RESEARCH ARTICLE

Interactive Effect of Bisphenol A (BPA) Exposure with -22G/C Polymorphism in LOX Gene on the Risk of Osteosarcoma

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Abstract

Background: Osteosarcomas have many established risk factors, both genetic and environmental, but by themselves these explain only part of the total cancer incidence. Bisphenol A (BPA) is an environmental estrogen associated with risk of several kinds of tumour. The lysyl oxidase gene (LOX) may also contribute to risk of tumours including osteosarcomas. Here, we investigated possible interactions of BPA and a LOX polymorphism on the risk of osteosarcoma. Method: The present hospital-based case-control study included 106 cancer patients and 112 controls from a Chinese population. Internal burden of BPA exposure was assessed using high-performance liquid chromatography–mass spectrometry (HPLC-MS) method. Genotypes were determined using PCR-RFLP methods. Results: Compared with those in low BPA exposure group, subjects with BPA more than or equal to median value had significant increased risk of osteosarcoma among subjects who carried GC or CC genotypes. A significant interaction with BPA level and the -22G/C polymorphism was observed for osteosarcoma overall, osteosarcoma affecting knee and osteosarcoma affecting hip, as $P_{forinteraction}$ = 0.036 for osteosarcoma overall; $P_{forinteraction}$ = 0.024 for osteosarcoma affecting knee; and $P_{forinteraction}$ = 0.017 for osteosarcoma affecting hip. Conclusions: The results suggest that BPA exposure interacts with the -22G/C polymorphism of the LOX gene to increase the risk of osteosarcoma.

Keywords: Osteosarcoma - bisphenol A (BPA) - Lysyl oxidase gene (LOX) - gene polymorphism - interactive effect

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Introduction

Osteosarcoma is one of the most commonly diagnosed malignant diseases and the leading cause of pediatric cancer-related mortality worldwide(Hameed and Dorfman, 2011). It is well known that linear bone growth is under the influence of the sex steroid hormone control pathway (e.g., estrogen metabolism (ESTR) (Bland, 2000; Juul, 2001). Hence, any disturbance in estrogen pathways and physical function is reasonable candidates for exploration in osteosarcoma etiology. It has been reported that bisphenol A (BPA), an environmental estrogens which can mimic the action of normal estrogen (Melzer et al., 2011), is a definite risk factor associated with several tumors (Fernandez et al., 2006; Ericson et al., 2007; Duan et al., 2012). Although BPA accounts for the pathogenesis of most of tumors and osteosarcoma possibly, BPA itself explain only a small part of the total tumor incidence. In another prospective, non-environmental factors such as genetic variants comprised a high proportion of tumors including osteosarcoma, which implies influence of host genetic factors in individual susceptibility. In the past few years, genome wide association studies (GWASs) have successfully identified several novel genetic susceptibility variants involved in osteosarcoma etiology (Sandberg and Bridge, 2003; Mirabello et al., 2010; Caronia et al., 2011; Martin et al., 2012). However, even the most significant single nucleotide polymorphism (SNPs) identified in GWASs can not fully contributed to the total estimated incidence. So both genetic and environmental risk factors can not explain the whole part of osteosarcoma incidence. In such a case, gene and environment interaction is expected to be a possible explanation of the missing etiology that can not be explained by either established risk factors. BPA are environmental synthetic xenoestrogen involving in carcinogens can be widely detected in the urine samples of human population (Calafat et al., 2008). Although human population are widely exposure to environmental BPA and BPA exposure may plays an important role in osteosarcoma pathogenesis by disturbing estrogen metabolism pathways, few studies have focused on the potential effects of the genetic-BPA interactions following the initial findings of genetic polymorphism in osteosarcoma.

Lysyl oxidase gene (LOX) has been implicated in risk for osteosarcoma. Gene polymorphisms in LOX are suspected to have some relationship with response to chemotherapy and overall survival of advanced osteosarcoma patients (Liu et al., 2012). Among several SNPs in LOX, the variant genotype CC of -22G>C

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polymorphism showed the most significantly increased risk of developing osteosarcoma, compared to wild-type genotype GG validated by recent study. It is possible that genetic variation in LOX of -22G>C polymorphism may modify the association between BPA exposure and osteosarcoma risk. As such, we conducted a hospital-based, case-control study in Chinese population to test this hypothesis.

