Sutandyo et al., J Clin Exp Oncol 2017, 6:4
DOI: 10.4172/2324-9110.1000191

Journal of Clinical & Experimental Oncology

Research Article

A SCITECHNOL JOURNAL

Expression of Circulating Mir-206 in Patients with Lung and Head and Neck Cancers and its Association with Cancer Cachexia

Noorwati Sutandyo[1], Ririn Hariani[2], Puspita Eka Wuyung[3], Agus Rahmadi[4], Achmad Mulawarman[5], Cita Herawati[6] and Ramadhan Ramli[7]

Abstract

Background: Cancer cachexia is a common problem found in advanced stage cases. Pathophysiology of cachexia is complicated, involving cytokines and regulator molecules such as microRNA (miRNA). MiR-206, a specific miRNA in skeletal muscle cells was thought to play important role in regulating skeletal muscle loss but have not been studied well in cachectic patients.

Objective: To evaluate the clinical significance of circulating miR-206 in cancer patients presenting with cancer cachexia.

Method: A cross-sectional study was performed in Dharmais Cancer Hospital, Jakarta between September and December 2015. Patients enrolled were lung and head and neck cancers. Cachexia was defined as body mass index less than 20 kg/m^2. MiR-206 expression was assayed using quantitative real-time polymerase chain reaction (RT-PCR), whereas miR-16 served as internal control. The results were expressed as cycle threshold (C_T) and fold change (FC) which was calculated using the $2^{-\Delta\Delta C}{}_T$ method.

Results: Seventy patients were enrolled during the study period; consisting 37 (52.9%) lung 33 (47.1%) head and neck cancers. There were 31 (41.3%) patients presenting with cachexia. Serum miR-206 was overexpressed in cancer patients compare to normal healthy subjects. MicroRNA-206 expression was slightly up-regulated in cachectic patients than non-cachectic patients, i.e. FC=1.355 in lung cancers and FC=1.438 in head and neck cancers.

Conclusion: Circulating miR-206 is overexpressed advanced stage lung cancer as well as head and neck cancer patients. Increased circulating miR-206 in cachectic patients may reflect extensive skeletal muscle loss associated with cancer cachexia.

Keywords

Cancer cachexia; Circulating microRNA; Head and neck cancer; Lung cancer; MiR-206; Myo-miR

***Corresponding author:** Noorwati Sutandyo, Department of Hematology-Medical Oncology, Dharmais Cancer Hospital, Jl. Let. Jend. S. ParmanKav. 84-86, Slipi, Jakarta 11420, Indonesia, Tel: 2356 / 021-568 1570; Fax: 021-5696 9561; E-mail: noorwatis3@yahoo.com

Received: May 24, 2017 **Accepted:** June 05, 2017 **Published:** June 12, 2017

Introduction

Weight loss is a common finding in cancer patients, especially in advanced stage. Severe weight loss may lead to cancer cachexia, i.e. a multifactorial syndrome with ongoing loss of skeletal muscle mass that cannot be recovered by nutritional intervention [1]. Incidence of weight loss varies from 10-35% in breast cancer to 83% in pancreas or gastric cancer [2]. Other than cancers originated from gastrointestinal system, head and neck cancer rank the first, while lung cancer is the second most common cancer complicated by cachexia [2].

Muscle wasting during cachexia is a result of protein degradation after metabolic derangement during tumor progression [3]. Recent studies have found the role of microRNAs (miRNAs) expression during muscle wasting process. MiRNAs are small non-coding ribonucleic acids that play many important roles in regulating biological processes [4]. There are a group of miRNAs that specifically expressed by skeletal muscle cells called myomiRs [5]. They control skeletal muscle development, function and regeneration [6]. MicroRNA-206 (miR-206) is a myomiR which promotes myogenic differentiation since it is highly-expressed in skeletal muscle stem cells committed to differentiate [7,8]. MiR-206 was also identified in muscle-derived tumors (rhabdomyosarcoma) but was under-expressed in leiomyosarcomas and normal smooth muscle cells [9].

