Faculty of Medicine, Public Health, and Nursing
Universitas Gadjah Mada

INTERNATIONAL SYMPOSIUM ON CANCER BIOLOGY

"Opportunities for Research"

ABSTRACT BOOK

Tribute to 70th anniversary of
Prof. Sofia Mubarika Haryana, MD

Auditorium
Faculty of Medicine, Public Health, and Nursing
Universitas Gadjah Mada,
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24 – 25 August 2018

16.40-16.50	**Profiling of microRNA expression related to Warburg effect in luminal breast cancer and fibroadenoma mammae (FAM) using nanostring** Fathiya Rahma Arfira (Universitas Gadjah Mada, Yogyakarta, Indonesia)	OP-05

Day 2, 25 August 2018

TIME	ABSTRACT TITLE	ABSTRACT NUMBER
Topic: Development of biomarker in cancer		
09.15-09.25	**The Expression of miRNA-21 and miRNA-18a in Nasopharyngeal Cancer: a possibility as non-invasive biomarker** Hana Ratnawati (Universitas Kristen Maranatha, Bandung, Indonesia)	OP-06
09.25-09.35	**Clinicopathological characteristics and prognostic significances of Indonesian triple negative breast cancer** Irianiwati Widodo (Universitas Gadjah Mada, Yogyakarta, Indonesia)	OP-07
09.35-09.45	**The Role of MSH3 rs26279 Polymorphisms On Progressive Response Of First Line Platinum Based Chemotherapy In Non-Small Cell Lung Cancer Patients In Malang** Nur Annisa (Universitas Brawijaya, Malang, Indonesia)	OP-08
Topic: Cancer immunology and immunotherapy		
11.45-11.55	**PD-L1 Expression in Papillary Renal Cell Carcinoma** Arni Kusuma Dewi (Universitas Airlangga, Surabaya, Indonesia)	OP-09

OP-06

The Expression of miRNA-21 and miRNA-18a in Nasopharyngeal Cancer: a possibility as non-invasive biomarker

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Nasopharyngeal cancer, the most common head and neck cancer in Indonesia, is difficult to detect in the early stages due to unclear symptoms and hidden locations. This causes more than 70% of patients come at an advanced stage and with metastasis. Studies show that miRNA regulates more than 60% of protein coding genes, thus regulating almost all biological processes within the cell including the pathogenesis of cancer. It turns out that miRNA not only plays a role in the cell, but also can be found in blood circulation and other body fluids, thus it is a potential biomarker for early detection of various types of cancer. MiR-21 is one of the oncogene miRNA that regulates genes that play a role in cell proliferation, apoptosis and angiogenesis. MiRNA-18a is a miRNA associated with Epstein-Barr virus, which plays an important role in the pathogenesis of nasopharyngeal cancer. The purpose of this study was to determine the expression of miRNA-21 and mi-RNA-18a in nasopharyngeal cancer cell lines and analysis the possibility to become a non-invasive potential biomarker that could predict the presence of nasopharyngeal cancer at an early stage. This is an experimental laboratory study using EBV-positive nasopharyngeal cancer cell line, C666 and normal nasopharyngeal cell line, NP69. Total RNA was isolated from cell culture and RNA was transcribed back to cDNA then continued with real-time qPCR. The cycle threshold value is calculated to quantify the relative amounts of miR-21 and miR-18a.

Keywords: *nasopharyngeal cancer, miR-21, miR-18a, biomarker*

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The Expression of miRNA-21 and miRNA-18a in Nasopharyngeal Cancer: a possibility as non-invasive biomarker

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Abstract

Nasopharyngeal cancer, the most common head and neck cancer in Indonesia, is difficult to detect in the early stages due to unclear symptoms and hidden locations. This causes more than 70% of patients come at an advanced stage and with metastasis. Studies show that miRNA regulates more than 60% of protein coding genes, thus regulating almost all biological processes within the cell including the pathogenesis of cancer. It turns out that miRNA not only plays a role in the cell, but also can be found in blood circulation and other body fluids, thus it is a potential biomarker for early detection of various types of cancer. MiR-21 is one of the oncogene miRNA that regulates genes that play a role in cell proliferation, apoptosis and angiogenesis. MiRNA-18a is a miRNA associated with Epstein-Barr virus, which plays an important role in the pathogenesis of nasopharyngeal cancer. The purpose of this study was to determine the expression of miRNA-21 and mi-RNA-18a in nasopharyngeal cancer cell lines and analysis the possibility to become a non-invasive potential biomarker that could predict the presence of nasopharyngeal cancer at an early stage. This is an experimental laboratory study using EBV-positive nasopharyngeal cancer cell line, C666 and normal nasopharyngeal cell line, NP69. Total RNA was isolated from cell culture and RNA was transcribed back to cDNA then continued with real-time qPCR. The cycle threshold value is calculated to quantify the relative amounts of miR-21 and miR-18a. The result showed that there is a significant difference of miRNA-18a (p=0,000) and miRNA-21 (p=0,002) expression on nasopharyngeal cell (C666) and normal nasopharynx cell (NP69). It can be concluded that, miRNA-18a and miRNA-21 could play a role as non-invasive biomarker in nasopharyngeal cancer.

