Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2011, 23(4) 664–670
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Differential response of multiple zebrafish hepatic F-box protein genes to 17α-ethinylestradiol treatment

Hongping Zheng**, Shifeng Li**, Zhili Wu, Yunbin Zhang, Shengnan Hu, Yuanchang Yan, Yiping Li*

Shanghai Key Laboratory for Molecular Andrology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China.
E-mail: gt_19992000@yahoo.com.cn; happylishifeng@163.com

Received 28 October 2010; revised 21 December 2010; accepted 31 January 2011

Abstract

Estrogens are accumulating in environment and their effects on a variety of reproductive processes and tumorigenesis were reported by previous study, but the mechanism of estrogen promoting neoplasia was still not clear. F-box protein (FBP) is the component of E3 ubiquitin ligase which takes part in a variety of key biological processes. In this study, using mature male zebrafish, which are more sensitive to estrogen treatment, we examined influence of 17α-ethinylestradiol (EE2) exposure on the expression of a series of hepatic FBP genes, which take part in a variety of biological processes, including tumorigenesis. The influence of EE2 on the expression of hepatic mRNA concentrations of FBP genes were quantified based on the expression of the optimal internal control gene in male zebrafish after 7-day exposure to EE2, from a low-dose concentration (1 ng/L) to environmentally relevant concentrations (10, 100 ng/L). Our results showed that EE2 exposure reduced the expression of *fbxl14a, fbxl14b, fbxo25* and β-*TRCP2b*, but enchanced the expression of *skp2*. While the alterations in *fbxl2, fbxw7*, *fbxo9,* β-*TRCP2a, fbxl18* and *fbxo45* mRNA levels were not observed after EE2 exposure. Thus, our results showed that the expression of hepatic FBP genes exhibited differentially in male zebrafish exposed EE2. The changes of the expression level of FBP genes induced by EE2 may be an important clue to elucidate the correlations of estrogen and hepatic tumors.

Key words: estrogen; 17α-ethinylestradiol; F-box; hepatic tumor; zebrafish

DOI: 10.1016/S1001-0742(10)60578-5

Citation: Zheng H P, Li S F, Wu Z L, Zhang Y B, Hu S N, Yan Y C et al., 2011. Differential response of multiple zebrafish hepatic F-box protein genes to 17α-ethinylestradiol treatment. Journal of Environmental Sciences, 23(4): 664–670

Introduction

Endocrine disruptors (EDs) are a class of chemicals that can interfere with normal endocrine systems in vertebrates, including wildlife and humans (Colborn et al., 1993; Sonnenschein and Soto, 1998; Crews et al., 2000; Hayes et al., 2002). Among the various so-called EDs, those with estrogenic activity have attracted most research attention. One of the most potent of these xenoestrogens, which is widely used as the active ingredient in birth control pills and hormone replacement therapy, is 17α-ethinylestradiol (EE2) (Gutendorf and Westendorf, 2001). Its concentrations detected in effluents of some sewage treatment plants in different countries range up to 42 ng/L (Yin et al., 2002). A survey in the USA during 1999–2000 revealed a median EE2 concentration of 73 ng/L in more than 100 streams across 30 states (Kolpin et al., 2002). This concentration is high enough for EE2 as well as other estrogen mimics to introduce biological effects in fish, as estrogens can elicit response in aquatic organisms at concentrations as low as ng/L (Yin et al., 2002; Segner, 2009). Moreover, EE2 is more stable in environment than natural steroids such as estrogens (E1) and 17β-estradiol (E2) (Yin et al., 2002).

In comparison to the wealth of available literature on estrogens as EDs, little is known about their roles in other processes, such as cell cycle progression and tumorigenesis. Both natural and synthetic estrogens can produce adverse effects on supraphysiological or pharmacological concentrations, and these adverse effects include immunotoxicty, teratogenicity and carcinogenicity (Roy and Cai, 2002). It has been shown that concentrations of estrogen ranging from 0.1–1.0 ng/L to 0.01–100 μg/L could promote the growth of breast, pituitary, endometrial, and testicular Leydig cells, and in contrast, high concentrations ranging from 1 to 100 mg/L result in an inhibition of cell growth (DuMond et al., 2001; Roy and Cai, 2002; Welshons et al., 2003). In addition, estrogens have been shown to induce a variety of tumors in many species, such

* Corresponding author. E-mail: yipingli@sibs.ac.cn
** The authors contributed equally to this work.

as mammary tumors in mice and rats, ovarian tumors in dogs and kidney tumors in hamsters (Roy et al., 2007). In fish, estrogens are known to promote mutagen-induced hepatic neoplasia (Tilton et al., 2006), and estrogen exposure can decrease the transcription levels of nucleotide excision repair (NER) genes (Notch et al., 2007), which have been shown to be an important epidemiological marker for increased cancer risk.

