Table 2. Correlation of pesticide concentrations among breast milk, cord blood and maternal blood

	Breast milk						
	c-CHL	*t*-CHL	OxyCHL	*c*-Nonachlor	*t*-Nonachlor	*p,p'*-DDE	*p,p'*-DDT
Cord blood	0.543**	0.191	0.831**	0.836**	0.871**	0.837**	0.796**
Maternal blood	0.729**	0.291	0.943**	0.954**	0.959**	0.920**	0.878**
	Breast milk						
	Dieldrin	*c*-HCE	HCB	β-HCH	Mirex	Parlar-26	Parlar-50
Cord blood	0.821**	0.800**	0.879**	0.800**	0.673**	0.789**	0.778**
Maternal blood	0.819**	0.928**	0.921**	0.844**	0.894**	0.904**	0.917**

Pearson's r after log-transformed. n=68 for breast milk and cord blood, and n=49 for breast milk and maternal blood.

** $p<0.001$

Results and Discussion

Concentrations of pesticides in breast milk, cord blood and maternal blood are shown in Table 1. The highest values were observed for *p,p'*-DDE in all three materials. Since the use of DDT was prohibited in 1971 in Japan, this finding indicates the nature of intrinsic resistance to biological degradation of DDT/DDE. Mirex and toxaphene were measurable in most samples. Since neither chemical has ever been used in Japan, the route and the source of contamination are not fully understood.

High correlations of most chemicals among the three materials were observed as shown in Table 2. These findings indicated the usefulness of breast milk as a monitoring material for human exposure. Breast milk is rich in fat and therefore lipophilic chemicals such as POPs are accumulated, and it can be easily obtained from lactating women. In breast milk, most chemicals also correlate with each other (data not shown).

Since the working hypothesis on POPs-induced adverse effects is the disruption of the hypothalamus-pituitary-thyroid axis, associations of pesticides with TSH, T4 and T3 in maternal blood and cord blood were analyzed as shown in Table 3. It was confirmed that dioxins, PCBs and DDT/DDE were associated with TSH and thyroid hormones. In addition, other minor pesticides such as HCB, nonachlor, and toxaphenes were also associated with TSH, T4 and T3. Since there were clear multicollinearities among the chemicals, the causal relationships between the pesticide exposures and the levels of TSH and thyroid hormone remain to be clarified.

POPs exhibit bioaccumulation and biomagnification in the food chain, and therefore human exposure is thought to be mainly through the consumption of fish. However, maternal fish intake did not correlate with the concentrations of any of the chemicals (data not shown).

Further monitoring assessments and epidemiological examinations will make it possible to understand the exposure characteristics and biological effects of POPs on humans.

Acknowledgements

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Table 3. Relationship of POPs with maternal age, gestational age and concentrations of TSH and thyroid hormones

	Maternal age	Gestational age	TSH and thyroid hormones					
			Maternal blood			Cord blood		
			TSH	T4	T3	TSH	T4	T3
Breast milk (n=68, except for n=67 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.232§	ns	0.225§	ns	-0.224§	ns
PCB	ns	ns	0.205§	0.231§	0.257*	ns	ns	ns
trans-Nonachlor	ns	ns	ns	ns	0.281*	ns	-0.238§	ns
p,p'-DDE	ns	ns	ns	ns	ns	ns	ns	ns
p,p'-DDT	ns	ns	ns	ns	ns	ns	ns	ns
Dieldrin	0.229§	-0.265*	ns	ns	0.235§	ns	ns	ns
cis -Heptachlorepoxide	ns	ns	ns	ns	ns	0.243*	-0.212§	ns
HCB	ns	ns	ns	ns	ns	ns	-0.228§	ns
β-HCH	ns	ns	ns	ns	ns	ns	ns	ns
Mirex	0.422**	-0.232§	0.234§	ns	ns	0.233§	ns	ns
Parlar-26	ns	ns	ns	0.291*	0.395**	ns	ns	ns
Parlar-50	ns	ns	ns	0.287*	0.381**	ns	ns	ns
Maternal blood (n=49, except for n=48 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.264§	0.272§	0.375**	ns	ns	ns
PCB	ns	ns	0.277§	0.287*	0.402**	ns	ns	ns
trans-Nonachlor	ns	ns	0.274§	ns	0.429**	ns	-0.243§	ns
p,p'-DDE	ns	ns	0.242§	ns	0.252§	ns	ns	-0.251§
p,p'-DDT	ns	ns	ns	0.299*	0.426**	ns	-0.279§	ns
Dieldrin	0.351*	ns	ns	ns	0.247§	ns	ns	ns
cis-Heptachlorepoxide	ns	ns	ns	ns	ns	ns	ns	ns
HCB	ns	ns	0.251§	0.310*	0.391**	ns	ns	ns
β-HCH	ns	ns	ns	ns	0.267§	ns	ns	ns
Mirex	0.391**	-0.251§	0.342*	ns	0.324*	0.284*	ns	ns
Parlar-26	ns	ns	ns	0.267§	0.468**	0.261§	ns	ns
Parlar-50	ns	ns	ns	0.296*	0.500**	0.269§	ns	ns
Cord blood (n=68, except for n=67 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.203§	ns	ns	ns	-0.270*	ns
PCB	ns	ns	0.239§	ns	0.240§	ns	ns	ns
trans-Nonachlor	ns	ns	0.238§	ns	0.244*	ns	-0.217§	ns
p,p'-DDE	ns	ns	ns	ns	ns	ns	-0.208§	ns
p,p'-DDT	ns	ns	0.206§	ns	ns	ns	-0.238§	ns
Dieldrin	ns	-0.237§	ns	ns	ns	ns	-0.302*	-0.260*
cis -Heptachlorepoxide	ns	-0.278*	ns	ns	ns	0.262*	-0.335**	-0.208§
HCB	ns	ns	ns	ns	ns	0.202§	-0.293*	ns
β-HCH	0.206§	ns	ns	ns	ns	ns	ns	ns
Mirex	0.294*	-0.239§	0.234§	ns	ns	ns	ns	ns
Parlar-26	ns	-0.237§	ns	ns	0.300*	ns	ns	ns
Parlar-50	ns	-0.220§	ns	ns	0.324**	ns	ns	ns

