

Conference Paper

The Expression of miR-141 and mRNA PTEN with Cisplatin Therapy on NPC

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Abstract

The incidence of NPC in Yogyakarta province is 6.2/100 000 population and the tendency is increasing in the younger population. It has been known that drugs resistance also issues in NPC therapy. Cisplatin is drugs of choice that used in NPC therapy, besides miR-141 and mRNA PTEN are also had a role in chemotherapy resistance in some of cancer. We conducted the examination of the expression of miR-141 from blood plasma pre and post-therapy patient and blood plasma in healthy control. The sample collected from whole blood and then plasma separation. RNA extracted using RNA Isolation Kit miRCURY-Biofluid, synthesis cDNA with cDNA Synthesis kit. mRNA analysis with KAPA SYBR® FAST One-Step qRT-PCR Kit then detected by qRT-PCR. Data analysis with comparative analysis and statistical analysis. MiR-141 showed upregulation by 1.49 (p-value: 0.075) and mRNA PTEN showed down-regulation by 0.65 (p-value: 0.323) in patients NPC compared to healthy control and there is a relationship between miR-141 and mRNA PTEN (p-value:0.001). The expression levels of miR-141 in patients pre- and post-therapy experience down-regulated (fold change:0.61, two-tailed t-test: 0.09) and mRNA expression levels of PTEN experience down-regulated (fold change:0.5, t-test two-tailed:0.09). The level expression of mRNA PTEN is still down-regulated through the expression of miR-141 is up-regulated indicated that the possibility of drugs resistance in therapy with cisplatin. Then, miR-141 is one of the important factors in cisplatin drugs resistance with inhibition of mRNA PTEN.

Keywords: miR-141, mRNA PTEN, Cisplatin, Drugs resistance, NPC.

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1. Introduction

NPC is happening by insertion of EBV-DNA viruses. EBV infects B lymphocyte by expressed the LMP1 (oncogene) to increase B-cell proliferation by making a similar signal to activated B-cell by upper cell molecule B-cell, CD40. In another hand, LMP-1 is prevented apoptosis by activated BCL2. Then, the proliferation will be uncontrolled


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[1]. To achieve long-term in vivo, the EBV express the latent gene which causes latent infection [2].

The drugs of choice in NPC therapy was cisplatin, an inorganic metal drugs as an alkylating agent. As an alkylating agent, it works by inhibiting cross-linking in the N7 alkyl group of guanines and makes uncoil strand [3]. The issue of drugs resistance in cancer therapy have been investigated after cisplatin therapy [4]. Molecular mechanism of cisplatin resistance has been investigated caused by a kinase, such as Akt and YAP1. Alteration of the kinase expressions has been influenced by the expression of a tumor suppressor gene. MicroRNA, the single-strand non-coding RNA has been worked in post-transcriptional mechanism and could be suppressed the expression of tumor suppressor gene [5].

miR-141 have been investigated in NPC proliferation cells by downregulated PTEN, a tumor suppressor gene as an antagonist in the AKT/PI3K pathway [6]. miR-141 have been investigated a role in resistance to cisplatin in esophageal squamous cell carcinoma by targeting YAP1 [7]. In ovarian cancer have been investigated the role of miR-141 in cisplatin resistance by targeting KEAP1 [8]. Induction of NPC in Akt/FoxO pathway is known to lead to their resistance to cisplatin [9].

A research study in NPC cells has investigated the role of miR-141 and mRNA PTEN in NPC pathogenesis. miR-141 has been investigated as oncomiR and mRNA PTEN was targetted [6]. In order to support the noninvasive biomarker agent in drugs resistance, research on miR-141 and mRNA PTEN expression was conducted in blood plasma of NPC patients. At the same time, inhibition of EBV-miR to mRNA PTEN also done to investigate the miRNA-miRNA interaction by in silico study.

2. Materials and Methods

2.1. Sample collections

Sample collections used was some blood patients with NPC (pre and post therapy) and plasma of normal people from Dharmais Cancer Hospital and diagnoses of NPC has been done by Cita Herawati. All sample experiments were approved by the ethical committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

2.2. Material

2.2.1. Equipment

Micropipet tube, microRNA mini spin column BF (Exiqon), vortex, centrifuge, spin down, thermal cyder Biorad c1000, qRT-PCR Biorad.