The timely generation and degradation of proteins especially of cell cycle regulators are crucial for irreversible and error-free cell cycle progression, as well as other fundamental pathophysiology processes in eukaryotes. F-box proteins (FBPs) are a family of specificity factors and members of the FBP family are characterized by an approximately 40-amino-acid F-box domain on N-terminal (Cardozo and Pagano, 2004; Nakayama and Nakayama, 2006). There are more than 70 FBPs in mammals and most of them function through Skp1/cullin/FBP (SCF) ubiquitin E3 ligase complexes, which is formed by Skp1, cullin, and FBPs (Cardozo and Pagano, 2004; Nakayama and Nakayama, 2005; Ho et al., 2008). It has been demonstrated that FBPs take part in a variety of biological processes, most notable of which is their role in cell cycle control, signal transduction and carcinogenesis (Cardozo and Pagano, 2004; Nakayama and Nakayama, 2005; Nakayama and Nakayama, 2006). FBPs are also found to be evolutionarily conserved in various species (Ho et al., 2008).

Both estrogens and FBPs are involved in the common key processes, and thus we asked whether FBPs are related to the effects of estrogens, which has not been reported. Zebrafish (*Danio rerio*) has been suggested as an experimental model to study endocrine-disrupting chemicals, and also as a cancer model system (Amatruda et al., 2002; Stern and Zon, 2003; Lam et al., 2006; Segner, 2009). In this study, using mature male zebrafish, which are more sensitive to estrogen treatment, we examined influence of EE2 treatment on the expression of a series of hepatic FBP genes. The mRNA concentrations of these genes were quantified.

1 Materials and methods

1.1 Adult zebrafish exposures

One-year-old male AB strain zebrafish, raised in our facilities, were maintained in aquaria with recirculating dechlorinated municipal water at a constant temperature of 27–29°C, a 14 hr:10 hr light-dark cycle, and standard diet. For EE2 treatment experiments, 8 male fish per experimental group were placed in separate 3 L tanks and exposed to a low-dose EE2 concentration of 1 ng/L and environmentally relevant concentration of 10 and 100 ng/L (Kolpin et al., 2002; Yin et al., 2002; Welshous et al., 2003) in 0.05% EtOH. Control fish received 0.05% EtOH only. Experimental and control fish were exposed for 7 days in static water with complete renewal every 24 hr and fish received a renewed dose of either EE2/EtOH or EtOH in clean water.

1.2 RNA treatment and reverse transcription

After 7 days, zebrafish were anaesthetized by a brief immersion in ice water, sacrificed immediately, sex verified, and liver dissected and rapidly frozen at –70°C. Total RNA of each liver was extracted separately using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's protocol. Total RNA was treated with 10 U of RNase free DNase I (TaKaRa, Japan) according to the manufacture's protocol. The integrity of RNA after DNase treatment was analyzed by 1% agarose gel electrophoresis. The total RNA was examined using Biophotometer (Eppendorf, Germany). RNA with OD 260/280 ration between 1.8 and 2.0 was considered satisfactory and was used in next experiments. cDNA was synthesized from total RNA (1 μg; 20 μL final reaction volume) with oligo dT and random primers using PrimeScript RT reagent kit (TaKaRa, Japan) according to the manufacturer's instructions. Each reverse transcription reaction was accompanied by a control in which the reverse transcriptase was replaced with DEPC H_2O.

1.3 Real-time PCR

The quantitative real-time PCR was performed on a 7500 Fast Real-Time PCR system (Applied Biosystems, USA) with SYBR green fluorescent label. The relative quantification of gene expression among the treatment groups was normalized to 18S rRNA and analyzed by the 2-ΔΔCt method (Livak and Schmittgen, 2001). The amplicon specificity was verified by dissociation curve, gel electrophoresis and sequencing. No genomic DNA contamination of our samples was confirmed by no-reverse transcription controls. No signals were detected in no-template controls and no reverse transcriptase controls. All primers used in the present study are listed in Table 1.

1.4 Statistical analysis

Data generated by real-time PCR were compiled and collected using SDS 1.4 software (Applied Biosystems, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's test to validate significant difference between control and other groups. Data are mean ± standard deviation of the mean (SD). Calculations were performed using the 17.0 version of SPSS software (SPSS Inc.). A difference was considered statistically significant with $P < 0.05$.