Pearson's r after log-transformed. § $p<0.1$, * $p<0.05$, ** $p<0.01$

THE BIOLOGICAL MONITORING PROGRAM OF PERSISTENT ORGANIC POLLUTANTS IN JAPAN: 2. CONCENTRATIONS OF DIOXINS AND POLYCHLORINATED BIPHENYLS IN BREAST MILK, CORD BLOOD AND MATERNAL BLOOD

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Abstract

Persistent organic pollutants (POPs) such as dioxins and polychlorinated biphenyls (PCBs) are bioaccumulative chemical toxins that are resistant to degradation. POPs are thought of as hazardous contaminants. The Ministry of the Environment of Japan (MOE) has been conducting environmental monitoring of POPs since FY2002 on the basis of the Stockholm Convention on POPs. Since we provided some biological samples for the POPs biological monitoring project, we reanalyzed the report from the MOE. In this presentation, we summarize the data on dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood. We also analyze the associations of the concentrations of these compounds with thyroid-stimulating hormone (TSH) and thyroid hormones in maternal and cord blood, since disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects. Concentrations of dioxins and PCBs in each biological sample were at levels similar to those in previous reports on Japanese, and high correlations among the three biological samples were observed. Furthermore, single regression analysis showed a statistically significant correlation of dioxins and PCBs with TSH and thyroid hormones such as total thyroxine (T4) and triiodothyromine (T3).

Introduction

POPs such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and PCBs are bioaccumulative chemical toxins that are resistant to degradation. Generally, the main source of exposure to POPs for the general population is thought to be food because the physicochemical attributes of POPs such as lipophilicity and persistence cause bioaccumulation in the food chain, and consequently they can be found in humans at considerable concentrations. Although these concentrations tend to decrease and to be at the background level in industrialized nations, long-term exposure may cause potential risks to human health.

In humans, POPs have been claimed to possess endocrine-disrupting potency. Dioxins, expressed as toxic equivalent (TEQ) levels, were correlated significantly with lower T3 and T4 levels in maternal blood, and with higher blood concentrations in breast-fed infants[1]. There was a significant negative association between dioxin concentrations in breast milk and total T4 in the blood of breast-fed infants[2]. On the other hand, higher environmental background exposure to dioxins significantly increased the T4 concentration in the blood of infants[3]. These findings suggest that exposure to dioxins may affect the hypothalamus-pituitary-thyroid axis. A growing number of reports have demonstrated the association between adverse effects in children and exposure to POPs at low doses over a longer period. In particular, human perinatal exposure to PCBs has been shown to be associated with immunological changes[4], neural and developmental changes[5, 6], lower psychomotor development[7, 8], defects of short-term memory and spatial learning ability[9] and lower cognitive development[10]. Therefore, monitoring and epidemiological verification of exposure to POPs are necessary to assess the health risks to the Japanese population.

In Japan, the MOE has been conducting the POPs monitoring project[11] since FY2002 for the monitoring of chemicals in each of the environmental media and to obtain data that can contribute to effective evaluations in the Stockholm Convention on POPs. Recently the biological monitoring of human samples was added. We have been collaborating with the POPs biological monitoring project of the MOE by providing biological samples

from our prospective birth cohort study, The Tohoku Study of Child Development (TSCD)[12]. We reanalyzed the results and summarized the data of dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood[13]. We also analyzed the associations of the concentrations of these compounds with TSH and thyroid hormones in maternal and cord blood, since the disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects.

Materials and Methods

The biological samples analyzed were randomly selected from the participants in the TSCD, and provided anonymously to the MOE. These samples were measured by IDEA Consultants, Inc. (Tokyo, Japan) as part of the MOE project. This study protocol was previously reported[12]. Briefly, the maternal peripheral blood was collected using heparin as the anticoagulant agent in the morning when the pregnancy was at 28 weeks. The cord blood was collected immediately after delivery. These whole blood samples were frozen at –80°C until the chemical analysis. The breast milk was collected one month after delivery, and then frozen similarly. The TSCD was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine, and all mothers provided signed informed consent.

Chemical analysis was conducted following the methods in the environmental monitoring report on persistent organic pollutants (POPs) in Japan 2002-2004[14]. Briefly, the biological samples were spiked with ^{13}C-labeled POPs as internal standards before extraction. The samples were extracted with liquid-liquid extraction and then extracts were purified by multilayer silica gel column chromatography. Active carbon dispersed silica gel column chromatography was further used for purification of PCDD/Fs and dioxin-like PCBs (DL-PCBs). For the other POPs, extracts were purified by Florisil column chromatography except for silica gel column chromatography for toxaphene. Congener-specific determination of the compounds was performed by high resolution gas chromatograph-high resolution mass spectrometry (HRGC-HRMS) or negative ion chemical ionization mass spectrometry (GC-NICIMS) for toxaphene by isotope dilution quantification. Although control samples were analyzed for every 9-sample batch, they did not contain significant amounts of the target compound. TSH, total T4, and total T3 were measured from the plasma of cord and maternal blood by SRL, Inc. (Tokyo, Japan). The statistical analyses were performed using JMP ver. 5.1.2..

Results and Discussion

Concentrations of TEQ and total PCBs in breast milk, cord blood and maternal blood are shown in Table 1. TEQ was calculated by the WHO (1998) toxic equivalency factor[15] (TEF) assuming that the amount of congeners below the determination limit was zero. These data were roughly in agreement with previous studies[16-21]. In these biological samples, TEQ and PCB levels in breast milk were higher than in cord blood and maternal blood. Concentrations of TEQ and total PCBs among the three biological samples showed high correlations (Figure 1). Therefore, to predict the concentrations of dioxins and PCBs for the purpose of biological monitoring, it might be useful to measure the concentrations in breast milk. The homologue pattern of PCBs in breast milk was similar in composition to those of cord blood and maternal blood. The predominant homologues in the biological samples were HxCBs, followed by HpCBs, PeCBs and TeCBs.