Animal experiment showed that overexpression of miR-206, together with miR-21, was needed to induce muscle atrophy. *In silico* analysis found genes that regulate muscle loss and weakness, i.e. the Yin Yang 1 (YY1) and eukaryotic translate on initiation factor 4E (eIF4E3) genes, which are their potential targets [10]. Other study showed that Pax7 transcription factor was a target of miR-206; Pax 7 overexpression disturbs muscle stem cell differentiation causing muscle atrophy [11].

In human cancers, miR-206 was observed to suppress metastasis of breast cancer cells [12]. Human lung cancer cell lines showed down-regulated miR-206 while increased miR-206 expression lead to decreased cancer cell proliferation and invasion ability [13]. However, those experiments used tissue miR-206 and not the circulating one. Increased overexpression of serum miR-206 has been found Duchene muscular dystrophy which could potentially serve as an invasive biomarker for disease progression [14]. Serum miR-206 is also increased in rhabdomyosarcoma and potentially is used as a diagnostic marker [15].

Little is known about circulating miR-206 in cachectic cancer patients and its diagnostic values in overall patient's management. MicroRNA in the circulation exists in micro vesicles ranging from 30 nm to 1 μm [16]. They are resistant to enzymatic digestion by ribonucleases which make their presence stable in blood [17,18]. This study was aimed to evaluate the clinical significance of circulating miR-206 in cancer patients presenting with cancer cachexia.

Methods

Study design and subjects

This was a cross-sectional study in Dharmais Cancer Hospital between 2014 and 2015 and already accepted ethical clearance from Faculty of Medicine University of Indonesia. Study subjects were

adult patients diagnosed with lung cancer and head and neck cancer, including nasopharyngeal cancer. Diagnosis was confirmed by imaging and histopathological examination. Clinical data obtained was sex, age, histopathological diagnosis, body mass index, and routine peripheral blood test. Patients gave a written consent for the use of their specimens for research purpose. This study was supported by a research grant from the University of Indonesia.

Definition and classification of cachexia

Cachexia was diagnosed according to an international consensus (2011) when there was a weight loss >5% over the past 6 months or body mass index (BMI) less than 20 kg/m^2 and any degree of weight loss >2%. Patients with a weight loss ≤ 5% with anorexia and metabolic change are clinically regarded as pre-cachectic. In this study, we used BMI less than 20 kg/m^2 to indicate cachectic patients with significant muscle loss.

RNA isolation and miR-206 quantification

5 ml of blood specimens were drawn from cancer patients and healthy subjects and stored in EDTA-containing vacationers. Blood specimens were centrifuged to obtain the plasma and then were stored at -80°C until miRNA isolation. Total serum RNA was extracted using a commercial kit (miRNEasy Serum/Plasma Kit, Qiagen, Germany) according to the manufacturer's instruction for serum samples. Two-hundred µl of serum was used for RNA extraction. Total RNA was then reverse transcribed using the TaqMan miRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). The expression levels of miR-216 were quantified by real time polymerase chain reaction (PCR) method using commercial kit (TaqMan miRNA assay kit, Applied Biosystems) according to the manufacturer's instruction. The expression of serum miR-16 gene was used as internal control. The sequence of miR-206 was: 5'-CCACACACUUCCUUACAUUCCA-3'. The expression levels of miR-206 and miR-16 from healthy normal subjects were also assayed to calculate the relative expression or fold change (FC) of miR-206. Quantitative miR-16 and miR-206 expression data were acquired and analyzed using an Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The CT value of miR-216 was normalized to the internal control of miR-16 to obtain the CT difference (ΔCT). The fold change (FC) of miR-216 expression between cancer patients and normal subjects was calculated as 2-ΔΔCT method [19].

Results

Characteristics of the study subjects

Seventy patients were enrolled during the study period; consisting 37 (52.9%) lung 33 (47.1%) head and neck cancers (Table 1). Two-thirds of the patients were men. Based on BMI calculation, there were 31 (41.3%) patients presenting with cachexia during the study period (Figure 1).

Expression of serum miR-206

Serum miR-206 was overexpressed in cancer patients compare to normal healthy subjects. The value of CT miR206 was higher in cancer patients than the normal subjects (Table 2). MicroRNA-206 expression was slightly up-regulated in cachectic patients than non-cachectic patients (Table 3). When compared to normal healthy subjects, serum miR-206 expression levels were significantly higher in cachectic patients, i.e. 13 times higher in lung cancer and 19.8 times higher in head and neck cancer patients (Table 4).