2 Results

2.1 EE2 exposure reduces the expression of *fbxl14a*, *fbxl14b*, *fbxo25* and β-*TRCP2b*

Male fish exposed to 100 ng/L EE2 had a 11.9-fold decrease in *fbxl14a* and a 2.2-fold decrease in *fbxl14b* expression, which were significantly different from controls ($p < 0.01$ and $p < 0.05$) (Fig. 1a, b). However, the other two concentrations, 1 and 10 ng/L EE2 exposure had no significant influence on *fbxl14a* and *fbxl14b* expression. And in contrast, exposed to 1 and 10 ng/L EE2, male zebrafish exhibited a 4.2- and 3.8-fold decrease in

Table 1 Sequences of primers used in the real-time PCR reactions

Gene	Sequences of primers	Amplicon size (bp)
fbxl2	Forward primer 5′-GAGCTGTCAGCCAACCTCTAAAC-3′ Reverse primer 5′-CAGCCACGCCCCTCAAT-3′	64
fbxw7	Forward primer 5′-ACCGAGAGAGTTGGCTTTGC-3′ Reverse primer 5′-TGTGAACCCCGGCTTGAT-3′	190
fbxo9	Forward primer 5′-ACGACCTGCTGACATACTTCCA-3′ Reverse primer 5′-CTCATGCTCACACAGCTTCATG-3′	71
β-*TRCP2a*	Forward primer 5′-GGGATGGAGTCTCAGATTAACACA-3′ Reverse primer 5′-CTGCCTTGAGATGGCCTCTT-3′	110
β-*TRCP2b*	Forward primer 5′-CCCCTCTTCAGTCGTTTGGA-3′ Reverse primer 5′-CCCACCCTCATGTTCTCTGTTT-3′	130
fbxl14a	Forward primer 5′-CACCAAAAGAGGACTGGAGAGAA-3′ Reverse primer 5′-TCTGCCAAAGTCCCAAGTTCA-3′	71
fbxl14b	Forward primer 5′-ATGCCCAACATCGAGAGCTT-3′ Reverse primer 5′-CCAGGCCGTTATCCGTTAAG-3′	61
fbxl18	Forward primer 5′-GGTACTTTGGGACGCCTCACT-3′ Reverse primer 5′-TGGAGGAGATTGTGCTAATGCA-3′	72
fbxo25	Forward primer 5′-TTCAAACGGGATGACACTGA -3′ Reverse primer 5′-CTTCCACAGATGTCGGTCCT-3′	145
fbxo45	Forward primer 5′-CGGCCGTATATGGGAACACT-3′ Reverse primer 5′-ATGATGCTCTCCGGTGTTATGA-3′	110
skp2	Forward primer 5′-CGAAAACACCCCGAACGA-3′ Reverse primer 5′-AGCAGTAGTCAGGGCCTTCTTG-3′	67
p27	Forward primer 5′-AAAGAAGCTCCTGTCTCGACTCA-3′ Reverse primer 5′-GGCGTTCGGGTCACTTCAT-3′	76
18S rRNA	Forward primer 5′-GCCTGCGGCTTAATTTGACT-3′ Reverse primer 5′-TCTCGTTCGTTATCGGAATGAA-3′	160