The correlations between TEQ and PCBs in breast milk, cord blood and maternal blood were very high (Table 2). It was found that the contribution ratio of DL-PCBs to total TEQ was about 40% and the percentage of the DL-PCB concentration in total PCBs was 10% by the congener-specific analysis of PCBs in these biological samples. Because of the high correlation between TEQ and PCBs, the levels of exposure to dioxins for the population could be estimated from the results of PCB measurements. We may be able to simplify the monitoring method by eliminating the determination of dioxins from the analytical procedures.

The working hypothesis is that dioxins and PCBs cause adverse effects via disruption of thyroid hormone regulation and metabolism. Indeed, as shown in Table 3, there were significant correlations of PCBs with T3, and T4 in maternal blood ($p<0.05$). Similarly, there were correlations of TEQ and PCBs with several thyroid function indicators in breast milk and cord blood ($p<0.05$). Although the exact mechanisms by which dioxins and PCBs affect the levels of TSH and thyroid hormones are not fully understood, these results suggest that exposure to dioxins and PCBs could cause hormonal disturbance of thyroid function.

Table 1. Concentrations of TEQ (pg-TEQ/g-fat) and PCBs (ng/g-fat) in breast milk, cord blood and maternal blood

Compound names	Breast milk Median (Min - Max)	Cord blood Median (Min - Max)	Maternal blood Median (Min - Max)
Dioxins			
PCDD/Fs-TEQ	9.9 (2.0-25)	5.4 (0.28-16)	8.6 (2.8-26)
DL-PCBs-TEQ	6.8 (2.1-21)	2.9 (0.74-7.3)	4.8 (1.4-11)
Total TEQ	17 (4.2-45)	8.3 (1.1-22)	13 (4.8-33)
PCBs			
Total PCBs	102 (31-274)	40 (12-128)	76 (20-163)

n=68 for breast milk and cord blood, n=49 for maternal blood.
TEQ was calculated by the WHO (1998) TEF assuming that the amount of congeners below the determination limit was zero.

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We thank all the families who participated in the study. This presentation was based on the environmental monitoring report on POPs from the MOE, with support in part from the Japan Ministry of Health, Labour and Welfare (Research on Risk of Chemical Substances) and Japan Ministry of Education, Culture, Sports, Science and Technology (Grant-in-Aid for Scientific Research (B)).

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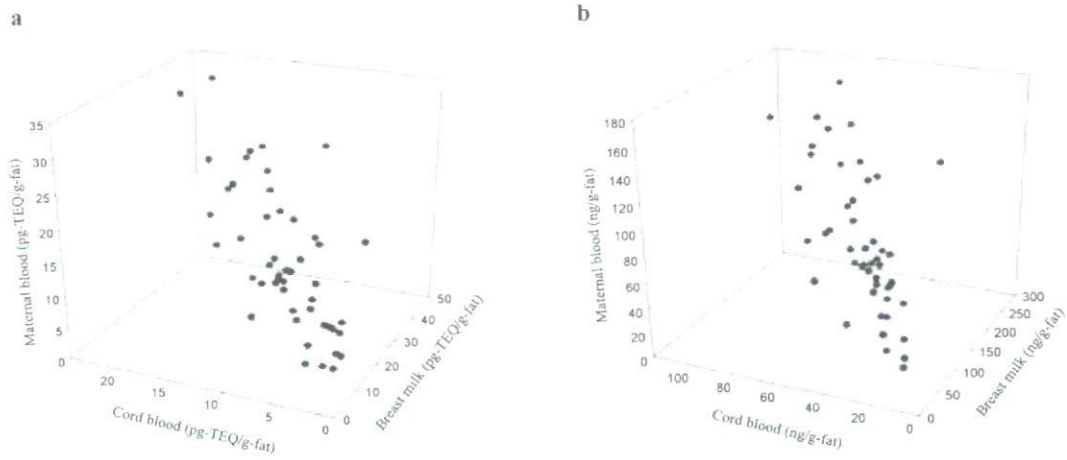

Fig. 1 Relationship of TEQ (a) and PCBs (b) among pair samples of breast milk, cord blood and maternal blood.

Table 2. Pearson correlation coefficients of TEQ and PCBs in breast milk with those of cord blood and maternal blood

	Breast milk		Cord blood		Maternal blood	
	TEQ	PCBs	TEQ	PCBs	TEQ	PCBs
Breast milk						
TEQ	-					
PCBs	0.901	-				
Cord blood						
TEQ	0.763	0.633	-			
PCBs	0.716	0.808	0.826	-		
Maternal blood						
TEQ	0.938	0.854	0.794	0.756	-	
PCBs	0.841	0.927	0.699	0.843	0.892	-

Pearson's r ($p < 0.001$) after log-transformed. n=49 for breast milk, cord blood and maternal blood.

Table 3. Pearson correlation coefficients of TEQ and PCBs with TSH, total T4 and total T3 in breast milk, cord blood and maternal blood

	Maternal blood			Cord blood		
	TSH	T4	T3	TSH	T4	T3
Breast milk (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.232	0.158	0.225	0.146	-0.224	-0.138
PCBs	0.205	0.231	0.257*	0.137	-0.163	-0.075
Cord blood (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.203	0.038	0.150	0.071	-0.270*	-0.051
PCBs	0.239	0.131	0.240	0.024	-0.144	0.085
Maternal blood (n=49, except for n=48 for maternal TSH/T4/T3)						
TEQ	0.264	0.271	0.375*	0.145	-0.238	-0.094
PCBs	0.278	0.287*	0.402*	0.185	-0.150	-0.038

n=68 for breast milk and cord blood, n=49 for maternal blood.
Pearson's r after log-transformed. * $p<0.05$

ASSOCIATIONS OF NEONATAL NEUROBEHAVIORAL STATUS WITH CORD BLOOD PCB, MATERNAL HAIR MERCURY, AND MATERNAL FISH INTAKE IN THE TOHOKU STUDY OF CHILD DEVELOPMENT

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Abstract

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposures to environmentally persistent organic pollutants and heavy metals on neurobehavioral development of offspring. In the present study, we examined the associations of the Neonatal Behavioral Assessment Scale (NBAS) with the total PCB concentrations in cord blood, maternal hair mercury (hair Hg), and maternal fish intake. Multiple regression analyses indicated some significant associations of the NBAS clusters with hair Hg, but there were no significant associations between total PCBs and any cluster of NBAS. These findings suggest that prenatal methylmercury exposure adversely affects neonatal neurobehavioral status.