Discussion

This study was the first to evaluate the role of circulating miR-206in cancer patients with cachexia in Indonesia. Lung and head and neck cancers ranked within the 10 most common cancers in Indonesia. Patients often came in advanced stage with cachexia. Using one indicator only (BMI less than 20 kg/m^2) we found over 40% patients with cachexia. In statistical analysis, patients with weight loss more than 5% but had normal or high BMI were not considered as cachectic since these patients might still have sufficient muscle mass.

The value of CT miR-206 was normalized against miR-16 which is commonly used as reference miRNAs. Expression of miR-16 is usually stable in malignant diseases, such as breast and colorectal cancers [20,21] Statistical analysis of CT values is not allowed, but the fold change of miR-206 was highly overexpressed in cancer patients. MiRNAs is present in the circulating blood in the form of exosomal particles or micro vesicles [22] Increased of circulating miR-206

Table 1: Characteristics of the study subjects (n=70).

Characteristics	Mean ± SD	N	%
Gender			
■ Male		47	67.1
■ Female		23	32.9
Mean age (years)	49.6 ± 21.16		
Diagnosis			
■ Lung cancer:			
o Adenocarcinoma		22	29.3
o Squamous cell carcinoma		6	8.0
o Small cell carcinoma		2	2.7
o Unspecified		5	6.7
o Others		2	2.7
■ Head and neck cancer:		33	47.1
o Nasopharyngeal cancer		22	29.3
o Tongue cancer		5	6.7
o Parotid cancer		2	2.7
o Others		4	5.3
Mean body mass index (kg/m^2)	20.7 ± 3.96		

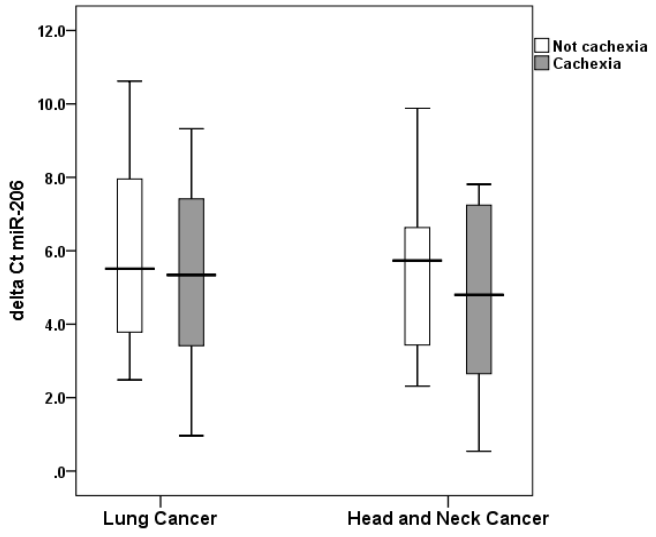

Figure 1: Comparison of ΔCt miR-206 between patients with and without cachexia.

Table 2: Cycle treshold (Ct) of miR-206 and miR-16 internal control among groups and miR-206 expression level.

Group	Ct miR-206	Ct miR-16	ΔCt miR-206	ΔΔCt miR-206	FC*
Lung cancer	33.137[a]	27.427	5.710	-3.527	**11.531**
Head &neck cancer	33.144[b]	27.925	5.219	-4.018	**16.204**
Normal subjects	36.553	27.316	9.237	0.000	1.000

***Note**: FC: fold change ($2^{-\Delta\Delta Ct}$); [a]p=0.016 and [b]p=0.018, as compared to normal subjects (one-way ANOVA).

Table 3: Expression levels of miR-206 between cachectic and non-cachectic patients.

Group	ΔCt miR-206			
	Cachectic	Non-Cachectic	ΔΔCt miR-206	FC*
Lung cancers	5.461	5.899	-0.438	**1.355**
Head &neck cancers	4.933	5.457	-0.524	**1.438**

*Note: FC: fold change ($2^{-\Delta\Delta Ct}$)

Table 4: Expression levels of miR-206 between cachectic patients and healthy subjects.

