Toxicology in Vitro 41 (2017) 133–142

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

An integrative transcriptomic analysis reveals bisphenol A exposure-induced dysregulation of microRNA expression in human endometrial cells

Wei-Chun Chou [a,b], Pei-Hsuan Lee [a], Yan-Yan Tan [a], Ho-Chen Lin [a], Chung-Wei Yang [a], Kuan-Hsueh Chen [a], Chun-Yu Chuang [a,*]

[a] Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan
[b] National Institute of Environmental Health Sciences, National Health Research Institute, Miaoli, Taiwan.

ARTICLE INFO

Article history:
Received 30 September 2016
Received in revised form 23 December 2016
Accepted 17 February 2017
Available online 24 February 2017

Keywords:
Bisphenol A
Endometrial cancer
MicroRNA
Gene network
Hedgehog signaling
Carcinogenesis

ABSTRACT

Bisphenol A (BPA) are commonly used in the manufacture of polycarbonate plastics. Higher BPA exposure levels have been found in patients with endometrial hyperplasia that is one of risk factors of endometrial cancer (EC). Aberrant microRNAs (miRNAs) regulation has been observed in the development of cancer. Thus, this study investigated whether BPA exposure can disrupt miRNA regulation and its gene expression regarding to EC carcinogenic progress. Microarray experiments of miRNA and mRNA were performed in human endometrial cancer RL95-2 cells with treatment of low-to-moderate (10, 10^3 and 10^5 nM) BPA to explore the aberrant genes corresponding to human EC progression. According to the analysis of KEGG pathway and Cytoscape gene network, this study identified that BPA exposure reduced miR-149 expression to down-regulate DNA repair gene ARF6 (ADP-ribosylation factor 6) and tumor protein p53 (TP53), and up-regulate CCNE2 (cyclin E2) potentially to interrupt cell cycle. BPA also increased miR-107 to suppress hedgehog signaling factors, suppressor of fused homolog (SUFU) and GLI family zinc finger 3 (GLI3) to activate hedgehog signaling for cell proliferation underlying carcinogenesis. Furthermore, the BPA-induced cell proliferation was attenuated by transfection with miR-149 mimic and miR-107 inhibitor. These findings provided an insight into potential epigenetic mechanism of BPA exposure on the risk of endometrial carcinogenesis.

1. Introduction

Endometrial cancer (EC), one common malignant carcinoma of gynecologic diseases, is the second most prevalent cancer among women following breast cancer. With over 140,000 cases occurring annually worldwide, it has become the fourth most common cancer in women (Nicolaije et al., 2013). The ascended incidence of EC is relevant to increasing age, body mass index and obesity or excessive estrogen exposure (Oldenburg et al., 2013). EC often develops in setting of endometrial hyperplasia, and presents with abnormal vaginal bleeding. Additionally, breast cancer drug Tamoxifen use (Leao et al., 2013), postmenopausal women hormone therapy (Ali, 2014), and lifestyle changes (canned food intake, plastic tableware and cosmetics use) may lead to increase the number of EC patients in the worldwide (Banno et al., 2012).

MicroRNAs (miRNAs) are a class of small non-coding and single-stranded RNAs that play important roles in regulation to gene expression. They regulate gene expression by targeting to the 3′ UTR of mRNAs, leading to their degradation or translation repression (Berezikov et al., 2016). Aberrant expression of miRNAs can either inactivate tumor suppressor genes or activate oncogenes thereby promoting tumor formation during tumor development (Sharma et al., 2010). For instance, down-regulation of miR-143, miR-145, miR-15 and miR-16 has been reported in colorectal cancer (Michael et al., 2003) and chronic lymphatic leukemia (Calin et al., 2002), while increased expression of members of the miR-17-92 cluster has been reported in lung cancer (Hayashita et al., 2005) as well as in diffuse B-cell lymphomas (Fassina et al., 2012). The miR-200 family (miR-200a/b/c, miR-141, and miR-429) is up-regulated in cases of EC (Snowdon et al., 2011). A combination survey of miRNAs in endometrial tissues has been identified that miR-182, 183, 200a, 200c and 205 was significantly higher in EC when compared with endometrial hyperplasia and normal endometrial tissue (Lee et al., 2012). Microarray studies have identified miR-27 (Mozos et al., 2014), miR-203 (Huang et al., 2014), miR-205 (Zhang et al., 2014), miR-141, and miR-200 (Wang et al., 2014) are up-regulated, whereas,

* Corresponding author at: Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, 101, section 2, Kuang-Fu Road, Hsinchu 30013, Taiwan.
E-mail address: cychuang@mx.nthu.edu.tw (C.-Y. Chuang).

http://dx.doi.org/10.1016/j.tiv.2017.02.012
0887-2333/

miR-30c (Kong et al., 2014), miR-101 (Konno et al., 2014), miR-449 (Ye et al., 2014), miR-143, miR-145 (Wang et al., 2014) and miR-424 (Li et al., 2015a, 2015b) are down-regulated in EC. In the endometrium throughout the menstrual cycle under normal and pathologic condition, miRNAs can regulate genes of cell cycle (Pan et al., 2007), inflammatory response (O'Connell et al., 2007), angiogenic factors (Suarez et al., 2007), extracellular matrix and apoptotic regulators (Horcajadas et al., 2007) infiltrating into the endometrium as well as in endometrial epithelial, stromal, and vascular endothelial cells (Pan and Chegini, 2008). The differentially expressed miRNAs can affect angiogenesis and proteolytic activities in endometriosis (Braza-Boils et al., 2014), and lead to tumor suppressor loss and disturb cell cycle in EC (Wang et al., 2014). Thus, dysregulation of miRNA may be an important consequence in the EC progressions.

Bisphenol A (BPA) is one of well-known endocrine disruptor compounds. Previous studies have been observed that exposure to BPA from plastic beverage containers significantly increases BPA concentrations in urine (Carwile et al., 2009; Calafat et al., 2008). High serum BPA levels are found in the women with endometrial hyperplasia (Hiroi et al., 2004) or polycystic ovarian syndrome (Kandaraki et al., 2011). BPA affects human endometrial endothelial cell angiogenic activity via estrogen receptor (ER) channel to disturb vascular endothelial growth factor (VEGF) (Helmestam et al., 2014).

Exposure to BPA has been found to lead to the alterations in miRNAs. In human placental cell lines (TCL-1, HTR-8, and 3A), BPA exposure alters miRNAs expression levels, particularly induces miR-146a overexpression, to result in slower proliferation rate and higher sensitivity to the DNA damaging agent bleomycin (Avissar-Whiting et al., 2010). BPA can up-regulate oncogenic miR-19a and miR-19b to induce cell proliferation in human breast cancer MCF-7 cells (Li et al., 2014). Evidences have shown that BPA affects miRNAs modulation, but the effect of BPA on miRNA expression for the progress of endometrial cancer is still not well known. Here, we performed a transcriptomic analysis by integrating mRNA and miRNA profiling to explore the aberrant miRNA-mediated gene network of BPA exposure corresponding to EC carcinogenesis, and verify the findings of transcriptomic analysis with miRNA transfection in human endometrial carcinoma RL95-2 cells.

2. Materials and methods

2.1. Cell culture and BPA treatment

Human endometrial carcinoma RL95-2 cells (ATCC) were maintained in DMEM/F12 medium supplemented with 10% (v/v) fetal bovine serum, 1× Antibiotic-Antimycotic and 5 μg/ml bovine insulin, and incubated at 37 °C under 5% CO_2. BPA (bis-(4-hydroxyphenyl)-propane; Sigma) was dissolved in DMSO (dimethyl sulfoxide; Sigma) to prepare the stock solution (100 mM), and diluted to different concentrations (10, $10^3$and 10^5 nM) by the culture medium. RL95-2 cells were seeded into 6-well plate (1×10^6) for 24 h, and treated with 10, $10^3$and 10^5 nM BPA and control group (DMSO) for 24 h.

2.2. RNA extraction and microarray processing

Total RNA was extracted from RL95-2 cells using Trizol reagent (Invitrogen, Carlsbad, CA). The RNA quantity and purity was assessed OD260/OD280 (≥1.8) and OD260/OD230 (≥1.5) using NanoDrop ND-1000 (Thermo, Wilmington, MA), and the RNA integrity number was determined ≥6 using Agilent RNA 6000 Nano assay (Agilent Technologies, Santa Clara, CA). gDNA contamination was evaluated by gel electrophoresis.

The 2.5 μg and 13 μg of total RNA were individually determined for miRNA profiles and mRNA profiles using Human miRNA OneArray v4 chip and Human OneArray v5 chip (Phalanx Biotech, Hsinchu, Taiwan). The chip uses two groups of probes respectively labeling with Cy3 and Cy5 fluorescent dyes to generate 532 nm and 635 nm excitation. The intensities of fluorescent were analyzed by GenePix 4.1 software (Molecular Devices, Sunnyvale, CA). The miRNA raw intensity of each probe was passed the criteria normalized by 75% median scaling normalization method. The normalized spot intensities with the cutoff values log2 ratio ≥ 0.60206 or ≤ −0.60206 (fold change ≥2) in BPA exposure groups compared to control were transformed as significant differentially expressed miRNAs (DEMs). miRDB online database was further used in the annotation analysis for miRNA target prediction and functional annotations.