Introduction

Several epidemiological studies have indicated some associations of perinatal exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg) with developmental deficits such as postnatal growth delay and poor cognitive functions. A common form of perinatal exposure is maternal fish intake; however, fish also contain some nutritive factors such as n-3 polyunsaturated fatty acids (n-3 PUFA) essential for normal brain development in the fetus and infant. From the perspective of risk assessment, these health hazard issues are important for fish-eating populations.

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to PCBs and MeHg on neurobehavioral development in Japanese children[1]. Previously[2], we reported some preliminary data about the associations of neonatal neurobehavioral status with total PCBs in cord blood and maternal fish intake. Since additional data on PCBs in cord blood have recently become available, in the present study we reexamined the associations of neonatal

neurobehavioral status with total PCBs in cord blood, maternal hair mercury (hair Hg), and maternal fish intake.

Materials and Methods

The subjects were 392 mother-infant pairs whose variables including the PCB concentration in cord blood, the NBAS, and other covariates were available. The mean maternal age at delivery was 31.9 (SD4.2) years. The infants consisted of 203 boys and 189 girls, and they were all singletons from full-term (36-42 weeks) gestation without congenital anomalies or diseases. Birth weight was 2400g or more. Information was obtained about pregnancy, delivery and infant characteristics from medical records.

The PCB concentration was measured from whole cord blood collected immediately after delivery. All 209 congeners were analyzed using HR-GC/MS (IDEA Consultants, Inc, Tokyo, Japan). The total PCB concentration represented the sum of the all measured congeners, expressed as ng/g-fat.

The hair Hg concentration was analyzed from maternal hair samples taken two days after delivery. The total hair Hg concentration was measured by cold vapor atomic absorption[4] at the National Institute for Minamata Disease (Minamata, Japan).

Maternal fish intake was estimated using a semiquantitative food frequency questionnaire (FFQ) for 122 individual foods and recipes[3] and 13 additional items regarding fish and shellfish. The FFQ was administered four days after delivery. Trained investigators showed life-size photographs of each food to the mothers, after which they were asked to answer questions about the frequency and the amount of intake per meal.

Thyroid hormones, including thyroid-stimulating hormone (TSH), total thyroxine (T4), triiodothyromine (T3), free T4 and free T3, were measured from plasma of cord blood by SRL, Inc. (Tokyo, Japan).

The NBAS was administered three days after delivery. Examiners of the NBAS were trained and certified at the training center for NBAS in Nagasaki University School of Medicine, Japan. Reliability checks were conducted throughout the data collection to maintain a 90% level of agreement.

In the statistical analysis, multiple regression analyses were performed for adjustment of covariates. The potential covariates were as follows: maternal age at delivery, maternal alcohol drinking during pregnancy, maternal smoking habit, maternal total energy intake, delivery type, parity, gestational age, sex, birth weight, Apgar score 1 min after delivery, TSH, T4 and T3 concentrations in cord blood, and the NBAS examiners. The significance level was set at 5%.

Results and Discussion

The mean total PCB concentration in cord blood was 55.9 ng/g-fat (SD 35.3) (median 48.4), the mean total hair Hg level was 2.2 μg/g (SD 1.1) (median 2.0), and total fish intake was 25.9 kg/year (SD 17.8) (median 22.7). Table 1 shows the results of multiple regression

analyses. The total PCBs in cord blood and the total fish intake were not significantly associated with any seven clusters of the NBAS. The total hair Hg was negatively associated with the motor score, and positively associated with the range of state and the reflex scores. For the motor and the range of state, higher scores mean more optimal behavioral status, and for the reflex, lower scores mean more optimal status because the reflex score indicates the number of unusual reflexes. Thus, the results suggested that prenatal MeHg exposure adversely affected neonatal neurobehavioral status. In early studies, an adverse effect of prenatal MeHg exposure on neurodevelopment was found in the Faroe Islands[5] and Boston[6], but not in the Seychelles[7]. Our findings are in line with the former two, although the types of examination were different.

Regarding the effects of PCB exposure, early studies demonstrated adverse effects of prenatal PCB exposure on neurodevelopment[8,9]. However, our findings do not agree with those studies. Several possibilities may account for this discrepancy. First, the levels of PCB exposure in Japanese pregnant women have decreased during the past several decades[10]. It is plausible that the level of PCB exposure in our cohort was too low to induce adverse effects on neonatal neurobehavioral status. Second, we used the value of the total PCB concentration as the exposure value. In the Oswego study, highly chlorinated PCBs (Cl7-9) were strongly associated with lower scores of the NBAS, but the total PCB level was not[7]. Therefore, we examined the associations of the NBAS with highly chlorinated PCBs, but found no significance (data not shown). Third, levels of toxicants such as PCB and MeHg and nutritive factors, including n-3 PUFA, vary among different fish types. The Japanese diet relies heavily on steamed rice, fish and vegetables. Indeed, the Japanese eat great amounts of fish, and they also eat many kinds of fish. This food lifestyle may contribute to the differences in the consequences of cohort studies. Further studies will require consideration of the potential risks of fish intake in the context of potential benefits. Since the TSCD study is a prospective cohort study, we will readdress these health issues when the children become older.

