Original Article

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Expression of miR-221/222 is affected by Triclosan in MCF-7 cells

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Abstract

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; TCS) is a broad-spectrum antibacterial compound commonly used in cosmetics, dentifrices, soap, and other consumer products. There is growing concern that estrogenic environmental compounds that act as endocrine disrupting chemicals such as TCS might potentially have adverse effects on hormone-sensitive organs. Overexpression of miR-221/222 has been observed in a number of advanced malignancies indicating that miR-221/222 could be potential therapeutic targets for epithelial cancer cells. In the present study we investigated whether TCS affects on miR-221/222 expression level in breast cancer cell line, MCF-7. In this experimental study, the expression level of miR-221 and miR-222 in MCF-7 through the quantificational real time polymerase chain reaction (QRT PCR) assay was detected. Our results showed that the expression level of miR-221 was increased by TCS and expression level of miR-222 was declined by TCS. Overall, TCS may act as an estrogen receptor agonist at lower concentrations via estrogen receptor pathway (ER) while inhibiting the growth of MCF-7 cells at higher concentrations via non-ER pathways.

Keywords: Estrogen receptor alpha (ERα), miR-221, miR-222, Triclosan.

1. Introduction

The general population is exposed to a number of hormonally active compounds on a daily basis. These compounds were introduced in the living environment during the last few decades and the majority of them are xeno-estrogens (1). Xeno-estrogens are estrogen-mimicking compounds that are commonly found in the personal care products, pesticides, and plastic bottles (2). Xeno-estrogens also are present in a number of substrates such as cigarette smoke, automobile exhaust, chemical industry pollutants, grilled meat, volcano dust, forest fire smoke, milk, water, as well as cosmetic products. This means that all human population may be exposed to the Xeno-estrogens (1). Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; TCS), a lesser-known xeno-estrogen, is a broad-spectrum antibacterial compound that are commonly used in the cosmetics, dentifrices, soap, and other consumer products (3). TCS exhibits lipophilic properties and also can be easily passed through biological barriers (4). The widespread use of TCS and its detection in human breast milk, urine, and serum have raised concerns regarding to its association with various health outcomes, including different types of cancer (3). There is growing concern that estrogenic environmental compounds that act as endocrine disrupting chemicals might potentially have adverse effects on hormone-sensitive organs such as the breast (5).

MiRNAs are non-coding RNA molecules,

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consisting of 18-24 nucleotides in length, that bind to the 3′ untranslated region (UTR) of target mRNAs to regulate gene expression (6). Many studies have demonstrated the important roles played by miRNAs in the occurrence, development and diagnosis of cancer (7). The location of miRNA genes in fragile sites and regions of deletion and amplification on chromo¬some supported its involvement in the development of cancer (8). MiRNAs are vital in regulating cell proliferation and apoptosis, in addition to functioning as either oncogenes or tumor suppressors in the cell cycle (9). Evidence is rapidly growing that miRNA regulation of gene expression may be affected by environmental chemicals. MiRNAs are newly emerged as a gene expression regulatory factor that may link environmental chemicals and their related diseases (10). TCS up-regulates endogenous levels of miR-22, miR-206 and miR-193b in MCF-7 cells. These results suggest that TCS might reduce ERα expression through inducing overexpression of these microRNAs (11). MiR-221 and miR-222 (miR-221/222) are two highly homologous microRNAs (12) and are expressed by single transcription that are found on the X chromosome (13). They contain identical seed sequences separated by 727 bases and are highly conserved in vertebrates (14). In healthy conditions, they have been found to regulate essential physiological vascular processes such as angiogenesis, neo-intimal hyperplasia, vessel wound healing, vascular aging, and atherosclerotic vascular remodeling (15-17). TCS activates AhR and has a similar chemical structure to dioxin. In the presence of sunlight, TCS can be transformed into as many as four dioxin compounds, such as 2,8-DCDD, 2,3,7-TCDD, 1,2,8-TriCDD, and 1,2,3,8-TCDD(18). Also TCS can be transformed to 2,8-DCDD without sun light at room temperature and under near dry conditions (19). Overexpression of miR-221/222 has been observed in a number of advanced malignancies indicating that miR-221/222 could be potential therapeutic targets for epithelial cancer (20). Falkenberg et al. reported that miR-221/222 were up-regulated in breast cancer tissues and were the predictor of distant metastases and poor prognosis (21). Some studies also showed that miR-221/222 promote breast cancer cell proliferative and invasive capabilities, through triggering epithelial-mesenchymal transition (EMT), or targeting, such as suppressor of cytokine signaling 1 (SOCS1) and cyclin-dependent kinase inhibit 1B (CDKN1B), β4 integrin, signal transducer and activator of transcription 5A (STAT5A) and of a disintegrin and metalloprotease-17 (ADAM-17)(22-26).