2.2.2. Material

Alcohol 70 %, isopropanol p.a, rDNase, miRCURY RNA isolation kit- Biofluid Exiqon (Lysis solution BF, Protein precipitation solution BF, Wash solution 1 dan 2 BF, RNase free water), kit universal cDNA synthesis kit II, 8-64 rxns (cat no. 2-33-1, exiqon), 5 times of reaction buffer, nuclease free water, spike in (Sp6), enzyme mix, ice pack. miRCURY LNA Universal RT micro RNA PCR, LNA PCR Primer Set, hsa-miR-141-3p UAACACUGUCUG-GUAAAGAUG

For mRNA: KAPA SYBR® FAST One-Step qRT-PCR Kit Universal (KAPA SYBR FAST qPCR master mix, ROX Reference Dye High, ROX Reference Dye Low, dUTP, KAPA RT mix), primer set mRNA PTEN (forward: GGGTCTGAGTCGCCTGTCA, reverse: CCGT-GTTGGAGGCAGTAGAAG), primer beta-actin (forward: GGGAATTCAAACCTGGAACG-GTGAAGG, reserve: GGAAGCTTATCAAAGTCCTCGGCCACA). The primer of mRNA PTEN was available from Vinciguerra, et al.[10].

2.3. Methods

2.3.1. Plasma isolation

Blood sample in vacutainer EDTA has been centrifuged at $1\,500\text{ rad} \cdot \text{s}^{-1}$ in 10 min and collect the plasma. Total RNA from blood plasma was extracted using the miRCURY RNA Isolation Kit-Biofluid (Cat No. 300112, Exiqon). Complementary DNA was prepared from DNase-treated RNA samples with Universal cDNA Synthesis kit II, 8-64 rxns (Cat. No. 203301, Exiqon). Quantitative PCR was performed on a StepOnePlus real-time PCR system (Applied Biosystems) using Exilent SYBR Green master mix (Cat No. 203402, Exiqon). mRNA PTEN was detected using KAPA SYBR®FAST One-Step qRT-PCR KIT Universal, All instructions according to the manufacturer's instructions.

2.3.2. Quantitative real-time PCR

Total RNA from blood plasma was extracted using the miRCURY RNA Isolation Kit-Biofluid (Cat No. 300112, Exiqon). Complementary DNA was prepared from DNase-treated RNA samples with Universal cDNA Synthesis kit II, 8-64 rxns (Cat. No. 203301, Exiqon). Quantitative PCR was performed on a StepOnePlus real-time PCR system (Applied Biosystems) using Excellent SYBR Green master mix (Cat No. 203402, Exiqon). mRNA PTEN was detected using KAPA SYBR[®]FAST One-Step qRT-PCR KIT Universal

2.3.3. Data analysis

Data have been analyzed with comparative quantitative, $2\Delta\Delta C_t$ method, using miR-16 and beta-actin Ct to reference gene.

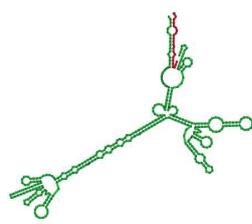
3. Results

Table 1 describes the expression of miR-141 and mRNA PTEN in the sample. The expression of miR-141 has been upregulated after cisplatin therapy, but the expression of mRNA PTEN have been downregulated after therapy. Its indicated that the therapy was not responded.

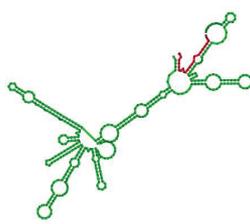
TABLE 1: The expression of miR-141 and mRNA PTEN in blood plasma sample by Fold Change ($2\Delta\Delta C_t$ method).

Sample	Before treatment	After treatment
miR-141	1.49	0.61
mRNA PTEN	0.69	0.5

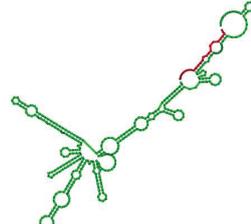
NPC happen because of EBV genome insertion. Then we conducted interaction between EBV-miR with mRNA PTEN by in silico study with Vienna Package Software (Figure 1). The study has been done for investigated the reason of down-regulated of mRNA PTEN after therapy. All of EBV-miR have been targeted mRNA PTEN, which means that its interaction may cause the down-regulated of mRNA PTEN.