Acknowledgments

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Table 1. Results of multiple regression analyses

	Total PCBs (ng/g fat)[1]		Total hair Hg (μg/g)[1]		Total fish intake (kg/year)[1]		R^2 of the model
	Standardized beta	F	Standardized beta	F	Standardized beta	F	
Habituation	0.10	0.15	0.37	3.48	-0.31	1.14	0.12
Orientation	0.08	0.21	0.06	0.10	0.17	0.65	0.26
Motor	0.01	0.01	-0.28	5.60*	0.18	2.10	0.16
Range of state	-0.02	0.02	0.38	6.85**	-0.08	0.28	0.11
Regulation of state	0.22	1.27	-0.29	1.70	0.27	1.39	0.08
Autonomic stability	0.02	0.02	0.19	1.07	-0.23	1.38	0.12
Reflex	-0.26	0.72	0.74	4.52*	-0.11	0.09	0.17

* $p < 0.05$ ** $p < 0.01$

[1] Log translations, $Log_{10}X$, were used on values of total PCBs, hair Hg, and total fish intake.

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REVIEW

Biological Roles of Estrogen and Progesterone in Human Endometrial Carcinoma — New developments in potential endocrine therapy for endometrial cancer —

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Abstract. Endometrial carcinoma is one of the most common female pelvic malignancies. It is well known that uterine endometrial cell proliferation is under the control of both estrogen and progesterone. In this review, results of the recent studies on the biosynthesis and action of estrogen and progestin in normal endometrium and its disorders will be summarized and the new aspects of hormonal therapies in the patients with endometrial carcinoma will be discussed including its future prospectives. We reported that the enzymes responsible for intratumoral estrogen metabolism and biosynthesis are markedly different between human breast and endometrial carcinoma, although both of them are considered "estrogen-dependent malignancies". In addition, the biological significance of Progesterone receptor (PR) isoforms is considered to differ between endometrial and breast carcinomas. Clinical data concerning Hormone replacement therapy (HRT) and estrogen-dependent cancer risk also support these findings. These basic and clinical findings help to understand the biology and provide the new knowledge for prevention, diagnosis and treatment of human endomerial carcinoma. Specific endocrine treatment of endometrial carcinoma should be explored in future, although aromatase inhibitors are the most effective endocrine treatments of estrogen-responsive breast carcinoma. Retinoid, metabolities of vitamin A, and synthetic peroxisome proliferator-activated receptor (PPAR) γ ligands, which have been used for the treatment of insulin resistance in type II diabetes mellitus, may be the important candidates as drugs not only for prevention but also for possible endocrine treatment of endometrial carcinoma.

Key words: Estrogen, Progesterone, Endometrial carcinoma, Organic cation transporter, Peroxisome proliferator-activated receptor

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1. Introduction

Endometrial carcinoma is one of the most common female pelvic malignancies and its incidence has recently increased [1]. It is well known that uterine endometrial cell proliferation is under the control of both estrogen and progesterone. The results of previous clinical, biological and epidemiological studies have all demonstrated that excessive and/or prolonged exposure to unopposed estrogens increases the risk of endometrial carcinoma, especially that of the endometrioid type [2, 3]. However, it is also true that the great majority of estrogen-dependent carcinomas occur during the post-menopausal period, when the ovaries cease to be functional or produce active sex steroids. Therefore, *in situ* estrogen metabolism and synthesis play cardinal roles in the development and progression of various human estrogen-dependent epithelial neoplasms, including breast and endometrial carcinomas in postmenopausal patients. Therefore, it is very important to investigate the enzymes responsible for intratumoral estrogen metabolism and biosynthesis. The results of recent studies have all demonstrated that complete blockade of *in situ* estrogen production could lead to an improvement in the prognosis of breast cancer patients [4, 5]. However, the precise roles of sex-steroid producing enzymes in endometrial carcinoma have remained unclear. On the other hand, the physiological roles of progesterone in the regulation of the glandular epithelium of the endometrium are, in general, considered to antagonize estrogen-mediated cell proliferation and to induce cellular differentiation [6]. Progesterone has been clinically demonstrated to provide some protection against the stimulatory effects of estrogenic agents. For example, hormone replacement therapy using combinations of estrogen and progestin yields a lower risk of endometrial carcinoma, despite an increment in the incidence of breast carcinoma [7, 8].

Both estrogen and progesterone exert their effects through intra-nuclear receptors, estrogen receptors (ER) and progesterone receptors (PR), respectively, which belong to the superfamily of steroid hormone receptors [9]. The expression of ER and PR is generally considered to be coordinated because transcription of the PR gene is induced by estrogen and inhibited by progesterone in the great majority of estrogen responsive cells [10]. Both ER and PR play important roles as the signal mediators of estrogen and progesterone, but the exact biological and clinical roles of these receptors in human endometrial carcinoma have also remained unknown. In this review, the results of the recent studies on the biosynthesis and actions of estrogen and progestin in normal endometria and its disorders will be summarized and the new aspects of hormonal therapies in patients with endometrial carcinoma will be discussed, including its future prospects.

2. Enzyme systems for local biosynthesis or Intracrinology of estrogen

1) Intracrinology: In situ estrogen metabolism and synthesis

Recently, a focus has been given to the importance of *in situ* estrogen metabolism and synthesis in the etiology and progression of various human estrogen-dependent epithelial neoplasms, including breast and endometrial carcinoma [11]. The results of several studies have demonstrated increased tissue estrogen content in human breasts, compared to serum and/or normal non-neoplastic tissues of the same patients [12–14]. In these studies, the tissue concentrations of estrone (E1), estradiol (E2) and their sulfates were generally several times higher than those found in the plasma or in the area of the normal breast tissues of the same postmenopausal patients, despite markedly low levels of circulating estrogens. These findings all indicated specific intratumoral biosynthesis and accumulation of these hormones.

