Expression of HSA-MIR-155-5P and mRNA Suppressor of Cytokine Signalling 1 (SOCS1) on Plasma at Early-stage and Late-stage of Nasopharyngeal Carcinoma

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Keywords: Plasma, Nasopharyngeal carcinoma, Hsa-miR-155-5p, mRNA SOCS1

Abstract: Nasopharyngeal carcinoma (NPC) is a head and neck tumor with high prevalence and recurrence rate in Asia. Accurate therapy based on carcinoma pathogenesis at molecular level is urgently needed. Overexpression of hsa-miR-155-5p has been identified in various carcinomas include NPC. In previous in-silico research, hsa-miR-155-5p are known to target mRNA SOCS1, as a transduction signal suppressor for gene transcription activator. This study aimed to analyze different expressions of hsa-miR-155-5p and mRNA SOCS1 on plasma patients at an early and late stage of nasopharynx carcinoma. Hsa-miR-155-5p and mRNA SOCS1 were isolated from blood sample plasma using miRCURY RNA Isolation Kit-Biofluid. cDNA synthesized using cDNA Synthesis kit II, 8-64 rxns running by PCR thermal cycler (Bio-Rad c1000), and Real-time qPCR (Bio-Rad CFX 96) for Hsa-miR-155-5p. mRNA SOCS1 was analyzed by One-Step qRT-PCR using KAPA™ SYBR® kit. Hsa-miR-155-5p expressions change was upregulated 1.13-fold (p value=0.713) on the late-stage compared to the early stage, while for mRNA SOCS1 downregulated 1.1-fold (p value=0.891) on late-stage compared with early stage. Hsa-mir-155-5p was overexpressed on late-stage nasopharynx carcinoma and aligned with mRNA SOCS1 downregulated expression, compared to early stage. Deregulation of -miR-155-5p dan mRNA SOCS1 may play important role in NPC progressivity.

1 INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a head and neck tumor with a high prevalence and recurrence in Asia, commonly in men (Wah et al., 2014; Wildeman et al., 2013). A better understanding of the molecular basis of tumorigenesis does improve clinical outcomes and useful to develop early detection, for more accurate prognosis, and developing cancer individualized therapy (Kong et al., 2012). miRNAs offer great potential as biomarkers for cancer detection because of their remarkable stability in blood and their characteristic expression in different diseases

MicroRNAs (miRNAs) are a family of small non-coding RNA molecules with 20–23 nucleotides in length. It has function to negatively regulate protein-coding genes at the post-transcriptional level by mRNA uncapping and deadenylation. This process will lead to increased mRNA turnover and decreased target gene expression (Abba et al., 2014; Calin & Croce, 2006; Koturbash et al., 2011; Lu et al., 2014; Vishwamitra et al., 2012). Deregulation of microRNAs (miRNAs) is indicated in several conditions such as inflammation, cancer development and tumor progression (Calin & Croce, 2006; Y. Huang et al., 2013; X. Liu et al., 2009; Sotiropoulou et al., 2009; Tang et al., 2012; H. Zheng et al., 2013; S.-R. Zheng et al., 2012).

Hsa-miR-155-5p is prominent in cancer biology. Among microRNAs that have been linked to cancer, it is the most commonly overexpressed in human

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Indraswary, R., Haryana, S. and Surono, A.
Expression of HSA-MIR-155-5P and mRNA Suppressor of Cytokine Signalling 1 (SOCS1) on Plasma at Early-stage and Late-stage of Nasopharyngeal Carcinoma.
DOI: 10.5220/0010488000750080
In *Proceedings of the 1st Jenderal Soedirman International Medical Conference in conjunction with the 5th Annual Scientific Meeting (Temilnas) Consortium of Biomedical Science Indonesia (JIMC 2020)*, pages 75-80
ISBN: 978-989-758-499-2
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malignancies (Du et al., 2011; Jiang, Zhang, Lu, He, Li, Gu, et al., 2010; Palma et al., 2014; Zhang et al., 2013). Those miRNAs that lead to tumorigenesis and cancer are classified as oncomiRs. These oncomiRs are not only therapeutic targets but also important biomarkers for cancer detection and management (Du et al., 2011).