Materials and Methods

Population

This hospital-based case-control study was carried out in Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The cases with osteosarcoma were recruited from January 2009 to July 2011. All patients of osteosarcoma were diagnosed by histopathological examination of biopsy or surgically resected specimen. The controls were selected from a pool of cancer-free subjects recruited from individual participating early cancer detection based on physical examination. There were no age, sex and stage restrictions; however, patients with previous cancer or metastasis from other origins were excluded. The selection criteria of control included no prior history of cancer, and controls were frequency matched to the cases by age (±2 years) and sex. This case–control study included 106 patients with osteosarcoma and 112 cancer-free controls. The mean age of the osteosarcoma patients was 16.13 ± 2.80 years (mean ± SD) and the control group was 15.89 ± 2.13 years. At recruitment, informed consent was obtained from each subject who was then interviewed for detailed information on demographic characteristics, alcohol consumption, family history and lifetime history of tobacco use. This study was approved by approved by Ethics committee of Tongji Medical College, Huazhong University of Science and Technology.

Genotyping

Genomic DNA from all the subjects was extracted from the peripheral blood leukocytes according to a previous report and stored at -80 °C until use for genotyping. LOX genotype was analyzed by PCR-RFLP methods as described previously. The PCR primers for amplifying DNA fragment containing the -22G/C was: F 5'-GAGGAAACGCTCGTTGCTAAG-3'/R 5'-TCCTCATTAATTCCCTCACGTC-3'. A 10% random sample of cases and controls was repeated by different persons, and the results were found to be 100% concordant for all of the masked duplicate sets.

Exposure assessment

Urinary concentration of BPA was determined at School of Public Health, Tongji Medical College (Ministry of Education Key Laboratory of Environment and Health) using high-performance liquid chromatography–mass spectrometry (HPLC-MS) according to previous report. Briefly, 2.0 mL urine of each sample was used for the detection assay. The identification and quantification of BPA were based on retention time and peak area measured using a linear regression curve obtained from internal standard solutions. The detection limit of BPA was 0.5 ng/mL; for measurements below 0.5ng/mL, we used 0.35 ng/mL (70% of the detection limit) as the default. The valid urine BPA concentrations were expressed as micromoles per mole creatinine.

Table 1. Frequency Distribution of Selected Characteristics

Variable	Patients (n=106)		Controls (n=112)		*P*-value*
	No.	(%)	No.	(%)	
Sex					0.128
Male	74	69.7	76	67.9	
Female	32	30.3	36	32.1	
Ethnicity					0.15
Chinese Han	91	86	95	84.6	
Non-Chinese Han	15	14	17	15.4	
Age					
≤ 15	37	34.6	39	35.1	0.216
15-18	54	50.8	55	49.9	
> 18	15	14.6	18	15	
Smoking status					<0.0001
No	38	35.9	59	52.4	
Yes	68	64.1	53	47.6	
Site					
Knee	49	46.3			
Hip	38	35.9			
Others	19	17.8			

Table 2. Associations Between BPA and Risk of Osteosarcoma and Common Subtypes

1-OHP μmol/mol creatinine	<7.01	≥7.01
Osteosarcoma overall		
Cases	43	63
Control	55	57
OR(95%CI)	1	1.41 (1.01-1.72)
P		0.045
Osteosarcoma affecting hip		
Case	14	22
OR(95%CI)	1	2.00 (1.30-3.17)
P		0.078
Osteosarcoma affecting knee		
Case	21	36
OR(95%CI)	1	1.66 (1.14-2.49)
P		0.02
Other sites		
Case	5	8
OR(95%CI)	1	1.22 (0.71-1.41)
P		0.082