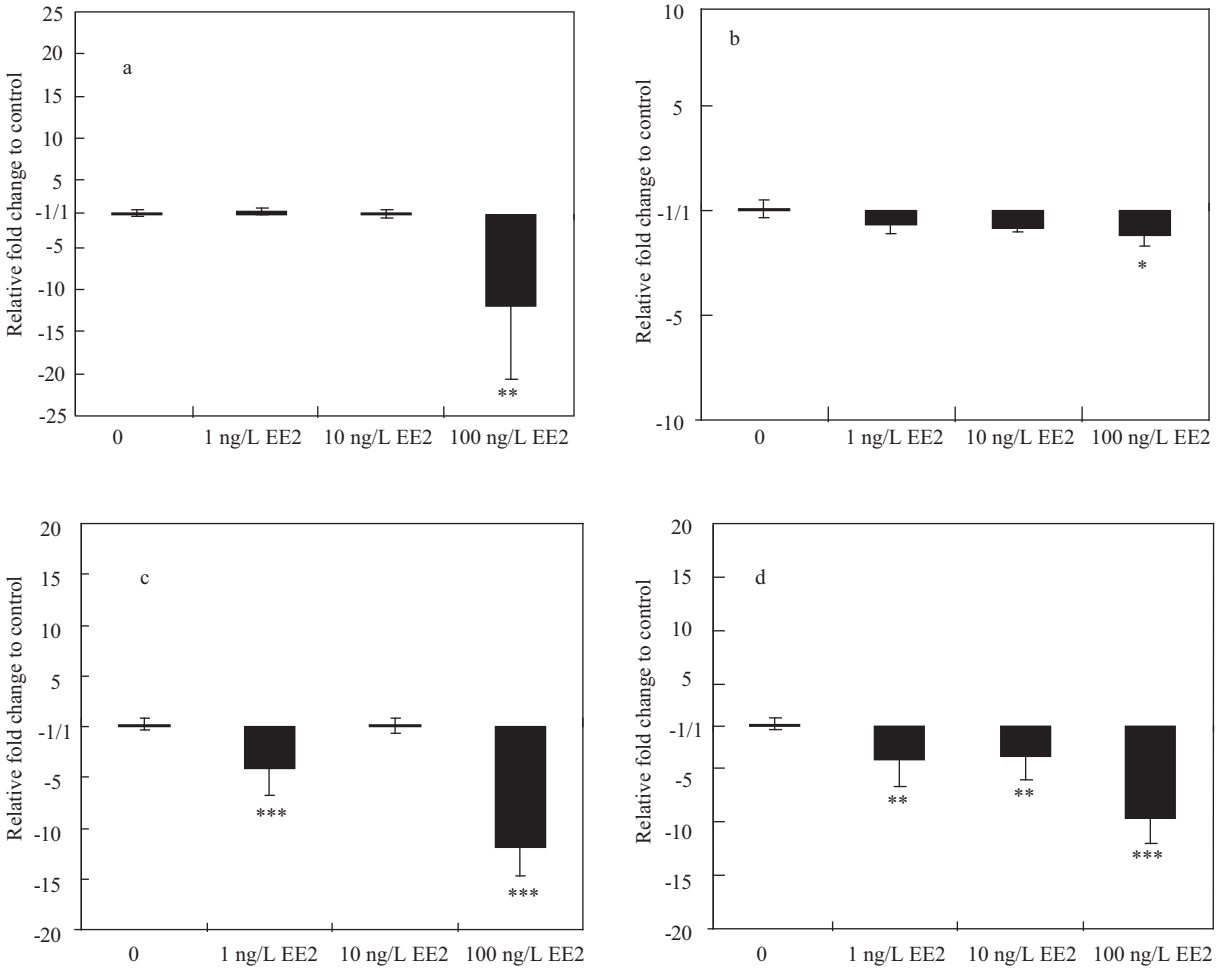

Fig. 1 Relative mRNA abundance *fbxl14a* (a), *fbxl14b* (b), *fbxo25* (c) and β-*TRCP2b* (d) in male zebrafish exposed to 0, 1, 10, 100 ng/L EE2 for 7 days, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with control group ($n = 6$).

β-*TRCP2b* transcript, respectively. Both decreases were significantly different from the expression levels of control fish ($p < 0.01$) (Fig. 1d). More significantly, 100 ng/L EE2 exposure resulted in a 9.7-fold decrease in β-*TRCP2b* message ($p < 0.001$). Male zebrafish exposed to 1 and 100 ng/L EE2 exhibited a 5.2- and 12.8-fold decrease in *fbxo25* transcript, respectively, which were significantly different from controls ($p < 0.001$) (Fig. 1c). While 10 ng/L EE2 exposure had no significant influence on *fbxo25* expression.

2.2 No change in *fbxl2, fbxw7, fbxo9,* β-*TRCP2a, fbxl18* and *fbxo45* mRNA levels after EE2 exposure

The transcript levels of *fbxl2, fbxw7*, *fbxo9, β-TRCP2a, fbxl18* and *fbxo45* were not observed to have significant alteration in male zebrafish exposed to the three concentrations of EE2 (Fig. 2).

2.3 Expression of *skp2* is enhanced after EE2 exposure

In contrast to *fbxl14a, fbxl14b, fbxo25* and *β-TRCP2b*, exposure of male zebrafish to 10 ng/L EE2 caused a 7.3-fold increase in *skp2* expression, which was statistically

