genes were demonstrated experimentally as direct target genes of miRNA-214, such as *LTF*, *Bim*, *Bax*, and *LINC0086*. Information about function and expression characteristics of these genes are shown in Table 3.

Table 3

Direct targets of miR-214 reviewed on previous studies

Study	Gene	Function of gene	Expression feature
Deng *et al*., 2013	*LTF*	Regulator of proliferation, invasion and metastasis	Down expression
Zhang *et al*., 2014	*Bim*	Regulator of apoptosis, proliferation and prognosis	Down expression
He *et al*., 2015	*Bax*	Regulator of cell proliferation and apoptosis	Down expression
Guo *et al*., 2017	*LINC0086*	Regulator cell proliferation and apoptosis	Down expression

4. Discussion

In this study, we found that miRNA-214 has an important role in NPC. By regulating target genes, miRNA-214 can participate in many different signaling pathways: Rap1 signaling pathway, pathways in cancer, cell cycle, cell proliferation, apoptosis signaling pathway, PI3K-Akt signaling pathway, Wnt signaling pathway, metabolic pathways, MAPK signaling pathway,... To test the hypothesis, an increase and decrease in expression of miR-214 can be experimentally performed for all mRNAs predicted.

In previous studies, many target genes have been demonstrated in relation to the expression of miR-214. For example, in the study of Deng et al (2013), the research team confirmed that the PI3K/ AKT signal pathway was related to *LTF*. *LTF* can inhibit AKT signals through two different mechanisms. Firstly, *LTF* reduced the expression level of PDK1 by inhibiting the MAPK signaling pathway and reducing c-Jun regulation, leading to inhibition of AKT activity. Secondly, *LTF*, through interaction with K18, inhibited the formation of complex K18-14-3-3. This caused separation of 14-3-3 in the nucleus and reduced AKT activity. Therefore, they confirmed that *LTF* expression levels were significantly reduced in NPC samples and negatively correlated with the levels of phosphorylation of AKT, which confirmed that *LTF* acts as a negative regulator of AKT in NPC development. In summary, *LTF* has an important role in preventing formation of NPC through inhibiting the AKT pathway. In addition, the expression level of *LTF* correlates with tumor metastasis and clinical stages, so *LTF* can be used as a biomarker of NPC in prognosis and early diagnosis. Another study, such as the study of Guo et al. (2017), *LINC0086* significantly reduced expression in biopsy and serum from NPC patients, and the expression of *LINC0086* was related to histopathological level, metastatic lymph nodes and clinical stage of the NPC. The overexpression of *LINC0086* has the effect of inhibiting cell proliferation both in the NPC cell lines and xenograft models. At the same time, Bim and Bax were direct target genes of miR-214, which have been demonstrated experimentally. *Bim* and *Bax* (belonging to the *Bcl-2* family) were all related to proliferation and apoptosis in NPC. Therefore, miR-214 acted as an oncogene in NPC, affecting major processes, such as: cell proliferation, apoptosis, metastasis and invasion through its target genes *LTF*, *Bim*, *Bax*, and *LINC0086*.

The expression of miRNA-214 in NPC patients still has not been clarified. Most studies confirmed that miRNA-214 increased

the expression in NPC patients. But there are a few studies that confirmed miRNA-214 reduced expression. It is worth noting that studies of miRNA-214 expression are still quite limited, mainly concentrated in Asia countries, by Chinese researchers. Therefore, there has not been a general observation about the expression properties of miRNA-214 in NPC patients. It was found that, in Vietnam, there have not been any studies related to the expression of miRNA-214, so the results of this study will be the scientific basis for experimental studies on NPC patients in Vietnam in the further research.

5. Conclusion

In summary, in current study, the systematic review and bioinformatics-based analysis showed that miRNA-214 acted as oncogenic roles in human NPC. Its up-expression was involved in many different signaling pathways, such as Wnt, JAK-STAT, tight junction, cell cycle,.... that has been reported as key pathway in tumorigenesis by interacted with many target genes, includes *YWHAZ*, *BCL2L11*, *Bax*, *PGF*, *PLCB4*, *PPP2CB*. These genes significantly contributed to the formation of NPC and their function has also been determined. In conclusion, this initial study will be the fundamental scientific platform for us to conduct further experiments to prove the role of miRNA-214 and its targets in Vietnamese NPC patients■

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