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Upregulation of p53 Gene Expression on Breast Cancer Stem Cells Treated with Trisindoline 5 Compound

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ABSTRACT

Background: Cancer stem cells (CSCs) are the subpopulation of cancer cells that have been demonstrated as the cause of tumor formation. The most common cancer for women and the second leading cause of death in breast cancer. Numerous genes have been involved in breast cancers, including p53. In cancer cells, p53 as well-known as the tumor suppressor gene plays an important role in directing cells with DNA damage into apoptosis. Trisindolines are heterocyclic nitrogen compounds consisting of an isatin core bearing two indole moieties that provide cytotoxic effects on cancer cells. Recently research had led to the development of the new modification of trisindoline compound into trisindoline 5

Objective: This study aims to investigate the effect of trisindoline 5 compounds against p53 gene expression in CSCs MDA-MB-231 in vitro.

Methods: CSCs MDA-MB-231 were divided into control and treatment groups which were further analyzed in gene expression using the qPCR Livak method.

Results: Based on gene expression analysis, trisindoline 5 increases the expression of p53 in CSCs MDA-MB-231.

Conclusion: This study informed that trisindoline 5 could upregulate the expression of tumor suppressor gene p53 in CSCs MDA-MB-231 in vitro.

Keywords: Breast cancer stem cells, p53, Trisindoline 5

INTRODUCTION

Cancer stem cells (CSCs) are the subpopulation of cancer cells that have been demonstrated as the cause of tumor formation.[1] CSCs within tumors holding stemness properties have been proposed to help drive tumorigenicity and inherent drug therapy resistance. CSCs could control the neighboring cells to provide the nutrients and collaborate in the elusion from the immune system, thus creating a favorable environment for tumor growth.[2] Many cancer patients with poor prognoses recurrence after treatment due to the permanence of CSCs.[3] The most common cancer for women and the second leading cause of death in breast cancer. The existence of CSCs in breast cancer cells helps maintain the incurable metastasis and the current optimal management of this disease remains unclear.[4] Thus, CSCs are emerging as a target for cancer therapy.[5]

Numerous genes have been involved in breast cancers, including P53.[6] In normal cells, P53 has a wide range of functions such as in cell homeostasis, including cell cycle, cell proliferation, DNA maintenance, and apoptosis[2]. It has also been referred to as the guardian of the genome that protects the cell from cellular damage under stress conditions.[7] Whether in cancer cells, P53 as well-known as the tumor suppressor gene plays an important role in directing cells with DNA damage into apoptosis.[6]

Apoptosis is a programmed cell death that results in the loss of cells without the release of harmful substances into the environment.[8] Previous study showed that a higher level of p53 expression in CSCs MCF-7 compared to CSCs MDA-MB-231 after being treated with doxorubicin.[9] Another research stated doxorubicin has been considered and approved as one of the most commercial drug administrations[3]. The treatment using doxorubicin leads to DNA alterations, cellular damage, and cell death[4]. However, this cellular damage not only occurs in cancer cells but also in healthy cells which are mainly a consequence of its toxicity.[10]

Using bioactive natural compounds from marine sponges as a new cancer therapeutic strategy has special attention because it has a rich source of secondary metabolites and is widespread in a tropical reefs.[11] Trisindolines are heterocyclic nitrogen compounds consisting of an isatin core bearing two indole moieties which were first isolated from the cultured marine bacterium *Vibrio* sp. symbiosis with marine sponge *Hyrtios altum* from Okinawa Japan.[12] Trisindoline was reported to have anticancer activity against many cancer cell lines, including colorectal adenocarcinoma cells (HCT-15), lung cancer cells (A-549), uterine sarcoma cells (MES-SA), and breast cancer cells (MCF-7).[13] Recently, a research had led to the development of the latest new modification of trisindoline compound into trisindoline 5-fluoro-3,3-di((methylindole-5-carboxylate)-3-yl)-2-indolon or trisindoline 5 which combines the isatin with a fluoro group and indole with a methyl ester group[5]. This compound was still tested in cytotoxicity in vitro on lung cancer cells (A-549), prostate cancer cells (DU-145), and normal heart cells of mice (H9C2) with IC_{50} 25.69 µg/ml, 22.23 µg/ml, and 201.65 µg/ml respectively.[14] Therefore, in this study, we explore the potential of trisindoline 5 to increase the expression of p53 in CSCs. This study aims to investigate the effect of trisindoline 5 compounds against p53 gene expression in CSCs MDA-MB-231 in vitro.

MATERIAL AND METHODS

Reagents and Chemicals

The Trisindoline 5 compound and doxorubicin as a positive control are obtained from the Chemistry of Natural and Synthetic Materials Laboratory, Department of Chemistry ITS Surabaya.