Several signaling pathways which contribute to cell proliferation, such as TGF-β, MAPK, Notch, and Wnt, were regulated by miR-221/222 (27). PTEN (phosphatase and tensin homolog deleted on chromosome ten) is one of the direct targets of miR-221/222 and these oncomiRs promote cell proliferation (8). The association of miR-221 and miR-222 for breast cancer is critical, but their detailed roles in its development and progression of breast cancer remain unclear (28) also TCS role as a xeno-estrogen and AhR-ligand in miR-221/222 expression is unknown. In the present study we investigated whether TCS affects on miR-221/222 expression level in breast cancer cell line, MCF-7.

2. Materials and methods

2.1. Chemicals

Chemicals were provided from the following suppliers: TCS from Sigma-Aldrich, Germany. All cell culture reagents and media were purchased from Invitrogen.

2.2. Cell culture and chemical treatments

In this experimental study, MCF-7 cells were maintained in 10% fetal bovine serum-supplemented Dulbecco's modified Eagle's medium (DMEM). 100 µg/ mL streptomycin, and 100 IU/ mL penicillin under an atmosphere containing 5% CO2 at 37 °C.

Desired concentrations of chemicals were treated after replacing the growth medium with fresh medium without FBS. The final concentration of DMSO was 0.1% (v/v). The MCF-7 cells were treated with TCS (100, 50 and 10 µM).

2.3. RNA extraction and cDNA synthesis for miRNAs

TThe TRizol reagent (Invitrogen, Carlsbad, CA, USA) was used for isolation of total RNA

Table 1. miRNAs related targets and their functions.

miRNA	Target or related genes/ pathway	Significant function	Ref
MiR-221/222	PTEN	Inhibition of cell proliferation	(8)
	ERα	Sensitivity to tamaxifen	(23)
	P27kip1	Inhibition of cell proliferation and self-renewal	(29)
	P57	Inhibition of cell proliferation and self-renewal	(18)
	GAS5	Induction of apoptosis	(28)
	SOCS1	Inhibition of metastais	(22-26)
	Notch-3	Inhibition of metastais	(30)
	CDKN1B	Inhibition of metastais	(22-26)

according to the manufacturer's instructions and then reversely transcribed into cDNA by using the RT microRNA Kit (EXIQON, Denmark). RT-PCR amplification consisted of 40 cycles (95 °C for 5 seconds, 63 °C for 20 seconds, and 72 °C for 30 seconds) after an initial denaturation at 95 °C for 5 minutes in an ABI Stepone real-time quantitative PCR system. The fold change of the miRNA expression was calculated by using the 2-ΔΔCt method after normalization to the 5S rRNA (as internal control) expression.

2.4. MiRNA target prediction

Prediction of miRNA targets was performed with the bioinformatics tools, TargetScan, PicTar, and miRanda (Table 1).

2.5. Statistical analysis

Statistical analyses were performed with Graphpad software. Statistical significance of differences between mean values were determined by Student's t-test or one-way ANOVA followed by Tukey post-hoc test. Statistically significant differences were assumed at $P<0.05$.

3. Results

3.1. Effect of of TCS on miR-221 and miR-222 expression level in MCF-7 cells

The quantitative RT-PCR detection analysis showed that the expression levels of miR-221 was higher in the cells treated with TCS 50 µM and 100 µM in comparison to control. Expres-

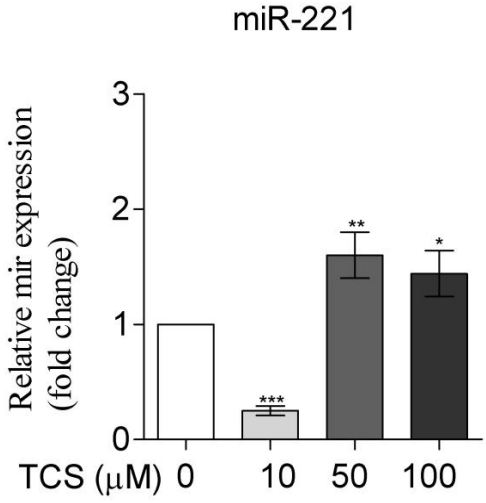

Figure 1. The expression of miR-221 was induced by TRC in MCF-7. The quantitative RT-PCR detection analysis showed that the expression level of miR-221 was much higher in the cells treated with TRC in comparison to control. Real-time RT-PCR was evaluated according to the "Materials and methods" section. Values are expressed as means±S.E; Asterisks denote significant differences (*$P<0.05$, **$P<0.01$ and ***$P<0.001$) between control and other treated groups.