Key words: nasopharyngeal cancer, miR-21, miR-18a, biomarker

INTRODUCTION

Based on data from the International Agency for Research on Cancer (IARC) 2012, it is known that there are 14 million new cases of cancer and 8.2 million deaths from cancer worldwide. It is estimated that more than 70% of cancer deaths occur in poor and developing countries (WHO and World Bank, 2005). Based on Riskesdas in 2013, the prevalence of cancer in Indonesia is 1.4 per 1000 population, and is the caused of death ranking 7 (Riskesdas, 2013).

In Indonesia, nasopharyngeal cancer is the most common head and neck cancer with an incidence of 6.2 / 100,000 and has a high mortality rate. Nasopharyngeal cancer suffered by many people in the productive age, therefore the incidence of cancer will affect the economic condition of the patient and also affect the health financing fund. The high prevalence of cancer in Indonesia, make cancer as one of the priorities of national health problems. Prevention, early detection, and appropriate treatment, is very important to overcome the problems due to cancer (Riskesdas, 2013). The high mortality rate in nasopharyngeal cancer are caused by late diagnose, as patients often come in advanced stage. This is due to unclear early symptoms of nasopharyngeal cancer and its location hidden behind the nose. That's why it is necessary to examine a biomarker which can detect a nasopharyngeal cancer in early stage and a new cancer therapy with less adverse side effects.

MicroRNA (miRNA) is a ribonucleic acid that does not encode proteins with a transcript of 18-25 nucleotides which interact with the target gene encoding mRNA. Research shows that miRNA regulates more than 60% of protein coding genes, so it regulates almost all biological processes within the cell. That is why deregulation of miRNA plays an important role in the pathogenesis of various diseases such as cancer.(Ma, 2012, Shen, 2013, Di Leva, 2014). MiRNAs control the development of cancer both against suppressor tumor genes, apoptosis, angiogenesis, and metastasis (Gottwein, 2008; Ventura, 2009).

It turns out that MiRNA not only plays a role in the cell, but also in blood circulation and other body fluids (Cortez et al, 2011). Several studies have shown that serum and plasma miRNAs give great hope as new non-invasive biomarkers for early detection of various cancers. Several studies of miRNA in

serum of colorectal and lung cancer patients showed that the concentrations of miRNA in the circulation may change according to the stage of cancer (Paranjape, 2009; Ferracin et al, 2015). This suggests that miRNA can also act as a prognosis biomarker of in cancer.

MiR-21 is one of the oncogene miRNA that regulates genes that play a role in the pathogenesis of cancer, such as cell proliferation, apoptosis and angiogenesis (Markou, 2016). MiRNA-18a is a miRNA associated with Epstein-Barr virus, which is the primary risk factor in the pathogenesis of nasopharyngeal cancer. The purpose of this study was to determine the expression of miRNA-21 and mi-RNA-18a in nasopharyngeal cancer cell lines and analysis the possibility to become a non-invasive potential biomarker that could predict the presence of nasopharyngeal cancer at an early stage.

METHODS

This is an experimental laboratory study using EBV-positive nasopharyngeal cancer cell line, C666 and normal nasopharyngeal cell line, NP69. Total RNA was isolated from cell culture and RNA was transcribed back to cDNA then continued with real-time qPCR. The cycle threshold value is calculated to quantify the relative amounts of miR-21 and miR-18a.

The C666 cell line is EBV-positive nasopharyngeal carcinoma cell lines, were cultured in H-DMEM medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with penicillin G (100U/ml), streptomycin (100 mg/ml) (Invitrogen, Carlsbad, CA, USA) and Glutamax I (1x) (Gibco-35050-061) and 10 % Fetal Calf Serum (FCS). Growth on BD falcon non-coated plate. The NP69 is a normal nasopharyngeal cell line, were cultured in Keratinocyte SFM (1 X) liquid 500 ml/pk (Gibco #17005-042) consists of 10724-011 SFM Medium and 37000-015 Supplement), supplemented with penicillin G (100U/ml), streptomycin (100mg/ml).