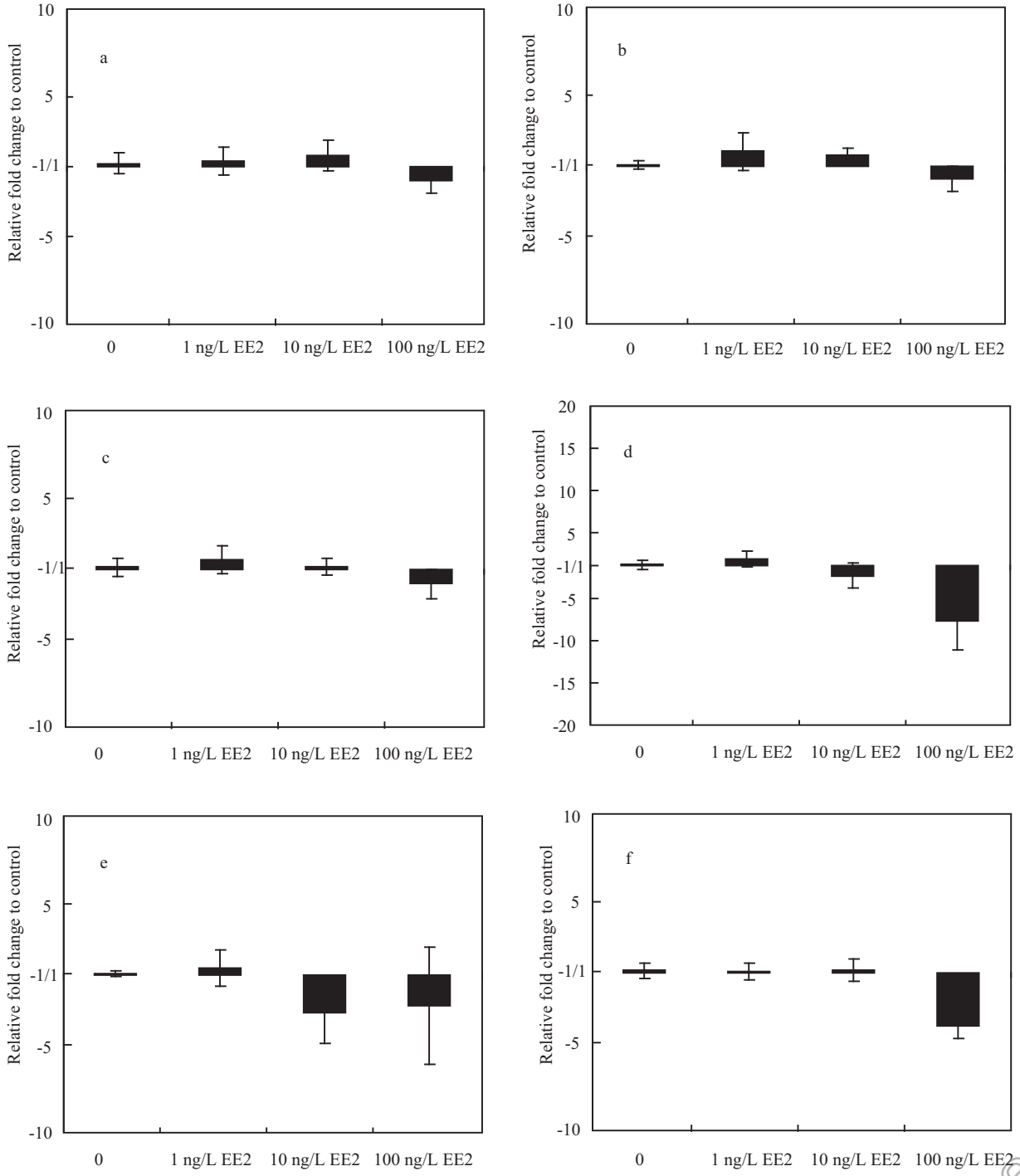

Fig. 2 Relative mRNA abundance of *fbxl2* (a), *fbxw7* (b), *fbxo9* (c), β-*TRCP2a* (d), *fbxl18* (e) and *fbxo45* (f) and in male zebrafish exposed to 0, 1, 10, 100 ng/L EE2 for 7 days, respectively.

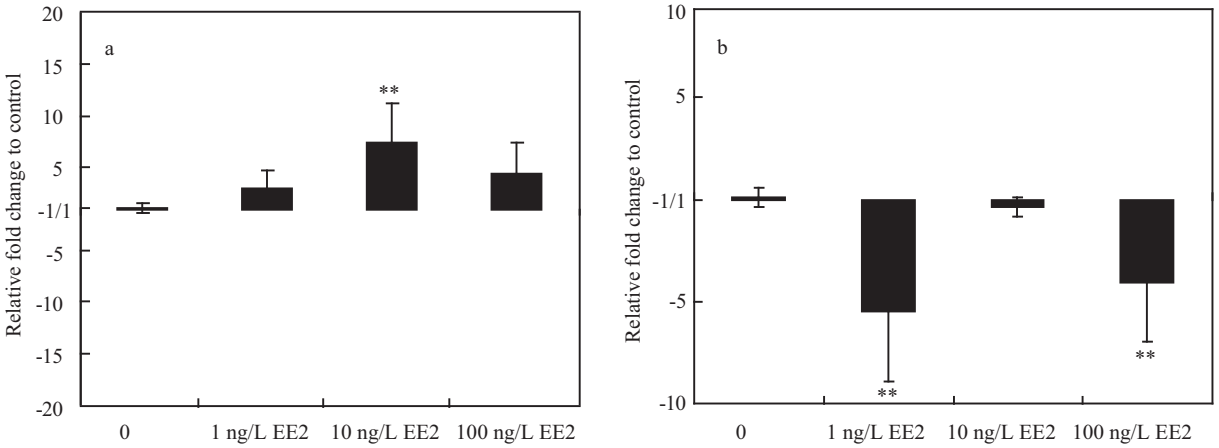

Fig. 3 Relative mRNA abundance of *skp2* (a) and *p27* (b) in male zebrafish exposed to 0, 1, 10, 100 ng/L EE2 for 7 days, respectively. ** $P < 0.01$, compared with control group ($n = 6$).