The mRNA raw intensity of each probe was loaded into Rosetta Resolver System (Rosetta Biosoftware) to process data analysis. The error model of Rosetta Resolver System can remove both systematic and random errors form the data. Those probes with background signals were filtered out. Probes that passed the criteria were normalized by the 50% median scaling normalization method. The significant differently expressed genes (DEGs) were cutoff with log2 ratio ≥ 0.39134 or ≤ −0.39134 (fold change ≥1.3) in BPA exposure group compared with control and further annotated in analysis of KEGG and Cytoscape.

2.3. Quantitative reverse-transcription real-time PCR

cDNA was synthesized from total RNA by a high-capacity cDNA reverse transcription kit following the manufacture's protocols (Applied Biosystems, Foster City, CA). A fragment of cDNA was quantified using 2× power SYBR Green PCR master mix (Applied Biosystems), forward and reverse primers, and RNase-free water by an ABI 7300 Sequence Detection System (Applied Biosystems).

Bulge-LoopTM miRNA qRT-PCR Primer Sets, miR-149, -107 and U6 (ssD809231415, ssD809230017, ssD0904071008; RiboBio, Guangzhou, China), were used to prepare cDNA for subsequent q-PCR applications involving quantification of mature miRNAs in parallel with precursor miRNAs, mRNAs, and other noncoding RNAs. ABI 2720 thermal cycler (Applied Biosystems) was used to amplify the cDNA with one cycle. A fragment of cDNA was determined by using 2× power SYBR Green PCR master mix, forward and reverse primers, and RNase-free water. The expression was used ABI 7300 Sequence Detection System to detect.

2.4. miRNA inhibition construct transfection

Human endometrial cancer RL95-2 cells were seeded in a 6-well plate. When reached 70% confluences, RL95-2 cells were transfected with synthesized miR-149 mimic (miR-10004609, 30 nM; RiboBio) or miR-107 inhibitor (miR-20000104, 50 nM; RiboBio). The miRNA mimics/inhibitor and lipofectamine (Invitrogen) were mixed to form miRNA mimics/inhibitor-lipo transfection complex. RL95-2 cells were cultured with DMEM/F12 medium and transfection complex in cultural plates at 37 °C under 5% CO_2. After the process, the transfection medium was removed and fresh DMEM/F12 medium with final concentration of 10, 10^3 and 10^5 nM BPA were respectively added in cultural plates at 37 °C under 5% CO_2. After treatment, the medium was switched by the FBS-containing culture medium at 37 °C under 5% CO_2. Total RNA was extracted for the determination of miRNAs and mRNAs expression using q-PCR.

2.5. Cell viability assay

RL95-2 cells and transfected RL95-2 cells (2×10^5 cells/well) were plated in 96-well plates for 24 h and treated with concentrations of 10, 10^3 and 10^5 nM BPA for 48 h, respectively. DMSO was used as a blank control. Cells were incubated with MTT (3-(4,5-dimethylthiazhol-2-yl)-2, 5-diphenyl-tetrazolium bromide) for 1 h at 37 °C. After removing MTT, DMSO was added to dissolve the purple formazan formed by viable cells. The optical density was detected at

570 nm within 20 min using VersaMax ELISA microplate reader (Molecular Devices).

2.6. Statistical analysis

The results of q-PCR were described as mean ± standard deviation (SD) of two independent experiments. The Student *t*-test was used to compare the differences of gene expression between BPA exposure group and control as well as between miRNA mimic/inhibitor transfection group and non-transfection group. A two-tailed p-value <0.05 was considered statistically significant.

3. Results

3.1. miRNA expression profile corresponding to BPA exposure and EC

All normalized miRNA signals in microarray analysis of RL95-2 cells treated with BPA (10, $10^3$and 10^5 nM) were visualized using heat map generated by unsupervised hierarchical clustering (Fig. 1A). Overall, a total of 82 miRNAs were considered differentially expressed (>2 fold

Table 1
List of significantly dysregulated miRNAs after BPA exposure.

miRNA	Fold change[a]	q-Value[b]
Up-regulated		
hsa-mir203	1.6, 2.3, 1.7	0.14, 0.14, 0.13
hsa-mir205	2.5, 2.9, 2.1	0.14, 0.07, 0.11
hsa-mir103a	1.8, 2.6, 2.2	0.17, 0.07, 0.12
hsa-mir107	2.0, 2.6, 1.4	0.13, 0.11, 0.18
hsa-mir200c	2.0, 4.4, 1.9	0.07, 0.10, 0.07
hsa-mir141	1.8, 2.6, 1.8	0.07, 0.19, 0.14
hsa-mir221	1.6, 3.5, 1.7	0.11, 0.12, 0.07
hsa-let_7a-5p	2.6, 2.5, 2.7	0.18, 0.14, 0.13
hsa-mir193b	1.6, 2.1, 1.6	0.10, 0.14, 0.07
hsa-mir423	1.6, 2.2, 1.6	0.07, 0.14, 0.12
Down-regulated		
hsa-mir513	3.2, 4.3, 4.8	0.08, 0.10, 0.10
hsa-mir149	2.4, 2.9, 1.8	0.10, 0.11, 0.11
hsa-mir765	3.3, 3.8, 4.9	0.19, 0.10, 0.11

[a] Number indicated significant fold change of miRNA expression compared to control after BPA exposure (10, 10^3 and 10^5 nM).
[b] q-Value: false discovery rate-adjusted p value calculated from BPA exposure referred to control.

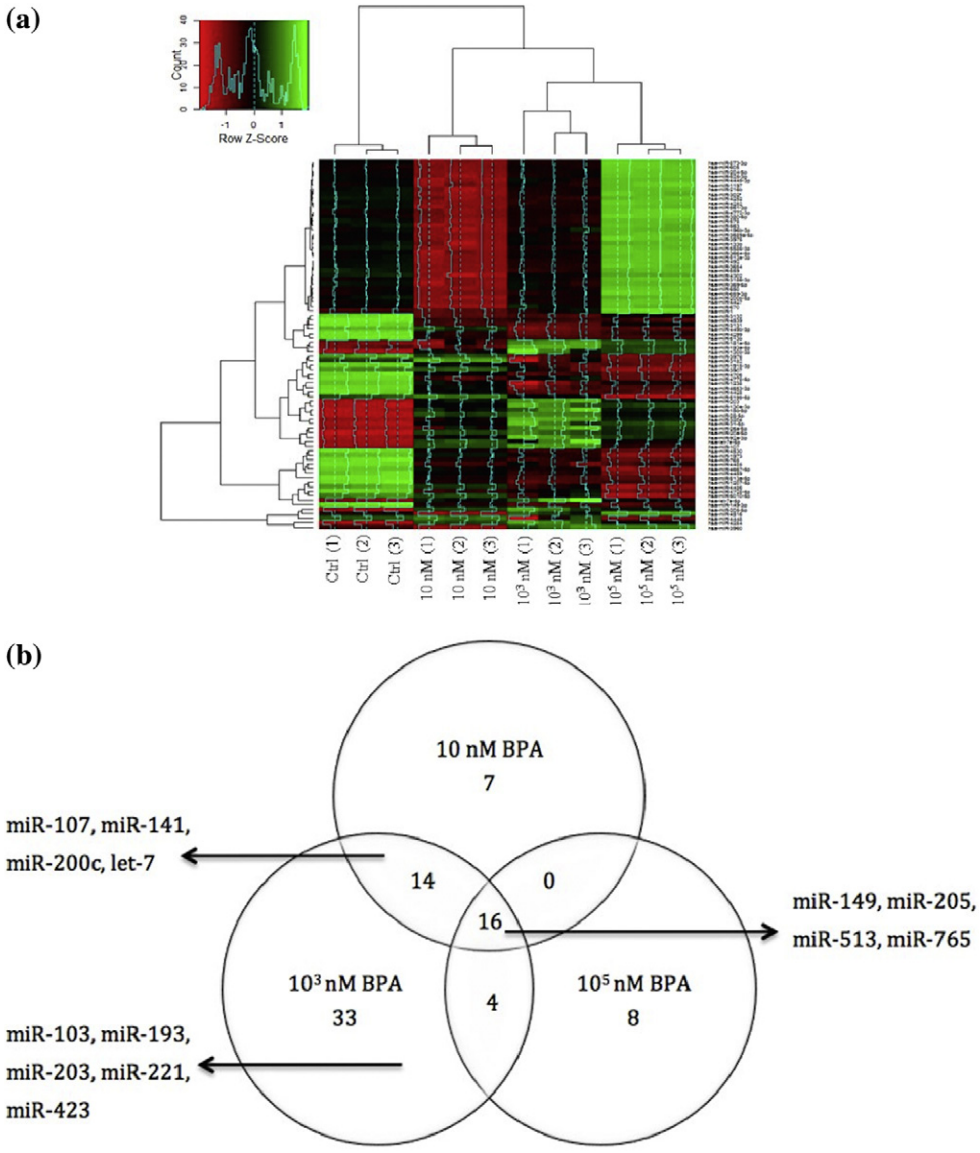

Fig. 1. Differentially expressed miRNAs in exposure to BPA exposure. (a) unsupervised hierarchical clustering of miRNA expression spotted on the microarray in analysis of RL95-2 cells treated with BPA (10, 10^3 and 10^5 nM). Columns represented triplicate samples, and rows were fold changes of intensity of miRNA expression labeled by colors (log2 transformed). (b) venn diagram showed 82 DEMs of RL95-2 cells between treatments with10, 10^3 and 10^5 BPA exposure.