Group	ΔCt miR-206			
	Cachectic	Healthy Subjects	ΔΔCt miR-206	FC*
Lung cancers	5.461	9.237	-3.776	**13.699**
Head &neck cancers	4.933	9.237	-4.304	**19.753**

***Note:** FC: fold change ($2^{-\Delta\Delta Ct}$)

might be due to an increase of leakage or secretion from the skeletal muscle [23]. In this study, it is possible that many patients have already suffered from muscle loss leading to increased serum miR-206 expression.

Increased specific miRNA in the serum may reflect disease burden of a specific tissue like skeletal muscle [24]. For example, circulating miRNAs including miR-1, miR-133a and miR-206 were increased in the mouse model of muscle dystrophy when compared to the wild-type controls [25]. In our study, increased serum miR-206 could reflect increased skeletal muscle loss in these advanced stage cancer patients.

Further analysis showed that the relative expression of miR-206 of cachectic patients showed a tendency to be higher than non-cachectic patients. However, the fold change was not significant (FC<2.0) since most patients have already high expression of serum miR-206. The fold difference was more obvious when we compare serum miR-206 of the cachectic patients with normal healthy subjects (FC>10). This finding supports the theory that circulating miR-206 is a reflection of muscle atrophy or high muscle turnover. The same condition was also observed in patients with chronic obstructive lung disease (COPD) who typically are associated with muscle wasting [26].

Profiling circulating miR-206 may have therapeutic potential. Currently, treatment using miR mimics and antago-miR has been introduced to restore its level in muscle tissues [27]. Experiment in rhabdomyosarcoma cell lines has showed that together with miR-1, miR-206 inhibits rhabdomyosarcoma carcinogenesis by targeting *c-Met* [28]. It was also suggested to improve muscle regeneration in muscle dystrophies [29]. Clinical studies are needed in the future to elucidate therapeutic benefit of anti-miR treatment to restore muscle loss in cancer patients.

Conclusion

Circulating miR-206 is overexpressed advanced stage lung cancer as well as head and neck cancer patients. Compare to normal subjects, miR-206 is highly expressed in cachectic patients. Increased circulating miR-206 in cachectic patients may reflect extensive skeletal muscle loss associated with cancer cachexia. Further clinical studies are needed to test the efficacy of antimiR-206 treatment to restore muscle loss in cancer patients.

References

1. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, et al. (2011) Definition and classification of cancer cachexia: an internal consensus. Lancet Oncol 12: 489-495.
2. Laviano A, Meguid MM, Inui A, Muscaritoli M, Rossi-Fanelli F (2005) Therapy insight: cancer anorexia-cachexia syndrome - when all you can eat is yourself. Nat Clin Pract Oncol 2: 158-165.
3. Sandri M, Carraro U (1999) Apoptosis of skeletal muscles during development and disease. Int J Biochem Cell Biol 31: 1373-1390.
4. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281-297.
5. McCarthy JJ (2008) MicroRNA-206: the skeletal muscle-specific myomiR. Biochim Biophys Acta 1779: 682-691.
6. Guller I, Russel AP (2010) MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. J Physiol 588: 4075-4087.
7. Kim HK, Lee YS, Sivaprasad U, Malhotra A, Dutta A (2006) Muscle-specific microRNA miR-206 promotes muscle differentiation. J Cell Biol 174: 677-687.
8. Chen J-F, Tao Y, Li J, Deng Z, Yan Z, et al. (2010) MicroRNA-1 and microRNA-206 regulate skeletal muscle satellite cell proliferation and differentiation by repressing Pax7. J Cell Biol 190: 867-879.
9. Subramanian S, Lui WO, Lee CH, Espinosa I, Nielsen TO, et al. (2008) MicroRNA expression signature of human sarcomas. Oncogene 27: 2015-2026.
10. Soares RJ, Cagnin S, Chemello F, Silvestrin M, Musaro A, et al. (2014) Involvement of microRNAs in the regulation of muscle wasting during catabolic conditions. J Biol Chem 289: 21909-21925.
11. He WA, Berardi E, Cardillo VM, Acharyya S, Aulino P, et al. (2013) NF-kappaB-mediated Pax7 dysregulation in the muscle microenvironment promotes cancer cachexia. J Clin Invest 123: 4821-4835.
12. Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, et al. (2008) Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451: 147-152.
13. Wang X, Ling C, Bai Y, Zhao J (2011) MicroRNA-206 is associated with invasion and metastasis of lung cancer. Anat Rec (Hoboken) 204: 88-92.
14. Hu J, Kong M, Ye Y, Hong S, Cheng L, et al. (2014) Serum miR-206 and other muscle-specific microRNAs as non-invasive biomarkers for Duchenne muscular dystrophy. J Neurochem 129: 887-883.