On the other hand, there have been limited and inconsistent data regarding tissue estrogen concentrations in endometrial carcinoma tissues, in contrast to that for human breast carcinoma [15–18]. Berstein *et al.* recently examined 78 endometrial carcinomas and detected higher concentrations of E2 in cancer tissue specimens compared with macroscopically normal endometrium [18]. The results of our recent study are generally consistent with those in previous investigations [19]. E2, E1 and testosterone levels in the tumor tissue were several times higher than those in serum. It then becomes important to evaluate the mechanisms and/or conditions responsible for such an intratumoral elevation of estrogens in post-menopausal patients with endometrial carcinoma. Numerous studies have demonstrated that human breast and endometrial carcinoma tissues contained the enzyme systems required

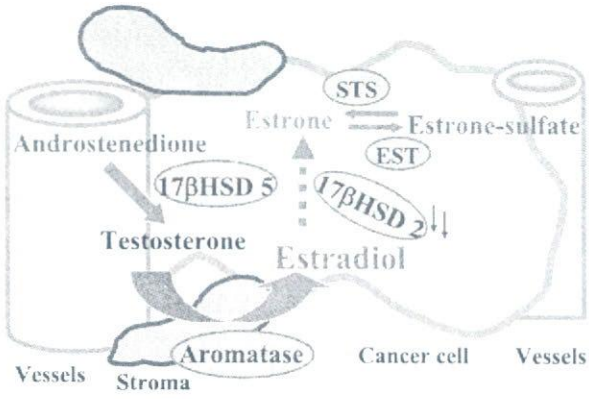

Fig. 1. Schema illustrating the possible cascade of local production of testosterone and estrogen in endometrioid endometrial carcinoma.

for local biosynthesis of estrogen. Among these enzymes, aromatase, 17β-hydroxysteroid dehydrogenases (17-HSDs), steroid sulfatase (STS) and estrogen sulfotransferase (EST) are the primarily involved in the formation of the biologically active estrogen, estradiol. Figure 1 represents the production/metabolism of sex-steroids in human endometrial carcinoma tissues.

2) Aromatase

Aromatase is an enzyme that is located in the endoplasmic reticulum of estrogen producing cells, and catalyzes the circulating androgens, mainly androstenedione and testosterone, into E1 and E2, respectively [4]. Aromatase is a key enzyme in the synthesis of estrogens and its levels in breast carcinoma tissues have been found to be significantly higher than those in benign breast lesions [5]. Expression of aromatase has also been detected in human endometrial carcinoma [20, 21]. Our laboratories previously reported marked aromatase immunoreactivity and mRNA, mainly in the stromal cells or fibroblasts of endometrioid endometrial carcinoma, but not in normal or hyperplastic endometrium [20] (Fig. 2). Aromatase expression was significant, both at the protein and mRNA levels, at the site of frank invasion in endometrial carcinoma, suggesting induction of aromatase expression by tumor-stromal interactions. Recently, Segawa *et al.* reported a significant correlation between aromatase immunoreactivity in stromal cells and poor prognosis in 55 patients with endometrial carcinoma [21]. This positive linkage indicates that local aromatase expression plays a role in tumor progression through the formation of *in situ* estrogens. In addition, we previously reported that an aromatase inhibitor suppressed the proliferation of endometrial carcinoma cells, which exhibited aromatase activities *in vitro* [22]. Aromatase is considered a key enzyme in the synthesis of estrogen in endometrial carcinoma as well as breast carcinoma. However, it is important to note that regulation of aromatase in endometrial carcinoma remains largely unknown.

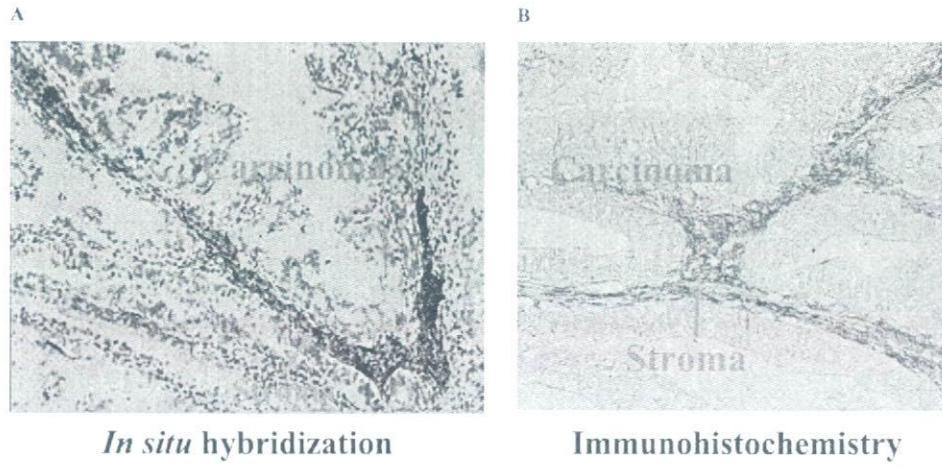

Fig. 2. Immunohistochemical staining and *in situ* hybridization for aromatase in endometrioid endometrial carcinoma. A, Accumulation of aromatase mRNA hybridization signals, appearing as *black dots* on the autoradiogram, was detected in the stromal cells, but not in carcinoma cells. (Ref. 20. Reproduced with permission from Watanabe *et al.*: *Am J Pathol* 146: 491–500. 1995) B, Immunohistochemical staining of aromatase was also detected in the stromal cells, but not in carcinoma cells. Original magnification, ×400.