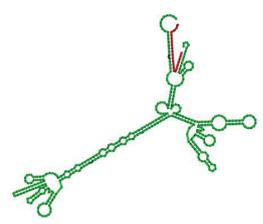
MFE Bart1-3p=-87.30 kcal · mol⁻¹



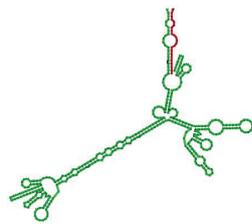
MFE Bart1-5p=-86.50 kcal · mol⁻¹



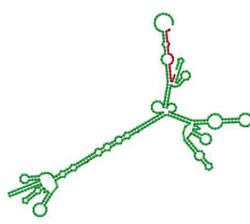
MFE Bart2-3p=-84.50 kcal · mol⁻¹



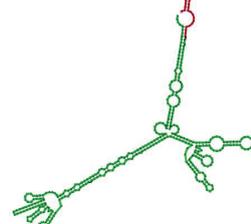
MFE Bart2-5p=-87.30 kcal · mol⁻¹



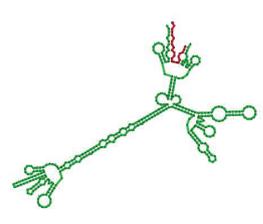
MFE Bart3-3p=-85.80 kcal · mol⁻¹



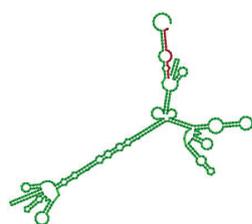
MFE Bart3-5p=-84.20 kcal · mol⁻¹



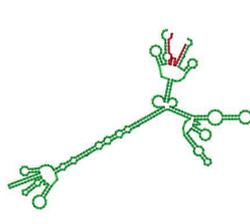
MFE Bart4-3p=-84.40 kcal · mol⁻¹



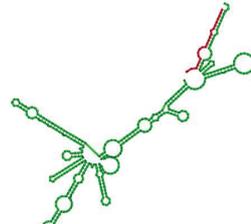
MFE Bart4-5p=-86.10 kcal · mol⁻¹



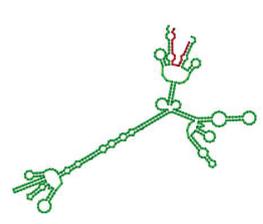
MFE Bart5-3p=-86.10 kcal · mol⁻¹



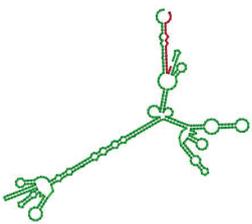
MFE Bart5-5p=-89.40 kcal · mol⁻¹



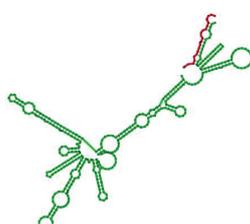
MFE Bart6-3p=-89.90 kcal · mol⁻¹



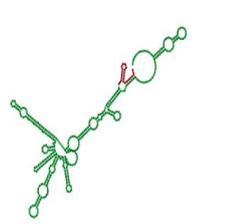
MFE Bart6-5p=-86.60 kcal · mol⁻¹