Table 2
Genes and genomes pathway analysis of miRAs target genes.

miRNA	Pathway[a]	Gene number	Genes[b]	p-Value[c]
miR107, miR149, miR200c, miR203, miR205, miR765	Cancer pathway	114	TP53, JUN, LAMB4, CCCDC6, PRKCA, STAT1, SUFU, DVL1, GLI3, MAPK1, CRK, LAMC1, MAPK9, CXCL8	1.0E-6
miR103a, miR107, miR205	MAPK signaling pathway	96	DUSP1, JUN, MAPK9, MAP3K1, MAP3K2, NF1, PKC-alpha, RPS6KA3, STMN1, CRKL	1.6E-4
miR103A, miR107, miR203, miR205	Focal adhesion	69	COL1A1, ITGAV, JUN, LAMB4, LAMC1, MAPK9, PDGFD, PKCα, CRKL, VAV3, VCL	9.4E-3
miR107, miR203, miR765	Hedgehog pathway	41	BTRC, CSNK1A1L, SUFU, SHH, SFRP1, WNT5A, GLI3, GLI2, GSK3B, PRKACA	2.7E-3
miR149, mIR141, mir205	Cell cycle	68	TP53, SMAD2, CCNE2, MYC, CDC14B, SMC1A, CDC14A, GSK3B, TGFB2, DBF4, PCNA, MAPK9, E2F5, TGF2, c-jun	1.4E-3
Overlapping		21	TP53, GLI3, CCNE2, CRK, KIF23, SAMD2, CCDC6, FZD3, ARF6, MAPK9, SUFU, PRC1, MDM2, SMAD4, DVL1, EGLN1, JUN, MYC, LAMC1, PRKCA, STAT1	

[a] KEGG database and DAVID Bioinformatics Resources 6.7 were used for pathway enrichment analyses of genes regulated by identified miRNA.
[b] Genes with significant fold change >2.0 compared with control.
[c] Significant enriched pathways at p-value <0.05.

change) at a false discovery rate of >0.2 in RL95-2 cells after BPA exposure (10, 10^3 and 10^5 nM), and 34 miRNA differentially expressed common in all BPA exposure groups (Fig. 1B). Interestingly, 13 miRNAs (miR-107, -141, -200c, -103, -193, -203, -221, -423, -149, -205, -513, -765 and let-7; Table 1) among 82 DEMs of BPA exposure were related with the progression of EC according to the survey from literatures (Table S1). These DEMs had moderate-to-high fold changes (1.6–4.9 fold changes referred to control, q-value <0.01), contributing to the pathways of oncogenic signaling, proliferation, angiogenesis and invasion metastasis in EC progression.

3.2. Gene network of BPA exposure corresponding to EC carcinogenesis

A regulatory gene network of DEMs was constructed to investigate the target genes for BPA exposure corresponding to EC carcinogenesis. First, the miRNA target computational prediction website (miRDB, version 4.0) was used to predict potential target genes of DEMs. The 4842 potential target genes were yielded corresponding to the 13 DEMs. To consider the context of the DEMs-gene regulatory correlation between BPA exposure and EC progressions, 696 of these 4842 target genes were selected corresponding to the EC-related DEGs following BPA

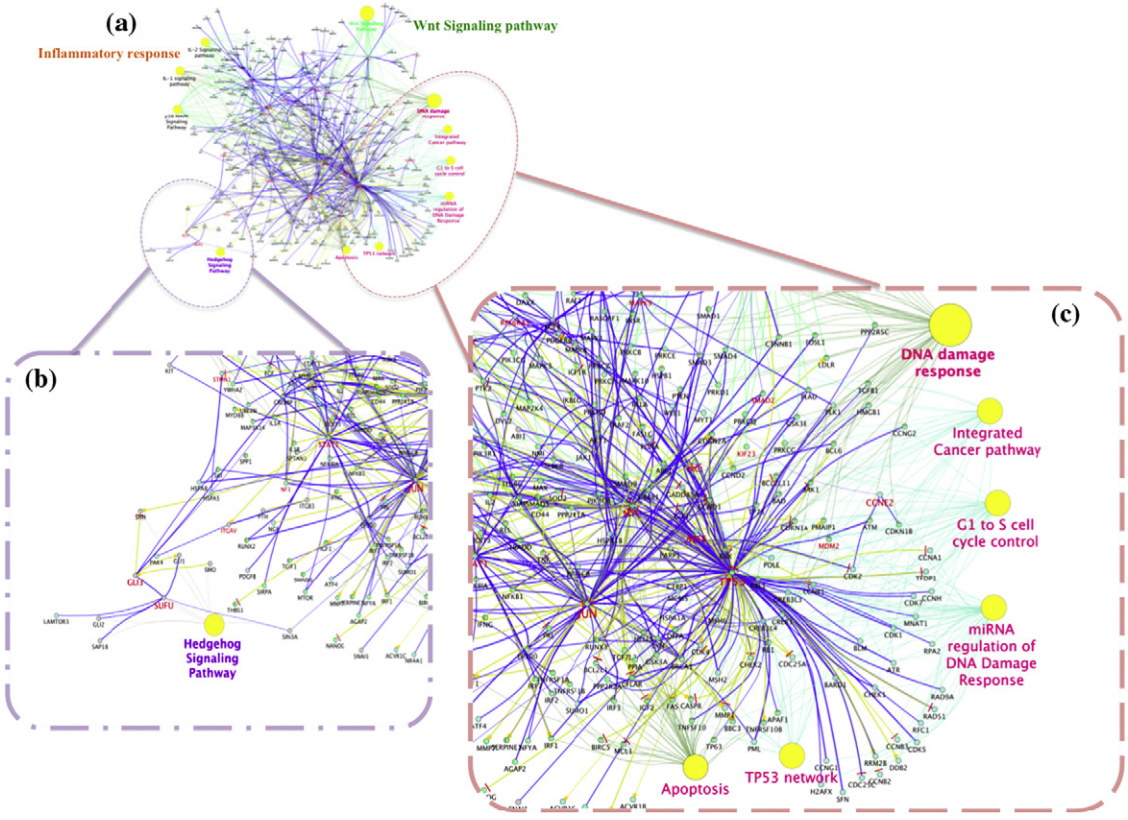

Fig. 2. Gene network analysis of differentially expressed genes mediated by miRNAs in response to BPA exposure. The gene network of 21 DEGs mediated by miRNAs in exposure to BPA illustrated by Cytoscape presented in (a) overview the biological function, and detailed view of (b) hedgehog signaling pathway, and (c) cancer pathway, cell cycle, DNA damage response, TP53 network and apoptosis. Yellow circle, blue line and yellow line respectively indicated function, gene-gene binding and gene-gene expression. Red labels were the genes chosen from KEGG pathway and black labels were genes co-expression or binding to the red labels.

exposure in our previous studies (Chou et al., 2014). This study performed the enrichment analysis in KEGG pathway, and identified five pathways relevant to the potential BPA-induced EC progressions. These pathways included (1) cancer pathway, (2) hedgehog pathway, (3) cell cycle, (4) adherens junction, and (5) MAPK signaling pathway. There were total 21 target DEGs (TP53, GLI3, CCNE2, CRK, KIF23, SAMD2, CCDC6, FZD3, ARF6, MAPK9, SUFU, PRC1, MDM2, SMAD4, DVL1, EGLN1, JUN, MYC, LAMC1, PRKACA, and STAT1) overlapped in these 5 pathways (Table 2).

We constructed the miRNAs-gene-pathway network to visualize the potential biological roles of miRNAs-regulated 21 DEGs by importing into Cytoscape software. In the network analysis, four major functions were classified into inflammatory response, WNT signaling, DNA damage response signaling, and hedgehog signaling, respectively (Fig. 2a). Among 21 DEGs, SUFU and GLI3 contributed to hedgehog pathway (Fig. 2b), and 9 DEGs (TP53, JUN, MYC, SMAD2, CCNE2, PRC1, KIF23, ARF6 and MDM2) contributed to DNA damage response performing in integrated cancer pathway, G1 to S cell cycle pathway, miRNA regulation of DNA damage response, TP53 network, and apoptosis (Fig. 2c). These pathways were enriched with the target genes of miR-149 and -107 (Table 2).