3) Steroid sulfatase (STS) and Estrogen sulfotransferase (EST)

A major circulating form of plasma estrogen is the biologically inactive form of estrogen, estrone sulfate (E1S). E1S exhibits a relatively long half-life in the peripheral blood, and the levels of E1S are 5 to 10 times higher than those of unconjugated estrogens, such as E1 and E2, during the menstrual cycle and in postmenopausal women [23]. STS hydrolyzes circulating E1S to E1, whereas EST (estrogen sulfotransferase) sulfonates E1 to E1S. It was recently reported that *in situ* estrogen activity in breast cancer may be primarily regulated by the status of intratumoral STS and EST [24]. Thus, the balance between the levels of intratumoral STS and EST may also play an important role in the regulation of *in situ* estrogen levels in estrogen-dependent neoplasms. Immunoreactivity to STS was not detected but that to EST was evident in normal human mammary glands. However, STS and EST immunoreactivity was detected in 74% and 44% of breast carcinomas, respectively. STS and EST immunoreactivity were associated significantly with an increased and decreased risk of recurrence, respectively [24]. In normal endometrium, immunoreactivity to STS was not detected but that to EST was evident during the secretory phase of the cycle. Both STS and EST immunoreactivity have been detected in 86% and 29% of endometrial carcinomas cases, respectively. In addition, the STS/EST ratio was associated significantly with poor prognosis in endometrial carcinoma patients [25]. Therefore, increased STS and decreased EST expression in both human breast and endometrial carcinomas may result in the increased availability of biologically active estrogens *in situ*.

4) 17β-Hydroxysteroid Dehydrogenase (17-HSD)

4.1. 17-HSD type 1 & 2

The 17β-hydroxysteroid dehydrogenases (17-HSDs) are enzymes involved in the formation of androgens and estrogens [26]. The enzymes, 17-HSD types 1 and 2 primarily catalyze the reversible interconversion of E1 and E2. Type 1 17-HSD catalyzes the 17β-reduction of biologically inactive E1 to E2 [27, 28], whereas the type 2 isozyme preferentially catalyzes the oxidation of E2 to E1 [29]. Both type 1 and type 2 17-HSD regulate tissue levels of E2 and modulate estrogenic actions in estrogen target tissues, such as the endometrium and breast [30].

Oxidative 17-HSD activity is the preferential biochemical reaction in normal breast tissues, but the reductive 17-HSD pathway generally predominates in breast carcinomas. 17-HSD type 1 immunoreactivity was detected in carcinoma cells in approximately 60% of breast carcinoma tissues, whereas 17-HSD type 2 was not expressed at all [31]. In addition, breast carcinoma patients exhibiting high levels of expression of 17-HSD type 1 mRNA also exhibited increased risk of recurrence of breast carcinoma [32]. Therefore, type 1 17-HSD is considered responsible for regulating the process leading to the accumulation of E2 in human breast carcinomas.

However, 17-HSD type 1 immunoreactivity was not detected in any of the cases with normal endometrium, endometrial hyperplasia or endometrioid endometrial carcinoma [33, 34]. 17-HSD type 1 mRNA expression and enzymatic activity were also absent in all carcinoma cases. In normal endometrium tissues, 17-HSD type 2 immunoreactive protein was detected only in the cytoplasm of glandular cells during the secretory phase. 17-HSD type 2 mRNA was also markedly expressed in the endometrial glandular epithelial cells during the luteal phase, but 17-HSD type 1 mRNA was not detected in any of the phases of the examined endometrium [35]. 17-HSD type 2 immunoreactivity was detected in 75% and 37% of cases of endometrial hyperplasia and endometrioid endometrial carcinoma, respectively. 17-HSD type 2 expression was decreased from normal endometrium (secretory phase) to hyperplasia and finally carcinoma [34]. In addition, there was a statistically significant inverse correlation between the intratumoral E2 concentration and the level of 17-HSD type 2 mRNA in endometrial carcinoma [19]. These results strongly suggest that type 2 17-HSD contributes to the regulation of "intratissue" estrogen levels in normal endometrium and that disruption of the control or regulatory mechanisms of intratissue estrogen levels may be related to the development of endometrial disorders.

4.2. Type 5 17-HSD

Intratumoral E2 concentration may be maintained primarily by aromatization of testosterone in endometrial carcinoma, since 17-HSD type 1 expression is negligible in human endometrioid endometrial carcinoma tissues. Recently, 17-HSD type 5, which catalyzes the reduction of androstenedione to testosterone,

was cloned [36]. 17-HSD type 5 is a member of the aldo-keto reductase (AKR) superfamily, and is formally termed AKR1C3. This enzyme is expressed in various peripheral tissues, liver, prostate, and ovary, and has been also detected in prostate and breast carcinoma tissues [26, 37, 38]. 17-HSD type 5 immunoreactivity was detected in normal mammary gland and breast carcinoma cells in 53% of such cases. Immunoreactivity of 17-HSD type 5 also correlated significantly with that of 5α-reductase, which catalyzes the reduction of testosterone to the biologically active and potent androgen, 5α-dihydrotestosterone (DHT). 17-HSD type 5 is considered to be involved with DHT production in breast carcinomas *in situ* [5, 37].

In normal human endometrium, 17-HSD type 5 immunoreactivity was detected in 19% and 25% of proliferative and secretory phase endometria, respectively. However, 17-HSD type 5 immunoreactivity was detected in 50% and 69% of cases of endometrial hyperplasia and endometrioid endometrial carcinoma, respectively. 17-HSD type 5 expression was increased significantly throughout normal endometrium, hyperplasia and finally carcinoma. In addition, there was a statistically significant inverse correlation between intratumoral testosterone concentration and aromatase mRNA level in endometrial carcinoma [19]. Testosterone produced by 17-HSD type 5 in the tumor tissue may be finally aromatized to E2 by aromatase, which is also overexpressed in human endometrial cancer tissues. Therefore 17-HSD type 5 is considered one of the key enzymes in the local regulation of estrogen concentrations in endometrial malignancy.