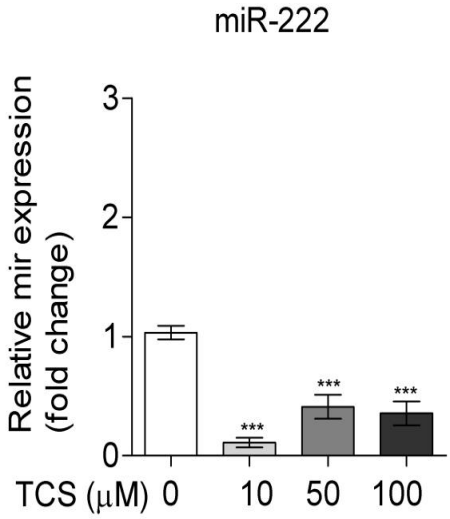

Figure 2. The expression of miR-222 was inhibited by TRC in MCF-7. The quantitative RT-PCR detection analysis showed that the expression level of miR-222 was much lower in the cells treated with TRC in comparison to control. Real-time RT-PCR was evaluated according to the "Materials and methods" section. Values are expressed as means±S.E; Asterisks denote significant differences (***P<0.001) between control and other treated groups.

sion level of miR-221 was much lower in the cells treated with TCS 10 μM in comparison to control (Figure 1). The results also showed that the expression levels of miR-222 were much lower in the cells treated with TCS 10 μM, 50 μM and 100 μM in comparison to control (Figure 2).

4. Discussion

Breast cancer is a malignant tumor that seriously affects life and health of females. Incidence and mortality of breast cancer rank in the top area among all malignancies. Breast cancer at early stage shows no obvious symptoms and most patients are diagnosed at advanced stages, and thus missing the best treatment time, leading to poor prognosis (31). Therefore, novel and minimally invasive techniques, which are adequately sensitive for patients, might offer a valuable alternative or complement to the existing methods as mammography (32). MiRNAs are emerging as innovatively promising biomarkers for the detection of breast cancer at an early stage, prediction of prognosis, and monitoring of the effect of therapy (33). The miR-221/222 cluster has been discovered to function as oncogene in human malignancies including breast cancer (34). Silencing miR-221/222 could represent a promising approach for therapeutic studies (35). Inhibition of miRNA-221/222 in ER-positive human breast adenocarcinoma cell line (MCF-7) can also increase the sensitivity to tamoxifen through the upregulation of tissue inhibitor of metalloproteinases-3 (TIMP3). Therefore, miR-221/222 might serve as potential therapeutic targets for drug resistance in breast cancer (36). Transfection of miR-221/222 synthetic mimetic into an immortalized and non-transformed mammary cell line (MCF10A) resulted in a significant enrichment of genes involved in EMT and the RAS pathway (37). MiR-221/222 has also been shown to act as oncogenes by repressing cell cycle inhibitor proteins p27/Kip1 and p57 and thus facilitating cell proliferation and self-renewal (38). There are evidence for a pivotal role of Notch3 in the suppression of EMT and metastasis via transactivating ERα in breast cancers. MiR-221/222 promote epithelial-mesenchymal transition by targeting Notch-3 in breast cancer cell lines (30).

In present study our data showed TCS (50 μM and 100 μM) enhanced miR-221and decreased miR-222 expression levels in MCF-7 cell line. TCS could regulate estrogen-responsive genes expression levels, in the same manner as E2 (39).The positive results of reporter gene assays, indicating TCS is ERα agonist (11). TCS might perform estrogenic activity at lower concentrations, and exhibit anti-estrogenic activity and/or cytotoxicity at

high concentrations (11). TCS like phytosterogens might act as an estrogen receptor agonist at lower concentrations through the ER pathway while inhibiting the growth of MCF-7 cells at higher concentrations via non-ER pathways (40).Finally, deeper investigations of the relevant mechanisms underlying the biphasic effects of TCS on the MCF-7 cells is needed.

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Conflict of Interest

None declared.

5. References

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