significantly different from controls ($p < 0.01$) (Fig. 3a). While exposure to lower or higher concentrations of EE2 did not change the expression of hepatic *skp2* in adult males. On the contrary, male zebrafish exposed to 1 ng/L and 100 ng/L EE2 exhibited a 5.4- and 4.0-fold decrease in *p*27 transcript, which were significantly different from controls ($p < 0.01$) (Fig. 3b). While 10 ng/L EE2 exposure had no significant influence on *p*27 expression.

3 Discussion

There is increasing concern on the presence and accumulation of the environmental chemicals with estrogenic activity. The activity can lead to disruption of the endocrine system, alteration of development, impairment of reproduction, as well as induction of tumors. One of the most potent and most studied of these chemicals is EE2, which belongs to semisynthetic estrogen mimics. In the present study, we examined the effects of EE2 on transcription of multiple hepatic FBP genes using real-time PCR. Previous studies showed that the expression of some housekeeping genes can be altered by EE2 exposure in fish (Hoffmann et al., 2006; Filby and Tyler, 2007; Martyniuk et al., 2007). Thus, housekeeping genes were evaluated in our experiment system and those with altered expression level after EE2 exposure were excluded, and left 18S rRNA as the internal control gene in our study. Hepatic response to EE2 exposure in this study was confirmed by altered expression of *vtg-1*, which is a known biomarker of estrogen exposure (Navas and Segner, 2001; Martyniuk et al., 2007).

In the studied system, our analysis identified hepatic FBP genes as a group of genes, whose expression is differentially regulated by EE2 exposure to zebrafish. This result may pull FBPs closer to processes of estrogen-related carcinogenesis. In addition to function as an ED, estrogens also produce many adverse effects, including immunotoxicty, teratogenicity and carcinogenicity, and estrogens have been shown to induce a variety of tumors in many species (Roy and Cai, 2002; Roy et al., 2007). In fish, estrogens are known to promote mutagen-induced hepatic neoplasia (Tilton et al., 2006). However, the mechanisms involved in estrogen-related tumorigenesis are still elusive. Results from previous studies confirmed that liver is responsive to estrogens, and screened out a large number of hepatic genes that are significantly changed in their transcript levels in both males and females after estrogen exposure (Hoffmann et al., 2006; Martyniuk et al., 2007; Notch et al., 2007). Among these genes whose expression is significantly altered are the common biomarkers for estrogen exposure, such as *vtg*-1, *vtg*-3, estrogen receptor alpha (*esr1*), *cyp1A1* and *apolipoprotein A1* (*apoA1*). Many other genes are involved in a variety of biological processes and molecular events, including genes functioning in the processes related to transport, sterol biosynthesis, translation, regulation of growth, and hormone metabolism, genes with monooxygenase, steroid dehydrogenase, cyclin-dependent kinase, and ligase activities, genes involved in vitamin A biosynthesis, metabolism and transport, genes involved in cell apoptosis, regulating cell growth and proliferation, as well as genes for NER (Hoffmann et al., 2006; Martyniuk et al., 2007; Notch et al., 2007). These results indicate that estrogens have the potential to cause far more other deleterious effects than what have known. And alteration of some genes' expression level from the results, such as the upregulation of genes involved in apoptosis and downregulation of genes of NER, may explain the processes of estrogen-induced tumorigenesis.

Despite the large number of genes with altered expression level after estrogen exposure, our analysis identified hepatic FBP genes as a group of genes, whose expression is differentially regulated by EE2 exposure to male zebrafish. EE2 exposure reduces the expression of *fbxl14a, fbxl14b, fbxo25* and β-*TRCP2b*, but enhances that of *skp2*, and does not alter the transcript levels of *fbxl2, fbxw7*, *fbxo9,* β-*TRCP2a, fbxl18* and *fbxo45*. FBPs are a family of specificity factors and most of them function through SCF ubiquitin E3 ligase complexes (Cardozo and Pagano, 2004; Ho et al., 2008). It has been demonstrated that FBPs take part in a variety of biological processes, most notable of which is their role in cell cycle control, signal transduction and carcinogenesis (Cardozo and Pagano, 2004; Nakayama and Nakayama, 2006). Among the numerous FBPs, Skp2, Fbxw7 and β-transducin repeat-containing