3.3. miRNA expression in exposure to BPA

The expression of the two annotated human miRNAs (miR-107 and -149) that were identified from the microarray analysis as differentially expressed were assayed in RL95-2 cells after exposure to BPA using quantitative real-time PCR. The expression of miR-107 (2.05, 2.53 and 1.43 fold changes; Fig. 3a) was up-regulated and miR-149 (0.63, 0.71 and 0.64 fold changes; Fig. 3b) was down-regulated with increasing dose of BPA exposure (10, $10^3$and 10^5 nM).

3.4. Target gene expression after transfection of miR-149 mimic in exposure to BPA

miR-149 was down-regulated in RL95-2 cells exposure to BPA accompanying with the decreased gene expressions of ARF6 (Fig. 4a) and TP53 (Fig. 4b), and increased expression of CCNE2 (Fig. 4c). After RL95-2 cells transfected with miR-149 mimic (30 nM), ARF6 expression was significantly increased in 10^5 nM BPA exposure (0.72 fold; Fig. 4d) as well as TP53 expression (0.98, 1.03 and 0.96 folds in 10, $10^3$and 10^5 nM BPA; Fig. 4e), and CCNE2 displayed no different changes (Fig. 4f). The cell viability was decreased in exposure to BPA (13.8%, 32.1% and 45.6% in 10, 10^3 and 10^5 nM BPA) (Fig. 4g).

3.5. Target gene expression after transfection of miR-107 inhibitor in exposure to BPA

After BPA exposure, gene expressions of SUFU (Fig. 5a) and GLI3 (Fig. 5b) were decreased in RL95-2 cells while miR-107 was significantly up-regulated (Fig. 3a). After transfection of miR-107 inhibitor (50 nM), the gene expressions of SUFU and GLI3 displayed the diminished changes (Fig. 5c) and no significant changes (Fig. 5d), respectively. Moreover, the proliferation of RL95-2 cells was decreased after transfection of miR-107 inhibitor in exposure to 10, 10^3 and 10^5 nM BPA (13.5%, 30.3% and 46.5%, respectively; Fig. 5e).

4. Discussion

Previous profiling studies have presented that miRNAs differently express in various types of cells with the treatment of BPA (Avissar-Whiting et al., 2010; Tilghman et al., 2012) and pregnant women (De Felice et al., 2015). Gene targets of dysregulated miRNAs in cancer development have been investigated (Chou et al., 2014; Jacobsen et al., 2013). This study found miR-149 and miR-107 were significantly differentially expressed in the potential BPA-induced EC development according to the combined analysis of high-throughput miRNA/mRNA microarray profiling of BPA exposure in RL95-2 cells with the literature review of miRNAs and their target genes corresponding to EC development. miR-149 and miR-107 were previously reported to be differentially expressed in human endometrial cancer and dysregulated in cancer pathways (Bischoff et al., 2014; Boren et al., 2008; Chung et al., 2009; Wu et al., 2009), which emphasized the importance of these miRNAs in EC progression. In this study, the gene network analysis of mRNA/miRNA microarray data of RL95-2 cells presented that BPA exposure potentially dysregulated two pathways corresponding to the literature survey of EC development: (1) miR-149 regulated ARF6, TP53 and CCNE2 for cell cycle regulation (Fig. 6); (2) miR-107 regulated SUFU and GLI3 in hedgehog signaling (Fig. 7). BPA exposure increased the gene expression of CCNE2 (Fig. 4c) and decreased SUFU, GLI3 (Fig. 5a–b), ARF6 and TP53 (Fig. 4a–b) resulting in cell proliferation (Fig. 4g and 5e) in RL95-2 cells. Furthermore, this study used miRNA mimic/inhibitor transfection to verify that BPA exposure did reduce miR-149 to down-regulate the gene expressions of ARF6 and TP53 and up-regulate CCNE2, and increase miR-107 to down-regulate SUFU and GLI3 expression for cell proliferation in RL95-2 cells (Fig. 6 and Fig. 7). These results suggested that BPA exposure would reduce miR-149 expression in ARF6-TP53-CCNE2 pathway to interrupt cell cycle arrest for aberrant cell proliferation, and increase miR-107 to attenuate hedgehog signaling SUFU-GLI3 pathway for endometrial cancer development and metastasis through decreasing expression of apoptotic genes and increasing cyclins.

This study found BPA exposure increase miR-107 to down-regulate SUFU and GLI3 expression for cell proliferation in RL95-2 cells. miR-

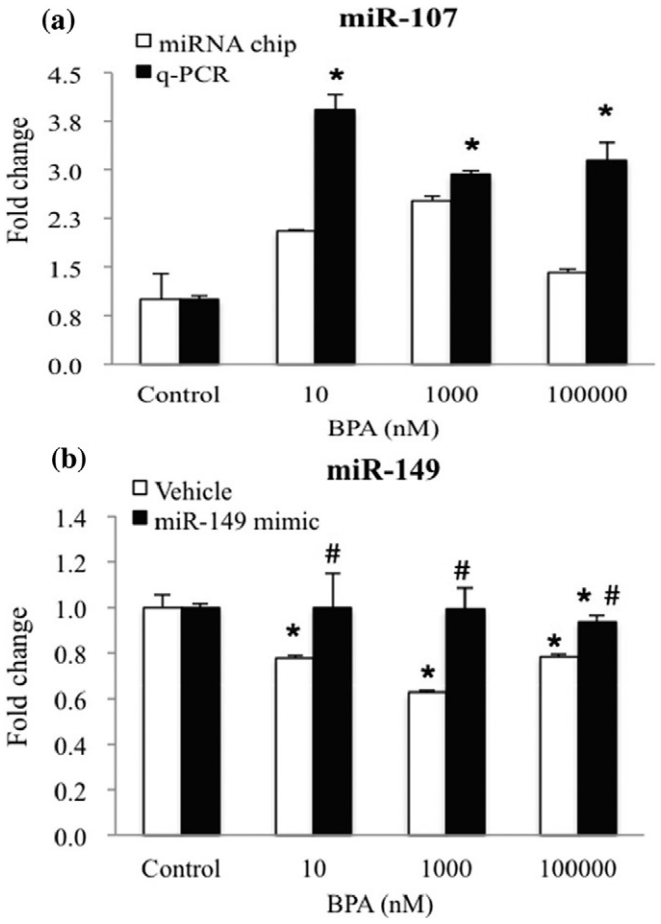

Fig. 3. miRNA expression in RL95-2 cells exposure to BPA. The expressions of (a) miR-107 and (b) miR-149 were determined using q-PCR. *$p < 0.05$ indicated significant difference between BPA exposure and control.

107 is involved in cell division, metabolism, stress response, and angiogenesis (Finnerty et al., 2010). Previous studies have demonstrated that miR-107 can be up-regulated in response to environmental exposure including estradiol (Cicatiello et al., 2010), and has both roles in regulation of proliferation and apoptosis in many types of cancers (Boren et al., 2008; Chen et al., 2011; Zhang et al., 2015). Two of down-regulated target genes of miR-107, SUFU and GLI3 are regulators in hedgehog signaling inactivation (Ruel and Thérond, 2009). SUFU is a repressor to GLI family (Szczepny et al., 2014), and GLI3 is known as a transcriptional repressor or have a positive transcriptional function (Taipale and Beachy, 2001). SUFU causes accumulation of GLI3 to form a complex with the reintroduced SUFU (Humke et al., 2010). Activation of the hedgehog pathway leads to decrease apoptotic genes to induce cellular proliferation and cause tumor growth (Athar et al., 2004). Thus, taken