Cytokines activate multiple intracellular signaling pathways to produce their physiological effects. One of the most studied pathways is involving the receptor-associated janus kinases (JAKs) and the latent cytoplasmic transcription factors signal transducers and activators of transcription (STATs). Suppressors of Cytokine Signalling1 (SOCS1) is a negative regulator for STAT3. Activation of STAT3 will induce transcription of several genes which has a role in oncogeneses such as cell proliferation, differentiation, invasion, and angiogenesis (Y. Huang et al., 2013; Jiang, Zhang, Lu, He, Li, Gu, et al., 2010). Studies have proved that hsa-miR-155-5p target mRNA SOCS1(L. Liu et al., 2013). Even ribonuclease was found in human plasma and miRNAs have proven stable in blood plasma covering by lipid complex or lipoproteins like an apoptotic body, micro vesicle, or exosomes (Du, 2012; Kim et al., 2012; H. Zheng et al., 2013). This makes miRNAs has exciting prospect as powerful and minimal-invasive cancer biomarkers using plasma sample. Deregulation expressions of Hsa-miR-155-5p and mRNA SOCS1 can monitor disease progression and treatments. This research aimed to analyze difference expressions of hsa-miR-155-5p and mRNA SOCS1 on plasma at early and late stage of NPC.

2 MATERIALS AND METHODS

First, the insilico study was done by bioinformatic analysis through non-profit database of microRNA-based on notation sequences, such as miRbase, miRTarbase, Diana miR-Path and microRNAMap. The result shows that mRNA SOCS1 is one of hsa-miR-155-5p with mRNAs targets. Hsa-miR-155-5p binding site complementary occurs in along 401 nucleotides 3' untranslated region (UTR), at 15-34 nucleotides withal *Minimum Free Energy* (MFE) -15,50 kj/mol, or 218-242 nucleotides withal MFE -15,90 kj/mol, and also possible binding at nucleotides 404-425 withal MFE -9,13 kj/mol (figure1).

This initial study included 37 nasopharyngeal carcinoma patients of early stage and late stage (Table 1). samples were obtained from Venous blood. Hsa-miR-155-5p and mRNA SOCS1 were isolated from blood sample plasma using miRCURY RNA Isolation Kit-Biofluid. cDNA was synthesized using cDNA Synthesis kit II, 8-64 rxns running by PCR thermal cycler (Bio-Rad c1000), and Real-time qPCR (Bio-Rad CFX 96) for Hsa-miR-155-5p. mRNA SOCS1 was analyzed by One-Step qRT-PCR using KAPA™ SYBR® kit. The Ct value for each product was determined. MiR-16 was used as a reference gene for has-miR-155-5p quantification, and mRNA Beta Actin was used as a reference for mRNA SOCS1. Relative quantification for transcript accumulation was performed according to the comparative Livak Method: $2^{-\Delta\Delta Cq}$.