MDA-MB-231 Cells culture

CSCs were isolated from breast cancer cells MDA-MB-231 (ECACC #92020424) (Porton Down, Wiltshire, UK) using *magnetic-activated cell sorting* (MACS) (Miltenyi Biotec, Bergisch Gladbach, Germany). CSCs validation is known based on the expression of $CD44^+/CD24^-$. CSCs MDA-MB-231 were plated in Dulbecco's Modified Eagle Medium F-12 (DMEM F-12) (Gibco, USA) supplemented with 3 ml fetal bovine serum (FBS) (Gibco, USA) 20%, 186 µl penicillin-streptomycin 1,24% (Gibco, USA), 153 µl glutamine 1,02% (Gibco, USA), 37,5 µl fungizone (Amphotericin B) (Gibco, USA) 0,25% and MammoCult 20%.

p53 gene expression by qRT-PCR

About 5-10 x 10^6 cells/ well were plated into 6 well plates, treated with doxorubicin 10 µg/ml and trisindoline 5 compounds with a concentration of ½ IC_{50} and 1 IC_{50} for 24 hours incubation. Total RNA was extracted using RNA Isolation Kit (*FavorPrep™ Tri-RNA*). Then, the isolated RNA was quantified using a Nanodrop instrument (Nanodrop ND-1000 Technologies). 2 µl of RNA was used to synthesize cDNA using the *TOYOBO ReverTra Ace™ qPCR RT Master Mix with gDNA Remover No. FSQ-301*. Quantitative reverse transcription-PCR (qRT-PCR) analyses were performed using *Eco™ Real-Time PCR System*. The effect of trisindoline 5 on the expression level of marker genes p53 and GAPDH was analyzed by RT-PCR. A comparative threshold cycle was used for the estimation of gene expression. The primer sequences used were shown in Table 1. In summary, the CT (threshold cycle) was obtained from triplicate amplification throughout the exponential stage of the amplification. Next,

the CT value obtained for the reference gene (GAPDH) was deducted from the CT values obtained for the p53 and was calculated for every single gene. Following the calculation of ΔΔCT for each sample, the relative expression of each gene was evaluated using the ratio formula (ratio=2-ΔΔCt).

Table 1. Primer's sequences used for real-time qPCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
p53	GCTCAGATAGCGATGGTCTGGC	AGTGGATGGTGGTACAGTCAGAG
GAPDH	CCAGCCGAGCCACATCGCTC	ATGAGCCCCAGCCTTCTCCAT

Statistical Analysis

Data were represented as mean ± standard deviation (SD) (SPSS statistical analysis software, version 20.0) of three independent assays. All variables were analyzed for normality, homogeneity, and parametric testing one-way analysis of variance (ANOVA), followed by post hoc analysis using Mann Whitney. The statistical significance difference is illustrated as asterisks (*, $p < 0.05$; **, $p < 0.01$; *, $p < 0.001$).

RESULTS

Effect of Trisindoline 5 on p53 gene expression

The result showed that there was an increase in p53 gene expression significantly in all treatment groups compared to the control. The optimum increasing p53 gene expression occurs in trisindoline 5 with IC_{50} dose and doxorubicin. This study showed a significant effect of trisindoline 5 on p53 gene expression in CSCs MDA-MB-231.