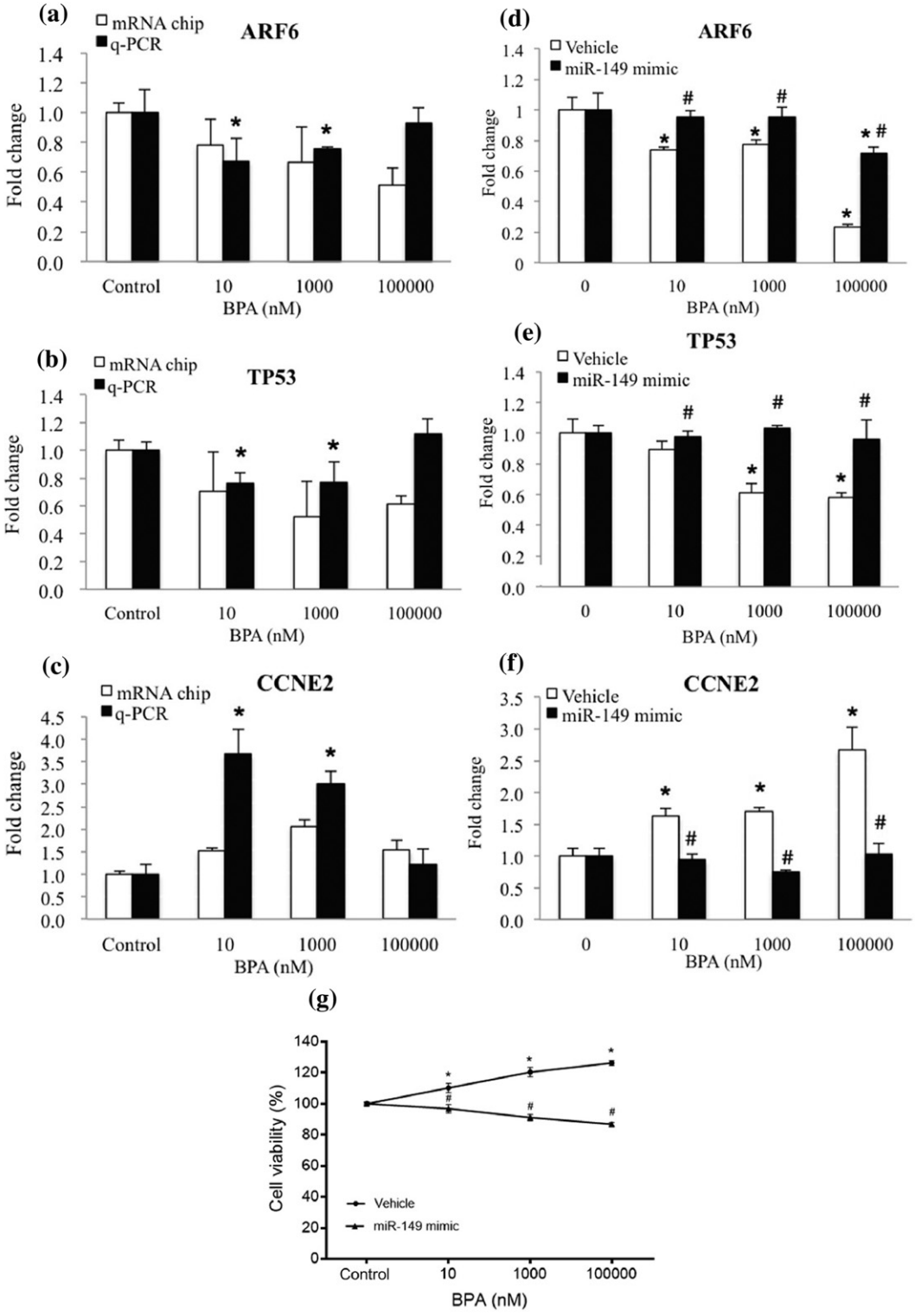

Fig. 4. Gene expression in RL95-2 cells transfected with miR-107 inhibitor in exposure to BPA. Fold change of (a) SUFU and (b) GLI3 after BPA exposure, as well as (c) SUFU (d) GLI3 in cells transfected with miR-107 inhibitor. (e) Cells viability of RL95-2 cells treated with BPA (10, 10^3 and 10^5 nM) for 48 h underlying transfection with miR-107 inhibitor. *$p < 0.05$ indicated significant difference between BPA exposure and control; #$p < 0.05$ indicated significant difference between transfection and vehicle.

together with our results, BPA exposure could induce miR-107 to attenuate SUFU and GLI3 expression for activating hedgehog signaling pathways, a hallmark of tumorigenesis in cancer development. Indeed, the activation of hedgehog signaling has identified to participate in gynecologic cancer development such as the ectopic tissue of endometriosis mice (Heard et al., 2014). Hedgehog signaling also can communicate with transforming growth factor β (TGFβ), fibroblast growth factor receptors (FGFR), Wnt and phosphoinositide-3-kinase-protein kinase B (PI3K-AKT) pathways in cancer process (Katoh and Nakagama, 2014). Thus, the results of this study suggested that BPA exposure might alter miRNA expression to activate the hedgehog signaling pathway for promoting cell proliferation and decreasing apoptosis in endometrial tumorigenesis.

This study discovered BPA exposure reduced miR-149 to down-regulate the gene expressions of ARF6 and TP53 and up-regulate CCNE2 in RL95-2 cells potentially underlying cell cycle interruption. MiR-149 is a tumor suppressor to control cancer cell migration and invasion, and considered as a regulator of ARF6-HDM2-TP53-CDKN1A pathway in gastric cancer (Wang et al., 2012). ARF6 serves as a DNA-binding protein to repair DNA damage specifically during the terminal stage of cytokinesis (Schweitzer and D'Souza-Schorey, 2005). TP53 regulates cell cycle as a tumor suppressor to prevent cancer progress (Strachan and Read, 1999). Human homolog of murine double minute oncogene 2 (HDM2) is an oncogene in TP53 suppression (Tong and Wu, 2014). ARF6-HDM2-TP53-CDKN1A pathway is important in cell cycle regulation controlled by zinc finger and BTB domain containing 2 (ZBTB2). ZBTB2 is a potent transcription repressor of cell cycle arrest, and can interrupt cell cycle arrest by inhibiting TP53 (Jeon et al., 2009). Therefore, BPA exposure might decrease miR-149 to attenuate ARF6 and TP53 expression for cell cycle arrest through ZBTB2 mediation, and the suppressed miR-149-ARF6-TP53-CCNE2 pathway in human endometrial cancer cells might promote tumor growth and cancer progression.

Overall, we found BPA exposure dysregulated miR-149 and miR-107 to affect the gene expression relevant to cell proliferation, cell cycle, repair system and hedgehog signaling underlying endometrial carcinogenesis. According to the findings of this study, the potential pathways of BPA-induced miRNA dysregulation were illustrated in Fig. 6 (miR-149-mediated cell cycle pathway) and Fig. 7 (miR-107-mediated hedgehog signaling). The general pathway of DNA damage as shown in Fig. 6a that miR-149 induced ARF6 and TP53 expression to further

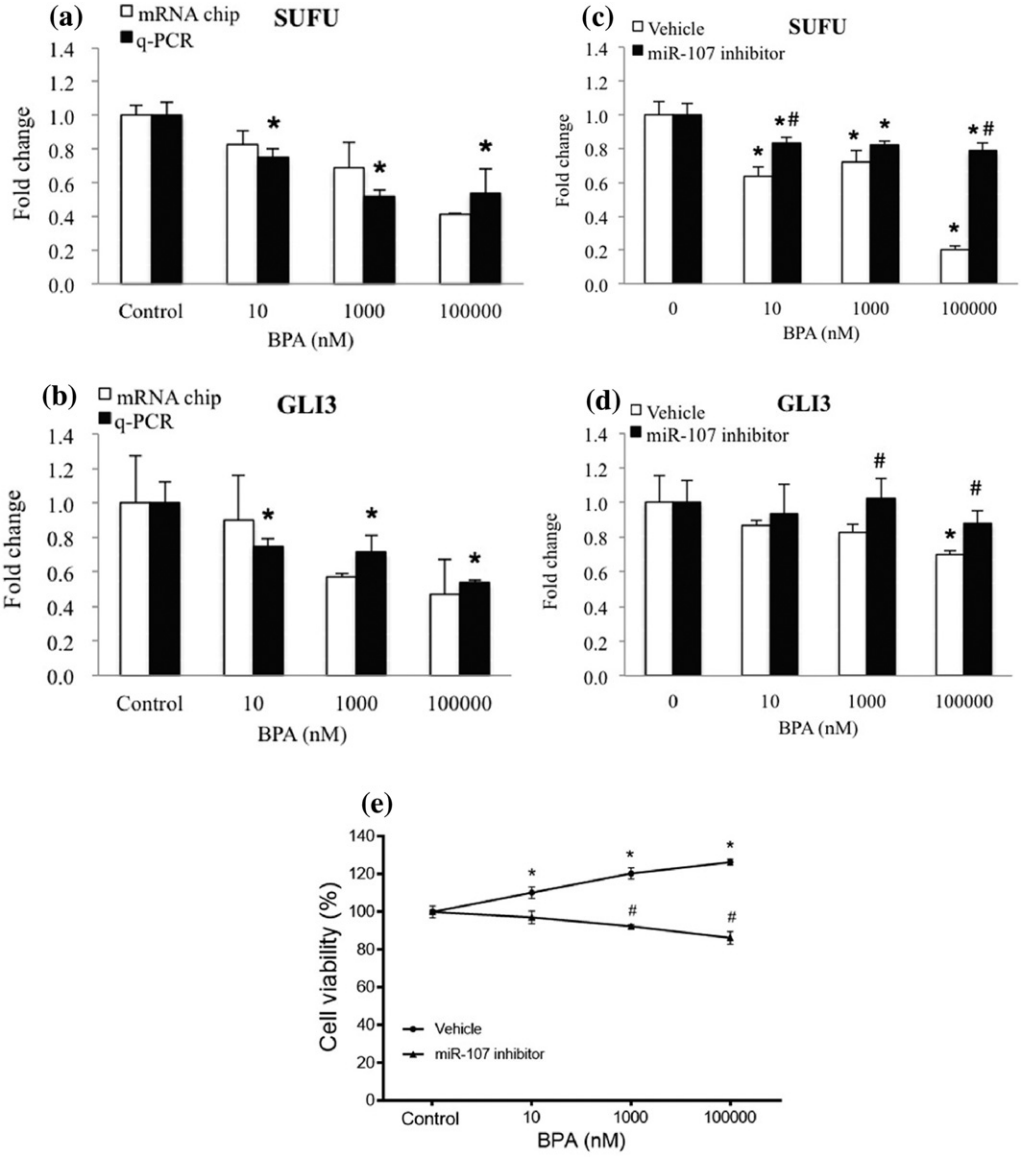

Fig. 5. Gene expression and cell viability of RL95-2 cells transfected with miR-149 mimic in exposure to BPA. Fold change of (a) ARF6, (b) TP53 and (c) CCNE2 between BPA exposure and control, as well as (d) ARF6, (e) TP53 and (f) CCNE2 in cells transfected with miR-149 mimic. (g) Cell viability of BPA exposure (10, $10^3$and 10^5 nM) for 48 h was determined with transfection of miR-149 mimic. *$p < 0.05$ indicated significant difference between BPA exposure and control; #$p < 0.05$ indicated significant difference between transfection and vehicle.