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TGACCGGCAGCGCCCGCCGTGCACGCAGCATTAACTGGGATGCCGTGTTATTTT
GTTATTACTTGCCTGGAACCATGTGGGTACCCTCCCCGGCCTGGGTTGGAGGGA
GCGGATGGGTGTAGGGGCGAGGCGCCTCCCGCCCTCGGCTGGAGACGAGGCCGC
AGACCCCTTCTCACCTCTTGAGGGGGTCCTCCCCCTCCTGGTGCTCCCTCTGGG
TCCCCCTGGTTGTTGTAGCAGCTTAACTGTATCTGGAGCCAGGACCTGAACTCG
CACCTCCTACCTCTTCATGTTTACATATACCCAGTATCTTTGCACAAACCAGGG
GTTGGGGGAGGGTCTCTGGCTTTATTTTTCTGCTGTGCAGAATCCTATTTTATA
TTTTTTAAAGTCAGTTTAGGTAATAAACTTTATTATGAAAGTTTTTTTTTT
```

Figure 1: hsa-miR-155-5p several binding site on 3'UTR region mRNA SOCS1.

Table 1: Characteristics of samples.

Characteristics	n (%)
Stages	
Early	6 (16)
Late	31 (84)
Sex	
Men	27 (73)
Women	10 (27)
Age	
20-30	5 (13.5)
31-40	10 (27)
41-50	11 (30)
51-60	6 (16)
61-71	5 (13.5)

3 RESULTS

Quantitative real-time PCR was performed to evaluate hsa-miR-155-5p and mRNA SOCS1 expressions in 37 NPC patients. From qRT-PCR examination, the amplification of hsa-miR-155-5p using hsa-miR-16 as a reference gene on samples were occurring (figure 2a). No shift in peak melt curve was validating product specificity (figure 2b).

Specific results mRNA SOCS1 obtained using the kit KAPA ™ SYBR® One-Step qRT-PCR (figure 3), where the optimal primer concentration at 10 pmol with annealing temperature 59.4°C.

Hsa-miR-155-5p and mRNA SOC1 expressions data quantifying were anlyzed statistically to determine whether the data were normally

distributed. Shapiro-Wilk test results indicate that the data quantifying of expression of hsa-miR-155-5p and mRNA SOCS1 both early-stage and late-stage normally distributed, Furthermore, the value of ΔΔCq hsa-miR-155-5p with hsa-miR-16 (table 2) and mRNA SOCS1 with Beta-actin mRNA (Table 3) analyzed used Livak method; *Fold change* = $2^{-\Delta\Delta Cq}$.

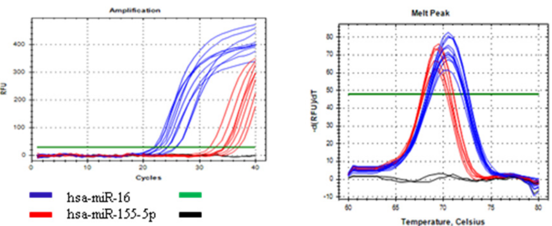

Figure 2: (a) hsa-miR-155-5p and miR-16 amplification curves on plasma NPC, analyzed by Bio-Rad CFX ManagerTM Software. (b) hsa-miR-155-5p and hsa-miR-16 melt peak.

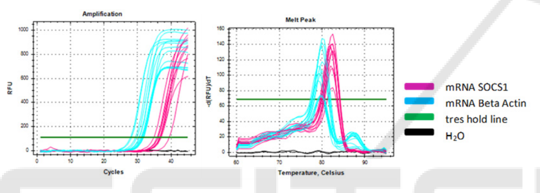

Figure 3: (a) mRNA SOCS1 and mRNA Beta-actin amplification curves done with one-step qRT-PCR using KAPA ™ SYBR® One-Step qRT-PCR kit. (b) mRNA SOCS1 and mRNA. Beta-actin Melt peak.

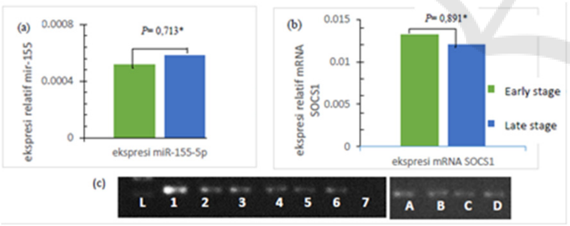

Figure 4. (a) Hsa-miR-155-5p expressions increased 1.13-fold. (b) mRNA SOCS1 expressions decreased 1.1-fold. Plasma samples of early-stage NPC compared with late-stage NPC (c) mRNA SOCS1 electrophoresis result on Agarose gel. L = Ladder (100bp), 1-3 = SOCS1 NPC early stage, 4-6 = SOCS1 NPC late-stage, 7 = negative control. A and B = β-actin early-stage NPC, C dan D = β-actin late-stage NPC.

Bio-Rad CFX Manager™ Software was used to analyzed qRT-PCR result. The results showed that hsa-miR-155-5p expressions fold change was upregulated 1.13 on the late-stage compared with early-stage (figure 4a). For mRNA SOCS1, it was downregulated 1.1-fold on late stage compared with early stage (figure 4b). qRT-PCR results were subsequently confirmed by electrophoresis. This was done using 2% Agarose gel electrophoresis, running by 100 volts for 30 minutes to determine the specificity and consistency of SOCS1 results of qRT-PCR (figure 4c).

4 DISCUSSION

This study was aimed to analyze different expressions of hsa-miR-155-5p and mRNA SOCS1 on plasma patients at an early and late stage of NPC. Livak method result showed that hsa-miR-155-5p were overexpressed in late-stage NPC plasma compared with early-stage NPC.

Overexpression's of hsa-miR-155-5p possibly aligned with NPC progressions. Hsa-miR-155-5p is a micro RNA that has been shown to be associated with the progression of carcinoma in human (Li et al., 2012; L. Liu et al., 2013). MiR-155 overexpression is also correlated with poor prognosis in cancer (H. Zheng et al., 2013). It also had been proven that miR-155 expressions in breast cancer patients serum were associated with clinical stages (Rawlings et al., 2004).