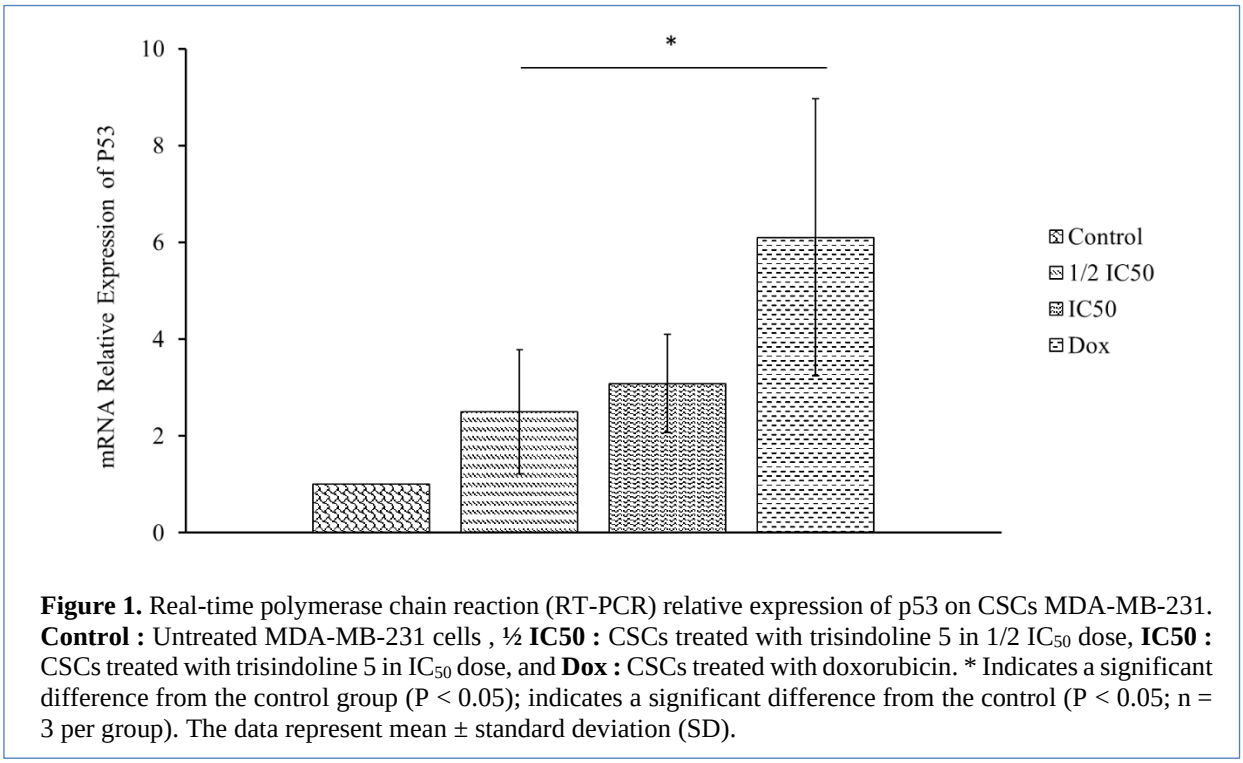

Figure 1. Real-time polymerase chain reaction (RT-PCR) relative expression of p53 on CSCs MDA-MB-231. **Control :** Untreated MDA-MB-231 cells , **½ IC50 :** CSCs treated with trisindoline 5 in 1/2 IC_{50} dose, **IC50 :** CSCs treated with trisindoline 5 in IC_{50} dose, and **Dox :** CSCs treated with doxorubicin. * Indicates a significant difference from the control group ($P < 0.05$); indicates a significant difference from the control ($P < 0.05$; n = 3 per group). The data represent mean ± standard deviation (SD).

DISCUSSION

The tumor suppressor p53 plays a crucial role in ensuring genomic integrity of embryonic stem cells and controls their proliferation, differentiation, and apoptosis.[15] p53 expression in normal cells is maintained at a low level due to the disruption of the activity of E3-ubiquitin ligases, such as MDM2, HDM2, and TRIM24 by ubiquitylation and protein degradation.[16] DNA damage, UV radiation, hypoxia, and hyper-proliferation in embryonic stem cells could lead to p53 activation.[6,17] In response to mild or severe DNA damage, p53 differentially regulates cell fate decisions. Under mild damage conditions, p53 is phosphorylated at Ser15 and Ser20 leading to cell cycle arrest and DNA repair. While under severe damage conditions, p53 is phosphorylated not only at Ser15 and Ser20, but also at Ser46 resulting in cell death.[18]

The activity of cyclin D and CDK4/6 which regulate the G1 to S phase is decreased when DNA is damaged by decreasing the amount of Rb protein. This results in the inhibition of the E2F transcription factor and triggers cell cycle arrest. The cyclin D will enter the damaged DNA and stimulate the activation of proteins involved in DNA repair mechanisms, such as RAD51 and BRCA2.[19,20]

The damaged DNA also will initiate a signaling pathway by activating the ATM/ ATR kinase.[21] These proteins then phosphorylate the Chk1/ Chk2 kinase to activate p53 as the main target.[22] The activated p53 will stimulate the transcription process of the p21 that acts as a CDK inhibitor.[23] The p21 protein then binds to G1/S-CDK to stop the cell cycle progression from G1 to the S phase.[20,24] On the other hand, p53 protein also could directly activate several pro-apoptotic target genes, such as BAX and BAK[25]. Then, BAX and BAK will release cytochrome c from mitochondria. The cytochrome c will bind to ATP, APAF-1, and procaspase-9 to form a complex called apoptosome[24,25]. The apoptosome then activates caspase 9 and will initiate the apoptosis.[26] Therefore, in this study, trisindoline 5 compounds might be potential as an alternative anticancer candidate on CSCs MDA-MB-231 to minimize the side effects from the chemotherapy agents through increasing the expression of tumor suppressor gene p53.

CONCLUSION

Our results demonstrated that Trisindoline 5 could upregulate the expression of tumor suppressor gene p53 in CSCs MDA-MB-231 in vitro.

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AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and interpretation of the work. All authors were involved in drafting the work, revising it critically for content, and approving the final version for publication.

COMPETING INTERESTS

The authors declare that there was no conflict of interest.

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