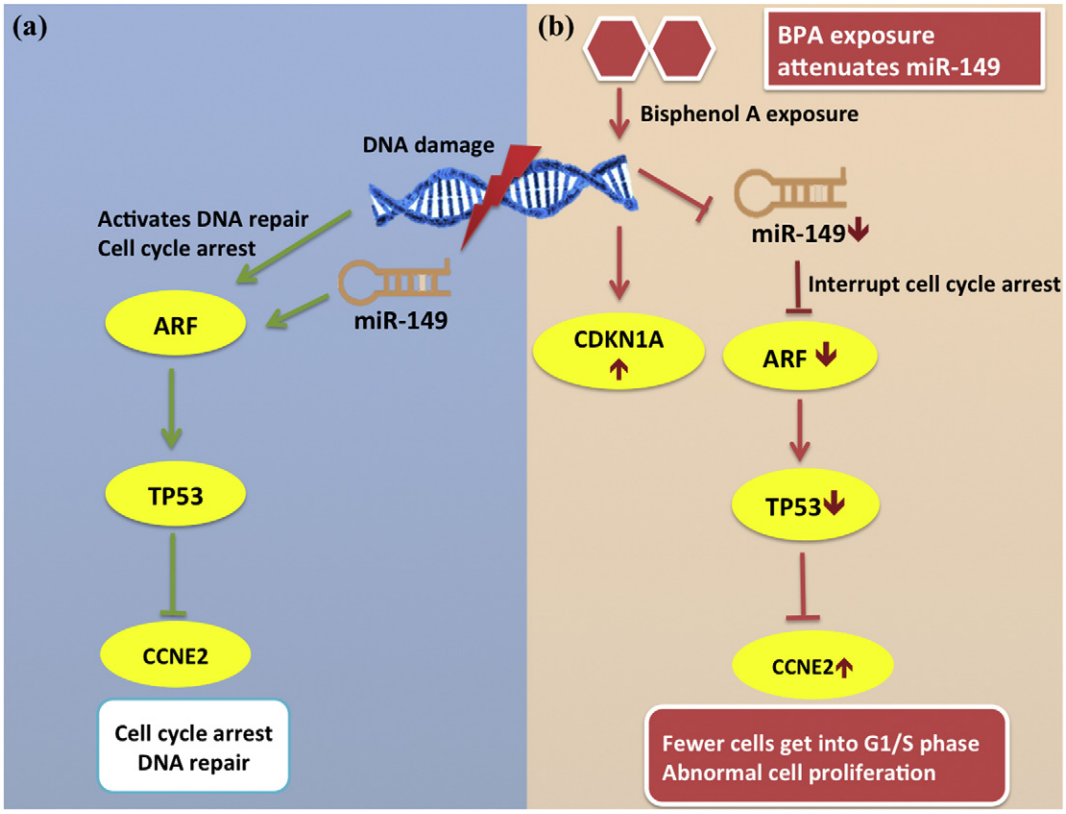

Fig. 6. Pathways of DNA damage response in regulation of BPA exposure. (a) a normal pathway in general situation, and (b) pathway dysregulated by BPA exposure. The figure displayed that miR-149 mediated ARF, TP53, CCNE2 and CDKN1A genes in cell cycle pathway. BPA decreased miR-149 to affect cell cycle arrest and interfere with DNA repair system. Green line was general direction of regulate path; red line was affected by BPA.

suppress CCNE2. ARF6, a tumor suppressor (Hemmati et al., 2002), can be induced by sustained mitogenic stimulation and activates tumor suppressor TP53 (Lohrum et al., 2003). TP53 plays a critical role in the maintenance of cell homeostasis through cell cycle arrest and apoptosis when cells are subjected to stressors such as hypoxia and DNA damage (Harris and Levine, 2005). TP53 is the upstream gene of CCNE2 and

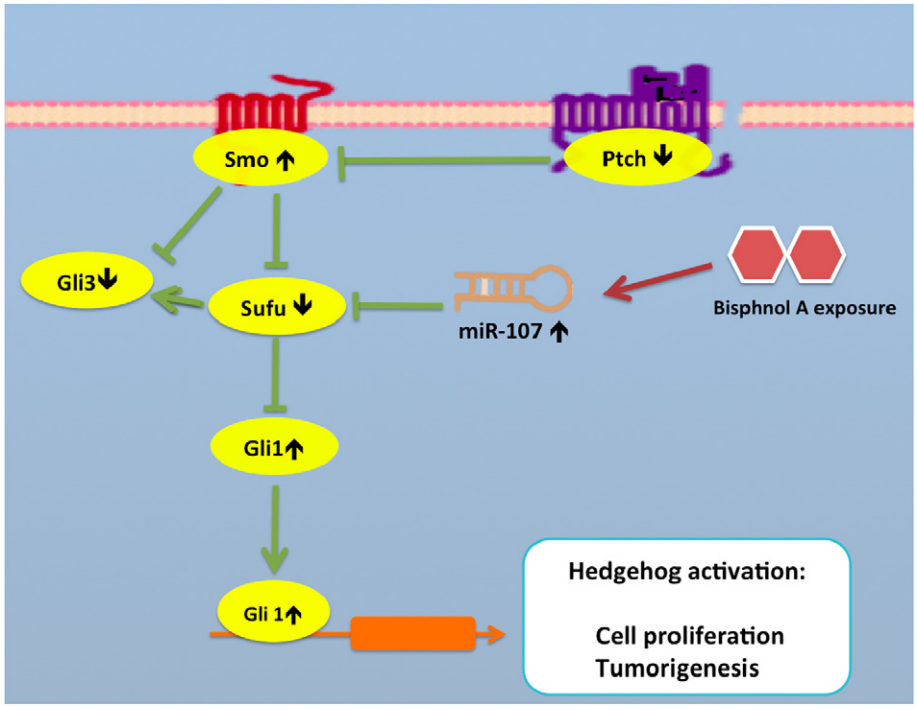

Fig. 7. Gene regulation of hedgehog signaling in response to BPA exposure. BPA exposure increased the expression of miR-107 to suppress SUFU and GLI3. The decreased SUFU and GLI3 could active hedgehog signaling to cause cell proliferation and tumorigenesis.

CDKN1A (Wang and Li 2013). CCNE2 and CDKN1A usually overexpress in cancers (Lu et al., 2013a; Lu et al., 2013b; Masamha and Benbrook, 2013). On the other hand, Fig. 6b displayed that BPA down-regulated miR-149 to interrupt cell cycle arrest by decreasing miR-149-mediated ARF6 and TP53 gene expression. BPA exposure also increased DNA damage related genes CCNE2 and CDKN1A expression to disturb cycle arrest and to result in abnormal cell proliferation. In Fig. 7, BPA exposure increased miR-107 to mediate SUFU and GLI3 expression decrease for activating hedgehog signaling. The activation of hedgehog signaling has been identified through increasing angiogenic factors, cyclins, and decreasing apoptotic genes to cause metastasis and tumor growth (Katoh and Nakagama, 2014), and can communicate with TGFβ, FGFR, Wnt and PI3K-AKT pathways for gynecologic cancer development (Heard et al., 2014). The above findings suggested BPA exposure might induce the progress of carcinogenesis and promote tumor growth in endometrial cells.

5. Conclusions

In summary, the results of this study revealed miRNAs may play important roles in tumorigenesis underlying BPA exposure. These miRNAs modulated unique gene targets but coordinately target a few common pathways, contributing on reprogramming critical pathways to facilitate tumor development. Our findings suggested that BPA would down-regulate miR-149 expression in ARF6-TP53-CCNE2 pathway to interrupt cell cycle arrest and initiate migration and invasion for cancer metastasis (described in the schema in Fig. 6). Meanwhile, BPA might up-regulate miR-107 to attenuate hedgehog signaling SUFU-GLI3 pathway, and disturb DNA repair function for cancer cell proliferation (described in the schema in Fig. 7). These findings provided the utility of miRNA expression profiles in response to BPA exposure, and the insight to the potential epigenetic mechanism of BPA exposure on endometrial carcinogenesis.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tiv.2017.02.012.