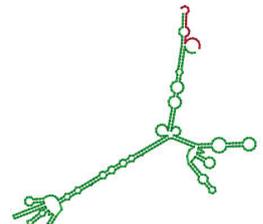
MFE Bart7-3p=-85.80 kcal · mol⁻¹



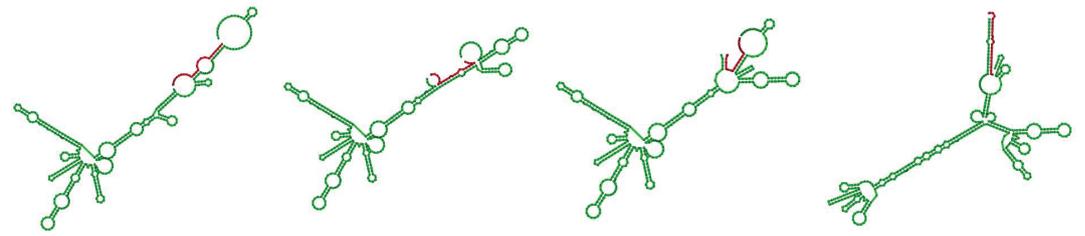
MFE Bart7-5p=-82.40 kcal · mol⁻¹



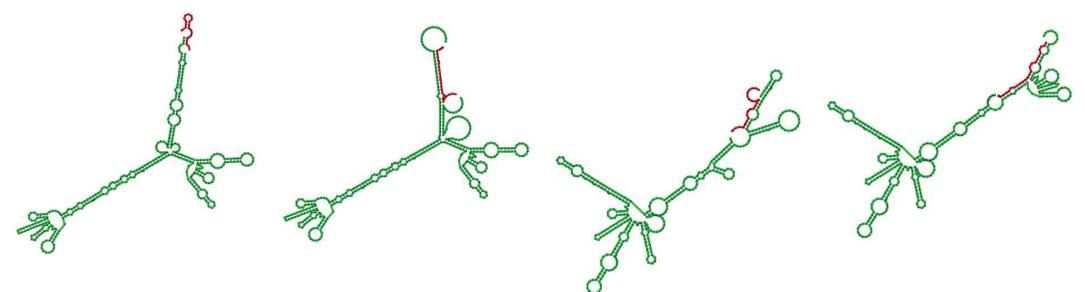
MFE Bart8-3p=-87.70 kcal · mol⁻¹



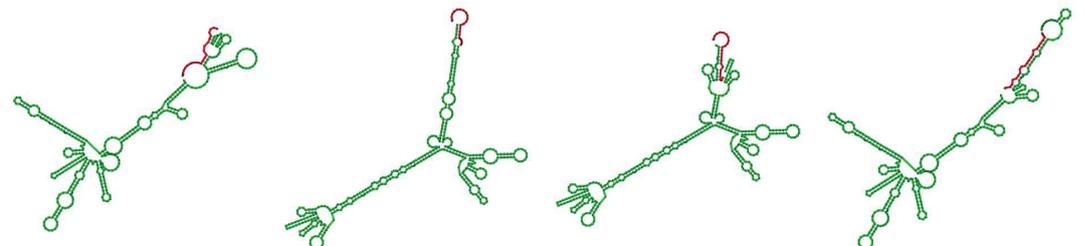
MFE Bart8-5p=-82.90 kcal · mol⁻¹



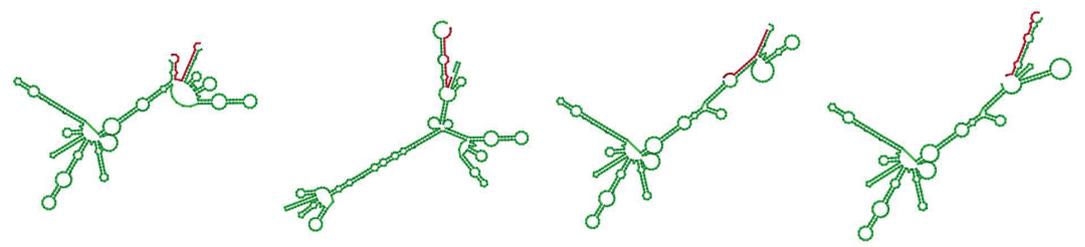
MFE Bart9-3p=-85.00 kcal · mol⁻¹ MFE Bart9-5p=-86.70 kcal · mol⁻¹ MFE Bart10-3p=-85.60 kcal · mol⁻¹ MFE Bart10-5p=-95.90 kcal · mol⁻¹



MFE Bart11-3p=-85.80 kcal · mol⁻¹ MFE Bart11-5p=-90.80 kcal · mol⁻¹ MFE Bart12=-85.40 kcal · mol⁻¹ MFE Bart13-3p=-86.50 kcal · mol⁻¹



MFE Bart13-5p=-82.90 kcal · mol⁻¹ MFE Bart14-3p=-86.00 kcal · mol⁻¹ MFE Bart14-5p=-86.30 kcal · mol⁻¹ MFE Bart15=-84.50 kcal · mol⁻¹



MFE Bart16=-91.10 kcal · mol⁻¹ MFE Bart17-3p=-87.00 kcal · mol⁻¹ MFE Bart17-5p=-87.70 kcal · mol⁻¹ MFE Bart18-3p=-86.00 kcal · mol⁻¹