protein (β-TRCP) are three most studied FBPs. It has been demonstrated that Skp2 is oncogenic, Fbxw7 functions more as a tumor suppressor and β-TRCP can regulate many pathways (Nakayama and Nakayama, 2006). Among the genes tested, Skp2 expression is inversely correlated with levels of the tumor suppressor p27 in many cancers, and with the malignant grade of certain human tumors. Moreover, estrogens down-regulate p27 in breast cancer cell partly through Skp2 (Foster et al., 2003). However, our results exhibit a differential alteration in expression of *skp2*, *fbxw7* and β-*TRCP*. The expression levels of *fbxw7* and β-*TRCP2a* were not changed after EE2 exposure. Increase of *skp2* mRNA abundance was seen after 10 ng/L EE2 exposure and in contrast, decreases of β-*TRCP2b* mRNA abundance were observed after the three concentrations of EE2 treatment. In addition, male zebrafish had a significant decrease in hepatic *p*27 expression after exposure to 1 and 100 ng/L EE2, while no change was observed after 10 ng/L EE2 exposure. Thus, the transcript levels of *p*27 were not inversely correlated with that of *skp2* in this condition. This could be due to that the short-term exposure and long-term exposure may lead to different results. Moreover, gene expression is only a potential biological response to toxicant stress, and EE2 exposure may induce biological effects by non-genomic way, such as alteration of protein level or functional modification of key proteins. Considering the functions of FBP family, the altered expression of FBP genes may to some way be used to explain increased rates of mutagen-induced neoplastic transformation by estrogen exposure and may indicate an involvement of FBPs in estrogen-related tumorgenesis (Roy and Cai, 2002; Tilton et al., 2006; Roy et al., 2007).

Although the mechanisms governing the regulation of FBPs transcript abundance by EE2 are not known at present, it can in part be attributed to the contribution of estrogen receptors (ERs), since the effects of estrogens are largely mediated by members of ERs (Pettersson and Gustafsson, 2001; Menuet et al., 2004). There are at least three ERs in zebrafish, but they are differentially regulated by estrogens and have different transcriptional capacities on estrogen target genes. On the other hand, the effects of EE2 on transcriptional level depend on the promoter of each gene, because ERs regulate the expression of estrogen target genes by direct binding to a specific palindromic DNA sequence called the estrogen-responsive element (Zilliacus et al., 1995; Pettersson and Gustafsson, 2001). Therefore, analysis the promoter sequences of the tested genes may be an important direction to understand the different responses of these genes, although the transcript levels of FBP genes under higher exposure concentrations (100 ng/L) may be not due to direct estrogenic effects, but a result of toxicity.

4 Conclusions

Although it is not yet known whether transcriptional increases of FBP genes are sufficient to increase cancer risk, the alteration should have the potential to cause alteration of certain cellular processes. As estrogens are accumulating in the environment and have been shown to involve in the development of human hepatocellular carcinoma, the altered expression of FBP genes may be a new factor in the process of estrogen-induced tumors. Whether FBPs are involved in the estrogen-induced tumorgenesis, and through what mechanism estrogen exposure affects the transcription of these genes should be the target of further study.

Acknowledgments

This work was supported by the Major State Basic Research Development Program (973) of China (No. 2007CB947100) and the Shanghai Municipal Commission for Science and Technology (No. 074319111, 07DZ22919).

References

Amatruda J F, Shepard J L, Stern H M, Zon L I, 2002. Zebrafish as a cancer model system. *Cancer Cell*, 1(3): 229–231.

Cardozo T, Pagano M, 2004. The SCF ubiquitin ligase: insights into a molecular machine. *Nature Reviews Molecular Cell Biology*, 5(9): 739–751.

Colborn T, vom Saal F S, Soto A M, 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives*, 101(5): 378–384.

Crews D, Willingham E, Skipper J K, 2000. Endocrine disruptors: present issues, future directions. *The Quarterly Review of Biology*, 75(3): 243–260.

DuMond JW Jr, Singh K P, Roy D, 2001. Regulation of the growth of mouse Leydig cells by the inactive stereoisomer, 17alpha-estradiol: Lack of correlation between the elevated expression of ERalpha and difference in sensitivity to estradiol isomers. *Oncology Reports*, 8(4): 899–902.

Filby A L, Tyler C R, 2007. Appropriate 'housekeeping' genes for use in expression profiling the effects of environmental estrogens in fish. *Bmc Molecular Biology*, 8: 1–13.

Foster J S, Fernando R I, Ishida N, Nakayama K I, Wimalasena J, 2003. Estrogens down-regulate p27Kip1 in breast cancer cells through Skp2 and through nuclear export mediated by the ERK pathway. *The Journal of Biological Chemistry*, 278(42): 41355–41366.