Finding sources

This work was supported by Ministry of Science and Technology of Taiwan 102-2918-I-007-011 and 104-2918-I-007-001.

Conflict of interest

The authors declared that they have no conflicts of interest to this work.

Abbreviations

AKT	protein kinase B
ARF6	ADP-ribosylation factor 6
CCDC6	coiled-coil domain containing 6
CCNE2	cyclin E2
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21Cip1)
CRK	v-crk sarcoma virus CT10 oncogene homolog
DAVID	database for annotation visualization and integrates discovery
DVL1	dishevelled gene
ER	estrogen receptor
FGFR	fibroblast growth factor receptors
FZD3	frizzled class receptor 3
GLI1	glioma-associated oncogene homolog 1
GLI3	GLI family zinc finger 3
HDM2	human homolog of murine double minute oncogene 2
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIF23	kinesin family member 23
MAPK9	mitogen-activated protein kinase 9
MDM2	mouse double minute 2 homolog
PRC1	polycomb repressor complex1
Ptch	patched tumor suppressor
Shh	sonic hedgehog
SMAD2	SMAD family member 2
SMAD4	SMAD family member 4
Smo	smoothened
SUFU	suppressor of fused homolog
TP53	tumor protein p53
VEGF	vascular endothelial growth factor
EGLN1	Egl nine homolog 1
JUN	jun proto-oncogene
MYC	v-myc avian myelocytomatosis viral oncogene homolog
LAMC1	laminin, gamma 1 (formerly LAMB2)
PRKCA	protein kinase C alpha
STAT1	signal transducer and activator of transcription 1

Transparency document

The Transparency document associated to this article can be found in the online version.

Acknowledgment

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Ali, A.T., 2014. Reproductive factors and the risk of endometrial cancer. Int. J. Gynecol. Cancer 24, 384–393.
Athar, M., Li, C., Tang, X., Chi, S., Zhang, X., Kim, A.L., et al., 2004. Inhibition of smoothened signaling prevents ultraviolet B-induced basal cell carcinomas through regulation of Fas expression and apoptosis. Cancer Res. 64, 7545–7552.
Avissar-Whiting, M., Veiga, K.R., Uhl, K.M., Maccani, M.A., Gagne, L.A., Moen, E.L., et al., 2010. Bisphenol A exposure leads to specific microRNA alterations in placental cells. Reprod. Toxicol. 29, 401–406.
Banno, K., Kisu, I., Yanokura, M., Masuda, K., Kobayashi, Y., Ueki, A., et al., 2012. Endometrial cancer and hypermethylation: regulation of DNA and microRNA by epigenetics. Biochem. Res. Int. 2012, 738274.
Berezikov, E., van Tetering, G., Verheul, M., van de Belt, J., van Laake, L., Vos, J., et al., 2016. Many novel mammalian microRNA candidates identified by extensive cloning and RAKE analysis. Genome Res. 16 (10), 1289–1298.
Bischoff, A., Huck, B., Keller, B., Strotbek, M., Schmid, S., Boerries, M., et al., 2014. miR149 functions as a tumor suppressor by controlling breast epithelial cell migration and invasion. Cancer Res. 74, 5256–5265.
Boren, T., Xiong, Y., Hakam, A., Wenham, R., Apte, S., Wei, Z., et al., 2008. MicroRNAs and their target messenger RNAs associated with endometrial carcinogenesis. Gynecol. Oncol. 110, 206–215.
Braza-Boils, A., Mari-Alexandre, J., Gilabert, J., Sanchez-Izquierdo, D., Espana, F., Estelles, A., et al., 2014. MicroRNA expression profile in endometriosis: its relation to angiogenesis and fibrinolytic factors. Hum. Reprod. 29, 978–988.
Calafat, A.M., Ye, X., Wong, L.Y., Reidy, J.A., Needham, L.L., 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ. Health Perspect. 116, 39–44.
Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., et al., 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. 99, 15524–15529.
Carwile, J.L., Luu, H.T., Bassett, L.S., Driscoll, D.A., Yuan, C., Chang, J.Y., et al., 2009. Polycarbonate bottle use and urinary bisphenol A concentrations. Environ. Health Perspect. 117, 1368–1372.
Chen, P.C., Su, J.L., Cha, S.T., Tan, W.Y., Wang, M.Y., Hsu, H.C., et al., 2011. miR-107 promotes tumor progression by targeting the let-7 microRNA in mice and humans. J. Clin. Invest. 121 (9), 3442–3455.
Chou, W.C., Cheng, A.L., Brotto, M., Chuang, C.Y., 2014. Visual gene-network analysis reveals the cancer gene co-expression in human endometrial cancer. BMC Genomics 15 (1), 300.
Chung, T.K., Cheung, T.H., Huen, N.Y., Wong, K.W., Lo, K.W., Yim, S.F., et al., 2009. Dysregulated microRNAs and their predicted targets associated with endometrioid endometrial adenocarcinoma in Hong Kong women. Int. J. Cancer 124, 1358–1365.
Cicatiello, L., Mutarelli, M., Grober, O.M., Parios, O., Ferraro, L., Ravo, M., et al., 2010. Estrogen receptor α controls a Gene network in luminal-like breast cancer cells comprising multiple transcription factors and microRNAs. Am. J. Pathol. 176 (5), 2113–2130.
De Felice, B., Manfellotto, F., Palumbo, A., Troisi, J., Zullo, F., Di Carlo, C., et al., 2015. Genome–wide microRNA expression profiling in placentas from pregnant women exposed to BPA. BMC Med. Genet. 8, 56.