All the cells cultures were maintained at 37°C in a humidified atmosphere of 95% air, 5% CO2. The viability of the cells was assessed by trypan blue exclusion assay and the cell pellets were stored at –80°C until they could be processed. In those cell lines will detect the expression of miRNA-18a and miRNA-21 and U6 as endogenous control.

miRNA-18 : 5’-UAAGGUGCAUCUAGUGCAGAUAG-3’

miRNA-21 : 5’-UAGCUUAUCAGACUGAUGUUGA-3’

U6 : 5’-CGCTTCACGAATTTGCGTGTCAT-3’

Total RNA from cultured cells was isolated using TRIZOL reagen (Invitrogen, NY, USA) following the manufacturer’s protocol. The differentially expressed amount of the miRNA-18 and miRNA-21 were validated in triplicate by two-steps quantitative reverse-transcription polymerase chain reaction (qRT-PCR), using SYBR PrimeScript miRNA RT-PCR kit. Then the data analyze with independent T Test.

RESULT AND DISCUSSION

After the cell confluenced, continued by isolated the total RNA and detect the expression of the miRNA. The expression of the miR-18a and miR-21 showed in the diagram below:

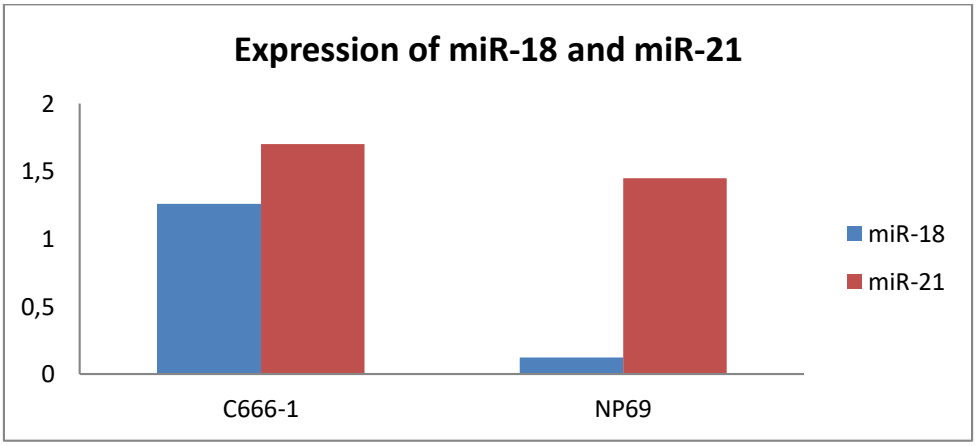

Fig. 1. Diagram Expression of miR-18a and miR-21

The highest miRNA-18 expression was in C666 cell (1,26±0,17) compare to NP69 cell (0,12±0,01). MiRNA-18 correlated to Epstein-Barr virus, that’s why the highest expression was in C666 cell. The highest miRNA-21 expression was also in C666 cell (1,70±0,24) due to miRNA-21 is an oncogene, but the miRNA-21 expression also high in NP69 cell. This can be caused by the immortal morphology of NP69 cell.

To analyze whether there was a significant difference between the expression of miR-18a in C666 and NP69 cells, an independent T-Test was performed, and was preceded by a Levene Test to ensure the normality of the data. The results of the Levene test showed that the data is normal, then it can be continued with the independent T Test.

Table 1. T Test Analysis on miRNA-18a

miR-18a Expression	t-test for Equality of Means						
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Equal variances assumed	69,810	4	0,000	1,140	0,016	1,094	1,185

Table 1 showed that the result of T test of miRNA-18a expression on C666 cell and NP69 cell showed a significant difference with p = 0.000. It means that the high miRNA-18a expression on nasopharyngeal cell (C666) have a significant difference with the miRNA-18a expression on normal nasopharynx cell (NP69). MiRNA-18a is associated with miRNA in Epstein-Barr virus that is closely related to the pathogenesis of nasopharyngeal cancer. MiRNA-18a plays a role in increasing tumor growth and metastasis (Bovell, 2011; Shen, 2013). The result of this study determined that the high expression of miRNA-18a can act as a biomarker in nasopharyngeal cancer.

MiRNA-21 is an oncogene miRNA that play a role in the pathogenesis of cancer. The T Test of the miRNA-21 expression on C666 and NP69 cell showed in table 2.

Table 2. T Test Analysis on miRNA-21

miR-21 Expression	t-test for Equality of Means						
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Equal variances assumed	7,319	4	0,002	0,250	0,034	0,155	0,345

MiRNA-21 expression on C666 cell and NP69 cell showed a significant difference with p = 0.002. It means that the high miRNA-21 expression on nasopharyngeal cell (C666) have a significant difference with the miRNA-21 expression on normal nasopharynx cell (NP69). MiR-21 is one of the most commonly expressed miRNAs in many cancers. Several studies have shown that miR-21 plays a role in the pathogenesis of cancer. In this study, the high expression of miRNA-21 as oncogene miRNA can act as a biomarker in nasopharyngeal cancer (Markou, 2016).

This cancer biomarkers, miRNA-18a and miRNA-21, could be a sensitive and specific biomarker to distinguish cell cancer from healthy cell.

CONCLUSION

The conclusion of this study is, miRNA-18a and miRNA-21 could play a role as biomarker in nasopharyngeal cancer.

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