4. Discussion

miR-141 have been investigated as oncomir by down-regulated mRNA PTEN in blood plasma NPC. PTEN plays a role in conducting dephosphorylation of PIP3 into PIP2 on

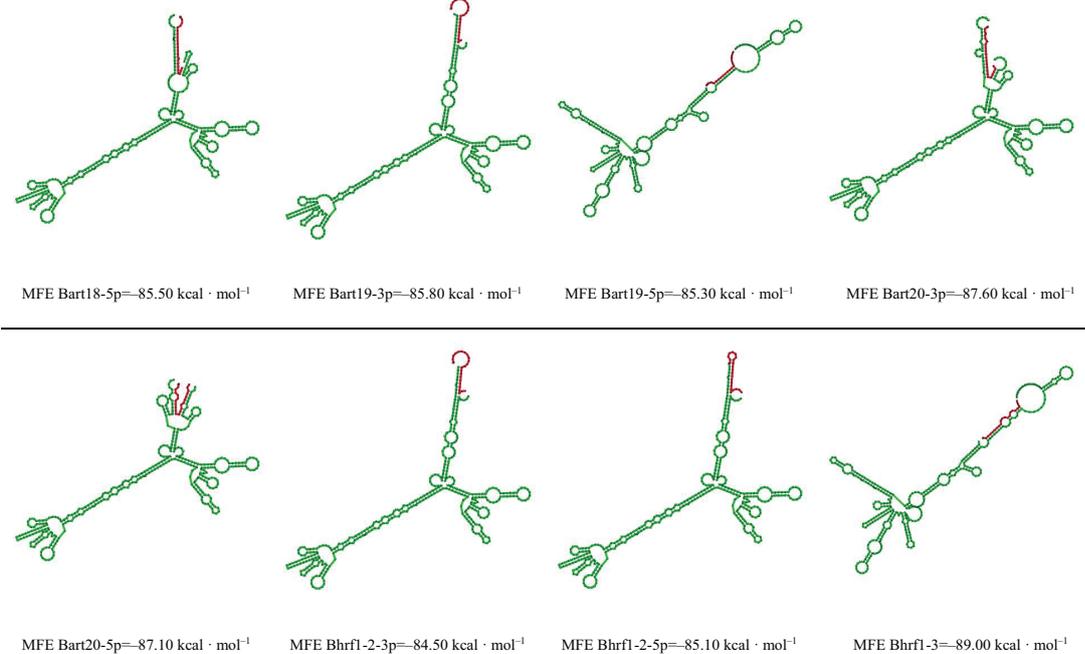


Figure 1: mRNA PTEN-EBV-miR interaction by Vienna Package Software (Green: PTEN. Red: EBV-miR). The strongest interaction was seen in PTEN-EBV-Bart10-5p (MFE=-95.90 kcal · mol⁻¹).

Akt phosphorylation of PI3K pathway [9]. However, miR-141 as oncomir can inhibit the expression of PTEN in post-transcriptional processes. The expression of oncomir also decreased after drugs therapy, which it can increase the expression of mRNA targeted. However, the expression of mRNA PTEN was upregulated after therapy although the expression of miR-141 was down-regulated. It has meant that the therapy was not responded and there might be cisplatin resistance.

In cellular respond, cisplatin can be interacted with secondary thiols, like glutathione and metallothionein. Glutathione-cisplatin conjugated exit from the cell by ATP-dependent pump. After cisplatin interacted with DNA, it will inhibit proliferation by activated DNA damage. Kinase such as Akt, PKC, and ERK is also involved in the regulation of cell death that induced by cisplatin. miR-214 was promoted cisplatin resistance by downregulating PTEN and Akt [11]. miR-141 might be promoted cisplatin resistance by downregulating PTEN and Akt activated similar as miR-214.

The inhibition in mRNA PTEN by another oncomir, like EBV-miR, makes mRNA PTEN down-regulated and not responded to cisplatin therapy. miRNA-miRNA interaction may be potential on complex network regulation of biological processed [12]. Another miRNA can influence any miRNA by regulated their targeted and modulated their activity [13].

5. Conclusions

miR-141 is one of the important factors in cisplatin drugs resistance through inhibition of mRNA PTEN and Akt activated signaling. mRNA PTEN in NPC might be bound to EBV-miR that makes mRNA PTEN down-regulated and not responded to cisplatin therapy

Acknowledgments

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