Gutendorf B, Westendorf J, 2001. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. *Toxicology*, 166(1-2): 79–89.

Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A, 2002. Herbicides: feminization of male frogs in the wild. *Nature*, 419(6910): 895–896.

Ho M S, Ou C, Chan Y R, Chien C T, Pi H, 2008. The utility F-box for protein destruction. *Cellular and Molecular Life Sciences*, 65(13): 1977–2000.

Hoffmann J L, Torontali S P, Thomason R G, Lee D M, Brill J L, Price B B et al., 2006. Hepatic gene expression profiling using Genechips in zebrafish exposed to 17alpha-ethynylestradiol. *Aquatic Toxicology*, 79(3): 233–246.

Kolpin D W, Furlong E T, Meyer M T, Thurman E M, Zaugg S D, Barber L B et al., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environmental Science & Technology*, 36(6): 1202–1211.

Lam S H, Wu Y L, Vega V B, Miller L D, Spitsbergen J, Tong Y et al., 2006. Conservation of gene expression

signatures between zebrafish and human liver tumors and tumor progression. *Nature Biotechnology*, 24(1): 73–75.

Livak K J, Schmittgen T D, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 25(4): 402–408.

Martyniuk C J, Gerrie E R, Popesku J T, Ekker M, Trudeau V L, 2007. Microarray analysis in the zebrafish (*Danio rerio*) liver and telencephalon after exposure to low concentration of 17alpha-ethinylestradiol. *Aquatic Toxicology*, 84(1): 38–49.

Menuet A, Le Page Y, Torres O, Kern L, Kah O, Pakdel F, 2004. Analysis of the estrogen regulation of the zebrafish estrogen receptor (ER) reveals distinct effects of ERalpha, ERbeta1 and ERbeta2. *Journal of Molecular Endocrinology*, 32(3): 975–986.

Nakayama K I, Nakayama K, 2005. Regulation of the cell cycle by SCF-type ubiquitin ligases. *Seminars in Cell and Developmental Biology*, 16(3): 323–333.

Nakayama K I, Nakayama K, 2006. Ubiquitin ligases: cell-cycle control and cancer. *Nature Reviews Cancer*, 6(5): 369–381.

Navas J M, Segner H, 2001. Estrogen-mediated suppression of cytochrome P4501A (CYP1A) expression in rainbow trout hepatocytes: role of estrogen receptor. *Chemico-Biological Interactions*, 138(3): 285–298.

Notch E G, Miniutti D M, Mayer G D, 2007. 17alpha-Ethinylestradiol decreases expression of multiple hepatic nucleotide excision repair genes in zebrafish (*Danio rerio*). *Aquatic Toxicology*, 84(3): 301–309.

Pettersson K, Gustafsson J A, 2001. Role of estrogen receptor beta in estrogen action. *Annual Review of Physiology*, 63: 165–192.

Roy D, Cai Q, 2002. Estrogen, immunoactivation, gene damages and development of breast, endometrial, ovarian, prostate and testicular cancers. *Recent Advances in Steroid Biochemistry and Molecular Biology,* 3: 1–32.

Roy D, Cai Q, Felty Q, Narayan S, 2007. Estrogen-induced generation of reactive oxygen and nitrogen species, gene damage, and estrogen-dependent cancers. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews*, 10(4): 235–257.

Segner H, 2009. Zebrafish (*Danio rerio*) as a model organism for investigating endocrine disruption. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 149(2): 187–195.

Sonnenschein C, Soto A M, 1998. An updated review of environmental estrogen and androgen mimics and antagonists. *The Journal of Steroid Biochemistry and Molecular Biology*, 65(1-6): 143–150.

Stern H M, Zon L I, 2003. Cancer genetics and drug discovery in the zebrafish. *Nature Reviews Cancer*, 3(7): 533–539.

Tilton S C, Givan S A, Pereira C B, Bailey G S, Williams D E, 2006. Toxicogenomic profiling of the hepatic tumor promoters indole-3-carbinol, 17beta-estradiol and beta-naphthoflavone in rainbow trout. *Toxicological Sciences: An Official Journal of the Society of Toxicology*, 90(1): 61–72.

Welshons W V, Thayer K A, Judy B M, Taylor J A, Curran E M, vom Saal F S, 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environmental Health Perspectives*, 111(8): 994–1006.

Yin G G, Kookana R S, Ru Y J, 2002. Occurrence and fate of hormone steroids in the environment. *Environment International*, 28(6): 545–551.

Zilliacus J, Wright A P, Carlstedt-Duke J, Gustafsson J A, 1995. Structural determinants of DNA-binding specificity by steroid receptors. *Molecular Endocrinology*, 9(4): 389–400.