Fassina, A., Marino, F., Siri, M., Zambello, R., Ventura, L., Fassan, M., et al., 2012. The miR-17-92 microRNA cluster: a novel diagnostic tool in large B-cell malignancies. Lab. Investig. 92, 1574–1582.
Finnerty, J.R., Wang, W.X., Hebert, S.S., Wilfred, B.R., Mao, G., Nelson, P.T., 2010. The miR-15/107 group of MicroRNA genes: evolutionary biology, cellular functions, and roles in human diseases. J. Mol. Biol. 402, 491–509.
Harris, S.L., Levine, A.J., 2005. The p53 pathway: positive and negative feedback loops. Oncogene 24, 2899–2908.
Hayashita, Y., Osada, H., Tatematsu, Y., Yamada, H., Yanagisawa, K., Tomida, S., et al., 2005. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 65, 9628–9632.
Heard, M.E., Simmons, C.D., Simmen, F.A., Simmen, R.C., 2014. Kruppel-like factor 9 deficiency in uterine endometrial cells promotes ectopic lesion establishment associated with activated notch and hedgehog signaling in a mouse model of endometriosis. Endocrinology 155, 1532–1546.
Helmestam, M., Davey, E., Stavreus-Evers, A., Olovsson, M., 2014. Bisphenol A affects human endometrial endothelial cell angiogenic activity in vitro. Reprod. Toxicol. 46, 69–76.
Hemmati, P.G., Gillissen, B., Von, H.C., 2002. Adenovirus-mediated overexpression of p14(ARF) induces p53 and Bax-independent apoptosis. Oncogene 21, 3149–3161.
Hiroi, H., Tsutsumi, O., Takeuchi, T., Momoeda, M., Ikezuki, Y., Okamura, A., et al., 2004. Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocr. J. 51, 595–600.
Horcajadas, J.A., Pellicer, A., Simon, C., 2007. Wide genomic analysis of human endometrial receptivity: new times, new opportunities. Hum. Reprod. Update 13, 77–86.
Huang, Y.W., Kuo, C.T., Chen, J.H., Goodfellow, P.J., Huang, T.H., Rader, J.S., Uyar, D.S., 2014. Hypermethylation of miR-203 in endometrial carcinomas. Gynecol. Oncol. 133, 340–345.
Humke, E.W., Dorn, K.V., Milenkovic, L., Scott, M.P., Rohatqi, R., 2010. The output of Hedgehog signaling is controlled by the dynamic association between Suppressor of Fused and the Gli proteins. Genes Dev. 24 (7), 670–682.
Jacobsen, A., Silber, J., Harinath, G., Huse, J.T., Schultz, N., Sander, C., 2013. Analysis of microRNA-target interactions across diverse cancer types. Nat. Struct. Mol. Biol. 20 (11), 1325–1332.
Jeon, B.N., Choi, W.I., Yu, M.Y., Yoon, A.R., Kim, M.H., Yun, C.O., Hur, M.W., 2009. ZBTB2, a novel master regulator of the p53 pathway. J. Biol. Chem. 284, 17935–17946.
Kandaraki, E., Chatzigeorgiou, A., Livadas, S., Palioura, E., Economou, F., Koutsilieris, M., et al., 2011. Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. J. Clin. Endocrinol. Metab. 96, E480–E484.
Katoh, M., Nakagama, H., 2014. FGF receptors: cancer biology and therapeutics. Med. Res. Rev. 34, 280–300.
Kong, X., Xu, X., Yan, Y., Guo, F., Li, J., Hu, Y., et al., 2014. Estrogen regulates the tumour suppressor miRNA-30c and its target gene, MTA-1, in endometrial cancer. PLoS One 9, e90810.
Konno, Y., Dong, P., Xiong, Y., Suzuki, F., Lu, J., Cai, M., et al., 2014. MicroRNA-101 targets EZH2, MCL-1 and FOS to suppress proliferation, invasion and stem cell-like phenotype of aggressive endometrial cancer cells. Oncotarget 5, 6049–6062.
Leao, R.B., Andrade, L., Vassalo, J., Antunes Jr., A., Pinto-Neto, A., Costa-Paiva, L., 2013. Differences in estrogen and progesterone receptor expression in endometrial polyps and atrophic endometrium of postmenopausal women with and without exposure to tamoxifen. Mol. Clin. Oncol. 1, 1055–1060.
Lee, H., Choi, H.J., Kang, C.S., Lee, H.J., Lee, W.S., Park, C.S., 2012. Expression of miRNAs and PTEN in endometrial specimens ranging from histologically normal to hyperplasia and endometrial adenocarcinoma. Mod. Pathol. 25, 1508–1515.
Li, X., Xie, W., Xie, C., Huang, C., Zhu, J., Liang, Z., et al., 2014. Curcumin modulates miR-19/PTEN/AKT/p53 axis to suppress bisphenol A-induced MCF-7 breast cancer cell proliferation. Phytother. Res. 10, 1553–1560.
Li, Q., Qiu, X.M., Li, Q.H., Wang, X.Y., Li, L., Xu, M., et al., 2015a. MicroRNA-424 may function as a tumor suppressor in endometrial carcinoma cells by targeting E2F7. Oncol. Rep. 33 (5), 2354–2360.
Li, L., Wang, Q., Zhang, Y., Niu, Y., Yao, X., Liu, H., 2015b. The molecular mechanism of bisphenol A (BPA) as an endocrine disruptor by interacting with nuclear receptors: insights from molecular dynamics (MD) simulations. PLoS One 10 (3), e0120330.
Lohrum, M.A., Ludwig, R.L., Kubbutat, M.H., Hanlon, M., Vousden, K.H., 2003. Regulation of HDM2 activity by the ribosomal protein L11. Cancer Cell 3, 577–587.
Lu, W., Qu, J.J., Li, B.L., Lu, C., Yan, Q., Wu, X.M., Chen, X.Y., Wan, X.P., 2013a. Overexpression of p21-activated kinase 1 promotes endometrial cancer progression. Oncol. Rep. 29 (4), 1547–1555.
Lu, W., Xia, Y.H., Qu, J.J., He, Y.Y., Li, B.L., Lu, C., Luo, X., Wan, X.P., 2013b. p21-activated kinase 4 regulation of endometrial cancer cell migration and invasion involves the ERK1/2 pathway mediated MMP-2 secretion. Neoplasma 60 (5), 493–503.
Masamha, C.P., Benbrook, D.M., 2013. Cyclin D1 degradation is sufficient to induce G1 cell cycle arrest despite constitutive expression of cyclin E2 in ovarian cancer cells. Cancer Res. 69 (16), 6565–6572.
Michael, M.Z., O'Connor, S.M., van Holst Pellekaan, N.G., Young, G.P., James, R.J., 2003. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol. Cancer Res. 1, 882–891.
Mozos, A., Catasús, L., D'Angelo, E., Serrano, E., Espinosa, I., Ferrer, I., Pons, C., Prat, J., 2014. The FOXO1-miR27 tandem regulates myometrial invasion in endometrioid endometrial adenocarcinoma. Hum. Pathol. 45 (942–951), 2014.
Nicolaije, K.A., Ezendam, N.P., Vos, M.C., Boll, D., Pijnenborg, J.M., Kruitwagen, R.F., et al., 2013. Follow-up practice in endometrial cancer and the association with patient and hospital characteristics: a study from the population-based PROFILES registry. Gynecol. Oncol. 129, 324–331.
O'Connell, R.M., Taganov, K.D., Boldin, M.P., Cheng, G., Baltimore, D., 2007. MicroRNA-155 is induced during the macrophage inflammatory response. Proc. Natl. Acad. Sci. U. S. A. 104, 1604–1609.
Oldenburg, C.S., Boll, D., Nicolaije, K.A., Vos, M.C., Pijnenborg, J.M., Coebergh, J.W., et al., 2013. The relationship of body mass index with quality of life among endometrial cancer survivors: a study from the population-based PROFILES registry. Gynecol. Oncol. 129, 216–221.
Pan, Q., Chegini, N., 2008. MicroRNA signature and regulatory functions in the endometrium during normal and disease states. Semin. Reprod. Med. 26, 479–493.
Pan, Q., Luo, X., Toloubeydokhti, T., Chegini, N., 2007. The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. Mol. Hum. Reprod. 132, 797–806.
Ruel, L., Thérond, P.P., 2009. Variations in Hedgehog signaling: divergence and perpetuation in Sufu regulation of Gli. Genes Dev. 23 (16), 1843–1848.
Schweitzer, J.K., D'Souza-Schorey, C., 2005. A requirement for ARF6 during the completion of cytokinesis. Exp. Cell Res. 311, 74–83.
Sharma, S., Kelly, T.K., Jones, P.A., 2010. Epigenetics in cancer. Carcinogenesis 31, 27–36.
Snowdon, J., Zhang, X., Childs, T., Feilotter, H., 2011. The microRNA-200 family is upregulated in endometrial carcinoma. PLoS One 6, e22828.
Strachan, T., Read, A.P., 1999. Human Molecular Genetics. Wiley-Liss Bios Scientific Publishers, an imprint of Taylor & Francis Group, New York.
Suarez, Y., Fernandez-Hernando, C., Pober, J.S., Sessa, W.C., 2007. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ. Res. 100, 1164–1173.
Szczepny, A., Wagstaff, K.M., Dias, M., Gajewska, K., Wang, C., Davies, R.G., Kaur, G., Ly-Huynh, J., Loveland, K.L., Jans, D.A., 2014. Overlapping binding sites for importin beta1 and Suppressor of Fused on Glioma-associated oncogene homolog 1 (Gli1) regulate its nuclear localisation. Biochem. J. 461 (3), 469–476.
Taipale, J., Beachy, P.A., 2001. The Hedgehog and Wnt signalling pathways in cancer. Nature 17, 349–354.
Tilghman, S.L., Bratton, M.R., Segar, H.C., Martin, E.C., Rhodes, L.V., Li, M., McLachlan, J.A., et al., 2012. Endocrine disruptor regulation of microRNA expression in breast carcinoma cells. PLoS One 7 (3), e32754.
Tong, L., Wu, S., 2014. ROS and p53 in regulation of UVB-induced HDM2 alternative splicing. Photochem. Photobiol. 91 (1), 221–224.
Wang, S., Li, W., 2013. Molecular imaging of p53 signal pathway in lung cancer cell cycle arrest induced by cisplatin. Mol. Carcinog. 52 (11), 900–907.
Wang, Y., Zheng, X., Zhang, Z., Zhou, J., Zhao, G., Yang, J., et al., 2012. MicroRNA-149 inhibits proliferation and cell cycle progression through the targeting of ZBTB2 in human gastric cancer. PLoS One 7, e41693.
Wang, Y., Adila, S., Zhang, X., Dong, Y., Li, W., Zhou, M., et al., 2014. MicroRNA expression signature profile and its clinical significance in endometrioid carcinoma. Zhonghua Bing Li Xue Za Zhi 43, 88–94.
Wu, W., Lin, Z., Zhuang, Z., Liang, X., 2009. Expression profile of mammalian microRNAs in endometrioid adenocarcinoma. Eur. J. Cancer Prev. 18, 50–55.
Ye, W., Xue, J., Zhang, Q., Li, F., Zhang, W., Chen, H., et al., 2014. MiR-449a functions as a tumor suppressor in endometrial cancer by targeting CDC25A. Oncol. Rep. 32, 1193–1199.
Zhang, G., Hou, X., Li, Y., Zhao, M., 2014. MiR-205 inhibits cell apoptosis by targeting phosphatase and tensin homolog deleted on chromosome ten in endometrial cancer Ishikawa cells. BMC Cancer 14, 440.
Zhang, J.J., Zhang, J.J., Wang, C.Y., Hua, L., Yao, K.H., Chen, J.T., et al., 2015. miR-107 promotes hepatocellular carcinoma cell proliferation by targeting Axin2. Int. J. Clin. Exp. Pathol. 8 (5), 5168–5174.