Statistical analysis

The frequency distributions of categorical variables were examined both in case and control groups by Pearson χ^2 test. The urine BPA exposure was divided into two groups according to the concentration examined. We defined persons as low exposure if their BPA level was less than median value and high exposure if their BPA level was more than or equal to median value. Unconditional logistic regression model was used to estimate the odds ratios (ORs) and 95% confidence intervals (Francis et al., 2003) for associations between BPA level, and risk of osteosarcoma and its subtypes in different genotype strata. Significance of gene-BPA exposure interaction was assessed by adding an interaction term in the logistic

Table 3. Associations Between LOX -22G/C Polymorphism, BPA, and Risk of Osteosarcoma

-22G/C polymorp-hisms	Osteosarcoma						Osteoscroma affecting knee				Osteoscroma affecting hip			
	BPA < 7.01			BPA ≥ 7.01			BPA < 7.01		BPA ≥ 7.01		BPA < 7.01		BPA ≥ 7.01	
	Case	Control	OR(95%CI)	Case	Control	OR(95%CI)	Case	OR(95%CI)	Case	OR(95%CI)	Case	OR(95%CI)	Case	OR(95%CI)
GG	33	40	1	42	35	1.37(1.00-7.15)	15	1	20	1.38(1.01-7.21)	10	1	12	1.67(1.13-2.12)
GC or CC	10	15	1	21	22	1.48(1.06-7.37)	6	1	16	1.72(1.23-2.24)	4	1	10	2.4(1.45-3.36)
$P_{forinteraction}$	0.036						0.024				0.017			

models. The Statistical Package for the Social Sciences (SPSS) software (version 13.0, SPSS, Inc., Chicago, Illinois) was used for the data analysis.

Results

Associations between BPA and risk of osteosarcoma and common subtypes

The study involved 106 patients with osteosarcoma and 123 non-neoplastic controls. The characteristics of the study population are provided in Table 1. The median value of urine BPA concentration in this study was 7.01 ng/ml. The association between BPA level and risk of osteosarcoma overall and osteosarcoma subtype are presented in Table 2. Compared with subjects in low exposure rank, those with BPA level more than 7.01 ng/ml had an increased risk of osteosarcoma overall (OR =1.41; 95% CI, 1.01-1.72). After stratification by subtypes, an increased risk was observed for osteoscroma affecting knee (OR =1.66; 95% CI, 1.14-2.49) and osteoscroma affecting hip (OR =2.00; 95% CI, 1.30-3.17), but not for other parts (OR =1.22; 95% CI, 0.71-1.41).

Associations between LOX -22G/C polymorphism, BPA, and risk of osteosarcoma

As shown in Table 3, a significantly increased risk of osteosarcoma was associated with urine BPA level among subjects who carried the variant of TC and CC polymorphism of LOX. Compared with the subjects whose BPA level were less than 7.01 ng/ml, subjects with higher BPA level had a more significantly increased risk of Osteosarcoma, if they carried TC or CC polymorphism of LOX. The $P_{forinteraction}$ of BPA level and LOX genotype is statistical significant, as $P_{forinteraction} = 0.036$. A similar pattern was also observed for osteoscroma affecting knee and osteoscroma affecting hip. The interaction of -22G/C polymorphism and BPA was statistical significant, as $P_{forinteraction} = 0.024$ for osteoscroma affecting knee and $P_{forinteraction} = 0.017$ for osteoscroma affecting hip.

Discussion

Our study provided the first comprehensive analysis of interaction BPA exposure, genetic polymorphism in LOX gene, and risk of osteosarcoma and its subtypes. Significant interactions were observed for -22G/C polymorphism and BPA exposure for osteosarcoma risk overall, as well as osteoscroma affecting knee and hip separately.

Consistent with our hypothesis, the present study suggested that high BPA exposure was associated with an increased risk of osteosarcoma overall. BPA, as the most significant kind of synthetic xenoestrogen, can cause the disturbance of normal estrogen metabolism and involved in several kinds of tumors. In such a case, variants in normal estrogen signaling pathways may affect the intervention effects commonly generated by exposure to the BPA, and subsequently modify the association between BPA and risk of osteosarcoma. LOX, which is essential for the structural integrity and function of bone tissue (Eyre et al., 1988; Knott et al., 1995), is also participated in estrogen signal pathway related-osteosarcoma pathogenesis (Liu et al., 2012).