15. Miyachi M, Tsuchiya K, Yoshida H, Yagyu S, Kikuchi K, et al. (2010) Circulating muscle-specific microRNA, miR-206, as a potential diagnostic marker for rhabdomyosarcoma. Biochem biophys Res Comm 400: 89-93.

16. Camussi G, Deregibus MC, Bruno S, Grange C, Fonsato V, et al. (2011) Exosome/ microvesicle-mediated epigenetic reprogramming of cells. Am J Cancer Res 1: 98-110.

17. Turchinovich A, Weiz L, Langheinz A, Burwinkel B (2011) Characterization of extracellular circulating microRNA. Nucleic Acids Res 39: 7223-7233.

18. Chen X, BaY, Ma L, Cai X, Yin Y, et al. (2008) Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 18: 997-1006.

19. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods 25: 402-408.

20. Davoren PA, McNeill RE, Lowery AJ, Kerin MJ, Miller N (2008) Identification of suitable endogenous control genes for microRNA gene expression analysis in human breast cancer. BMC Mol Biol 9: 76.

21. Chang KH, Mestdagh P, Vandesompele J, Kerin MJ, Miller N (2010) MicroRNA expression profiling to identify and validate reference genes for relative quantification in colorectal cancer. BMC Cancer 173.

22. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, et al. (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 105: 10513-10518.

23. Mizuno H, Nakamura A, Aoki Y, Ito N, Kishi S, et al. (2006) Identification of muscle-specific microRNAs in serum of muscular dystrophy in animal models: promising novel blood-based markers for muscular dystrophy. PLoS ONE 6: e18388.

24. Zacharewicz E, Lamon S, Russell AP (2013) MicroRNAs in skeletal muscle and their regulation with exercise, ageing, and disease. Front Physiol 4: 266.

25. Roberts TC, Godfrey C, McClorey G, Vader P, Briggs D, et al. (2013) Extracellular microRNAs are dynamic non-vesicular biomarkers of muscle turnover. Nucleic Acids Res 41: 9500-9513.

26. Donaldson A, Natanek SA, Lewis A, Man WD, Hopkinson NS, et al. (2013) Increased skeletal muscle-specific microRNA in the blood of patients with COPD. Thorax 68: 1140-1149.

27. Soriano A, Jubierre L, Almazan-Moga A, Molist C, Roma J, et al. (2013) microRNAs as pharmacological targets in cancer. Pharmacol Res 75: 3-14.

28. Yan D, Dong XA, Chen X, Wang L, Lu C, et al. (2009) MicroRNA-1/206 targets c-Met and inhibits rhabdomyosarcoma developments. J Biol Chem 284: 29596-29604.

29. Liu N, Williams AH, Maxeiner JM, Bezprozvannaya S, Shelton JM, et al. (2012) microRNA-206 promotes skeletal muscle regeneration and delays progression of Duchenne muscular dystrophy in mice. J Clin Invest 122: 2054-2065.

Author Affiliations

Top

[1]*Department of Internal Medicine, Dharmais Cancer Hospital, Jakarta, Indonesia*

[2]*Department of Clinical Nutrition, Dharmais Cancer Hospital, Jakarta, Indonesia*

[3]*Department of Anatomical Pathology, Faculty of Medicine, University of Indonesia, Depok, Indonesia*

[4]*Biomedical Science, Faculty of Medicine, University of Indonesia, Depok, Indonesia*

[5]*Department of Pulmonology, Dharmais Cancer Hospital, Jakarta, Indonesia*

[6]*Department of Ear Nose and Throat, Dharmais Cancer Hospital, Jakarta, Indonesia*

[7]*Department of Oncology Surgery, Dharmais Cancer Hospital, Jakarta, Indonesia*