mRNA SOCS1 expressions downregulated in the NPC plasma late stage compared to the early-stage. SOCS1 mRNA may be targeted by hsa-miR-155-5p. Other studies also proved there is a negative correlation between the expression level of miR-155 and SOCS1 (C. Huang et al., 2013). The results showed expression of SOCS1 decreased by 1.1-fold in the NPC plasma late stage compared to the early stage. Downregulated of SOCS1 may increase STAT3 expression. SOCS1 is a negative feedback regulator on the JAK / STAT pathway and these pathways stimulate cells to proliferate, migrate, or apoptosis (Chen et al., 2013).

STAT3 plays an important role in carcinoma invasion by inducing inflammatory pro-oncogenic pathways and tumor cell immunity, including nuclear factor-kappaB (NF-kappaB) and interleukin-6 (IL-6) -GP130-JAK pathway. SOCS1 acts as a negative regulator of this pathway by inhibiting STAT3 phosphorylation (Jiang, Zhang, Lu, He, Li, & Gu, 2010). The antiproliferative mechanism is carried by SOCS1 through inhibition of JAK2 kinase activity

Table 2. Hsa-miR-155-5p and miR-16 expressions in plasma NPC early-stage and late-stage.

NPC Plasma	Targets	Mean (ΔCq) ± Deviation	ΔΔCq	Fold change ($2^{-\Delta\Delta Cq}$)
Early stage	Hsa-miR-155-5p	10.92 ± 2.26	-0.175	1.13
	Hsa-miR-16			
Late stage	Hsa-miR-155-5p	10.74 ± 1.89		
	Hsa-miR-16			

Table 3. mRNA SOCS1 and mRNA Beta-actin expressions in plasma early stage and late stage of NPC.

NPC plasma	Targets	Mean (ΔCq) ± Deviation	ΔΔCq	$2^{-\Delta\Delta Cq}$	Fold change $-1/2^{-\Delta\Delta Cq}$
Early stage	mRNA SOCS1	6.24± 2.15	0.14	0.91	-1.102
	mRNA BA				
Late stage	mRNA SOCS1	6.38± 2.42			
	mRNA BA				

which prohibit the activation (phosphorylation) of STAT3 (Yoshimura et al., 2012).

There have been many studies proving that STAT3 is an oncogene in various malignancies. Hsa-miR-155-5p overexpressed in Human Laryngeal Squamous Cell Carcinoma late stage compared to early-stage followed by downregulated of mRNA SOCS1 expressions and overexpression of STAT3 in late stage. Other studies have shown a decrease of SOCS1 expressions in colonic adenocarcinoma progression and SOCS1 was found very low expressed on the late stage (David et al., 2014).

Until now the NPC classifications are based on TNM (Tumor, Node, Metastasis) as defined by the American Joint Committee on Cancer (AJCC) (Lee et al., 2004). On the late stage, TNM has a higher value than the early stage, reflecting the progression of cancer cell growth exceeded from the early stage. Hsa-miR-155-5p has an important role in tumor progression related to its ability to modulate epithelial-to-mesenchymal transition (EMT) (Bouyssou et al., 2014). EMT is a cell program, where epithelial cells transformed into mesenchymal cells characterized by loss of polarity, adhesion loss, motility, and increased potential ability. Cells able to move as metastasis incidence of malignancy. Knockdown on miR-155 resulted in the increase of SOCS1 and STAT3 decreases in malignancy. This is consistent with the results obtained, in which the hsa-miR-155-5p expressions increased in the late-stage followed by SOCS1 mRNA expression decreased.

5 CONCLUSIONS

The identification of carcinoma molecular mechanisms is substantial to obtain successful therapy. Plasma samples are feasible as media in NPC molecular examinations as shown by the results similarity in pattern with the tissues itself. Hsa-miR-155-5p and mRNA SOCS1 were proven stable in NPC blood plasma and able to be quantitatively analysed. This research shows that hsa-mir-155-5p was overexpressed on late-stage nasopharynx carcinoma, aligned with mRNA SOCS1 downregulated expression, compared to the early stage. Therefore, deregulation of -miR-155-5p dan mRNA SOCS1 are believed to have a significant role in NPC progressivity.

ACKNOWLEDGEMENTS

The authors thank the DIKTI funding research for providing financial support of this study. The authors thank to Universitas Gadjah Mada, Universitas Islam Sultan Agung, Prof. Dr. dr. Teguh Aryandono Sp.B (K) Onk, Dr. Med. dr. Indwiani Astuti, Prof. Dr. Mustafa, Apt., Kes, dr. Ahmad Hamim Sadewa, Ph.D., Drs. Zulaela, Dipl. Med. Stat., M.Si, Dr. dr. Cita Herawati, Risky Oktriani, S. Si, M. Biotech, dr. Sumadi Lukman A., Ph.D., dr. Zulrachman, Nihayatus Saadah, S. Si, and the entire members of the study group microRNA (genomiR) for their support to this research.

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