The study suggested that -22G/C polymorphism in LOX gene may have modified the relationship between BPA exposure and osteosarcoma risk. Especially, the result suggested the association of the C allele with increased risk of BPA related- osteosarcoma risk. Theoretically, if the LOX gene C allele has an increasing tumor triggering activity, individuals who carry this allele and are also exposed to BPA-induced hormone metabolism disorders will be at higher risk of osteosarcoma. This explanation was consistent with present study based on the analysis of interaction effect of BPA exposure and -22G/C polymorphism. However, the major limitation of our study is the modest sample size, for the interaction analysis. As such, chance can not be ruled out for some of the significant findings. So the interaction of the two established risk factors warrants further investigation in other larger population. Finally, the study was designed to investigate the interaction effect of BPA exposure and LOX pathway polymorphism onto osteosarcoma risk. Since only osteosarcoma patients were included in the study, in such a case, the results may not be generalizable to other tumor diseases

In conclusion, our study suggests that common genetic variation -22G/C polymorphism in LOX genes may modify the association between BPA exposure and osteosarcoma. The positive results in our study need to be replicated in larger population studies.

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The author(s) declare that they have no competing interests.

References

Bland R (2000). Steroid hormone receptor expression and action in bone. *Clin Sci (Lond)*, **98**, 217-40.

Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL (2008). Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol, 2003-2004. *Environ Health Perspect*, **116**, 39-44.

Caronia D, Patino-Garcia A, Perez-Martinez A, et al (2011). Effect of ABCB1 and ABCC3 polymorphisms on osteosarcoma survival after chemotherapy, a pharmacogenetic study.

PLoS One, **6**, e26091.
Duan B, Hu X, Zhao H, Qin J, Luo J (2012). The relationship between urinary bisphenol A levels and meningioma in Chinese adults. *Int J Clin Oncol*, **18**, 492-7.
Ericson U, Sonestedt E, Gullberg B, Olsson H, Wirfalt E (2007). High folate intake is associated with lower breast cancer incidence in postmenopausal women in the Malmo Diet and Cancer cohort. *Am J Clin Nutr*, **86**, 434-43.
Eyre DR, Dickson IR, Van Ness K (1988). Collagen cross-linking in human bone and articular cartilage. Age-related changes in the content of mature hydroxypyridinium residues. *Biochem J*, **252**, 495-500.
Fernandez SV, Russo J (2010). Estrogen and xenoestrogens in breast cancer. *Toxicol Pathol*, **38**, 110-22.
Francis DD, Szegda K, Campbell G, Martin WD, Insel TR (2003). Epigenetic sources of behavioral differences in mice. *Nat Neurosci,* **6**, 445-6.
Hameed M, Dorfman H (2011). Primary malignant bone tumors--recent developments. *Semin Diagn Pathol*, **28**, 86-101.
Ho SM, Tang WY, Belmonte de Frausto J, Prins GS (2006). Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res*, **66**, 5624-32.
Juul A (2001). The effects of oestrogens on linear bone growth. *Hum Reprod Update,* 7, 303-13.
Knott L, Whitehead CC, Fleming RH, Bailey AJ (1995). Biochemical changes in the collagenous matrix of osteoporotic avian bone. *Biochem J*, **310**, 1045-51.
Liu Y, Lv B, He Z, et al (2012). Lysyl oxidase polymorphisms and susceptibility to osteosarcoma. *PLoS One*, **7**, e41610.
Martin JW, Squire JA, Zielenska M (2012). The genetics of osteosarcoma. *Sarcoma*, **2012**, 627254.
Melzer D, Harries L, Cipelli R, et al (2011). Bisphenol A Exposure is Associated with In-Vivo Estrogenic Gene Expression in Adults. *Environ Health Perspect*, **119**, 1788-93.
Mirabello L, Berndt SI, Seratti GF, et al (2010). Genetic variation at chromosome 8q24 in osteosarcoma cases and controls. *Carcinogenesis*, **31**, 1400-04.
Sandberg AA, Bridge JA (2003). Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors, osteosarcoma and related tumors. *Cancer Genet Cytogenet*, **145**, 1-30.