3. Sex-steroid receptors

1) Estrogen receptors

The biological effects of estrogens are mediated through the estrogen receptors (ER). ER is expressed in a great majority of breast and endometrial carcinoma tissues. To date, two ERs (ERα and ERβ), which are encoded by different genes, have been detected [39]. ERα and ERβ differ markedly in the N-terminal A/B domains, exhibiting only about 20% amino acid identity. They also differ substantially in the hormone-binding domain. The differences in the A/B domains suggest that the transcriptional activation of different estrogen-responsive genes by ERα and ERβ may play different roles in carcinogenesis. It is well known that the presence of ERα in breast and endometrial carcinoma is associated with a less aggressive phenotype [39–41]. However, the roles of ERβ in the development and growth of these tumors have not been as completely elucidated. The ratio of ERα/ERβ differed between normal and cancerous tissues and a higher ERα/ERβ ratio was reported in breast and endometrial carcinoma [42–44]. ERβ mRNA was detected in 36% of endometrial carcinoma cases, whereas ERα mRNA hybridization signals were detected in 80% of those cases. ERβ was co-expressed with ERα and the estrogenic effects were considered to occur predominantly through ERα in endometrial carcinomas [43].

2) Progesterone receptors

The status of the progesterone receptor (PR) in endometrial and breast carcinoma has been considered an independent prognostic factor of the patients [40, 45]. Progesterone receptor (PR) is present in two isoforms, termed PRA and PRB. These isoforms are translated from the same gene, following initiation of transcription from different promoters [46]. There have been several studies, which have reported individual effects of PR isoforms. PRA can repress PRB activity in cells in which PRA was not transcriptionally active, and PRA might be associated with a cell- and promotor-specific repressor of PRB [47]. In addition, microarray analyses of human breast cancer cells expressing either PRA or PRB have confirmed that each of the PR isoforms has a unique set of target genes, with little overlap [48]. These functional and transcriptional differences suggest that the development, invasiveness, and metastatic potential of carcinoma cells can be influenced by the PR status of the tumor cells. In breast carcinoma, a significant proportion of tumors expressed very low levels of PRB and consequently exhibited a high PRA/PRB ratio [49]. PRA predominated in invasive ductal carcinoma [50]. In addition, breast carcinoma patients with PRA-rich tumors were generally associated with poorer disease-free survival rates or adverse clinical outcomes [51]. PRA overexpression was also associated with altered adhesive properties.

Reduced expression of either one or both of the PR isoforms has been observed in the great majority of endometrial carcinomas, compared with hyperplastic or

normal endometrium [52]. Several studies have demonstrated that PRB was more common than PRA in endometrial carcinoma [53, 54]. Very recently, we reported that cases negative for either one or both of the PR isoforms were associated significantly with shorter disease-free and overall survival of the patients [54]. In addition, multivariate analysis demonstrated that an absence of PRA immunoreactivity was an independent risk factor in disease-free survival of the patients. The results of our study indicated that the loss of expression of PR isoforms, especially that of PRA, may result in more aggressive biological characteristics in human endometrioid endometrial carcinoma that can play important roles in the prognosis and/or recurrence, in these patients.

In summary, these results all indicated that the biological significance of PR isoforms differ markedly between endometrial and breast carcinomas.

4. New Aspects with Hormone-Related Drugs

1) Progestin Therapy

The standard or conventional therapy for early endometrial carcinoma is established through staging laparotomy with total abdominal hysterectomy and bilateral salpingo-oophorectomy, which deprives these patients of any potential for fertility. Therefore, a more conservative medical treatment may be considered in young patients who may wish to preserve their fertility. Approximately 3–5% of patients with these neoplasms are under age of 40, some of whom have been treated with progestin, especially with high dose of MPA, alone as a primary endocrine therapy for both atypical endometrial hyperplasia and endometrioid endometrial adenocarcinoma. This approach is by no means a standard therapy and should not be recommended routinely, even when the patients desire. However, this approach has been supported by several reports in patients desiring to maintain fertility, despite the fact that the subsequent success rate of pregnancy is not necessarily high [55, 56]. The initial response rate of MPA ranged from 55% to 100% for endometrial carcinoma and 70 to 100% for atypical hyperplasia [55, 56]. In a recent multi-center study, Ushijima *et al.* reported the results of 17 cases of atypical endometrial hyperplasia and 28 cases of endometrioid endometrial carcinoma [57]. Complete response was detected in 64% of overall patients. Nine cases became pregnant and six cases delivered babies.

Atypical hyperplasia and endometrial carcinoma, especially of the well-differentiated endometrioid type, often express PR, and their growth is suppressed by progestin. In general, the effect of progestin is considered to be mediated through PR, because the response rate to MPA in PR-positive carcinoma was higher (70%) compared with PR-negative tumors (16%) [58]. We previously demonstrated that the *in situ* abundance of 17-HSD type 2, which catalyzes the conversion of the potent estrogen, E2, to an inactive form, E1, and PR, especially PRB, can predict the responsiveness of patients with endometrioid endometrial carcinoma to progestin treatment [59]. We also demonstrated that 17-HSD type 2 was only detected in the cytoplasm of the glandular cells during the secretory phase, but not in the proliferative phase endometrium [19, 34]. In addition, progestin stimulates the expression of 17-HSD type 2 in epithelial cells of human endometrial tissue [60]. Progestin may exert a potent anti-estrogenic effect in the endometrium by inducing 17-HSD type 2 and thereby promoting the regression of endometrial proliferative disease.

2) Progestin Therapy & the Organic Cation Transporter SLC22A16

Adriamycin is one of the key drugs for treatment of endometrial cancer. However, the molecular mechanisms by which anticancer drugs enter cells across the plasma membranes are much less clear. Recently, SLC22A16, which is one of newly-isolated organic cation transporters, was demonstrated to be responsible for uptake and transport of adriamycin into cells [61]. SLC22A16 mRNA, normally expressed in adult testis and bone marrow and fetal liver, has also been detected in various cancer cell lines, particularly cell lines derived from the liver and colon. Okabe *et al.* demonstrated that Xenopus oocytes injected with SLC22A16 cRNA imported adriamycin in a saturable and dose-dependent manner, and SLC22A16 overexpressing leukemic cells became significantly more sensitive to adriamycin treatment in cytotoxicity assays [61]. Very recently, we examined the expression of SLC22A16 in human endometrium and its disorders [62]. Immunohistochemical analysis demonstrated that the SLC22A16 protein was highly expressed in endometrium during the normal secretory phase, but

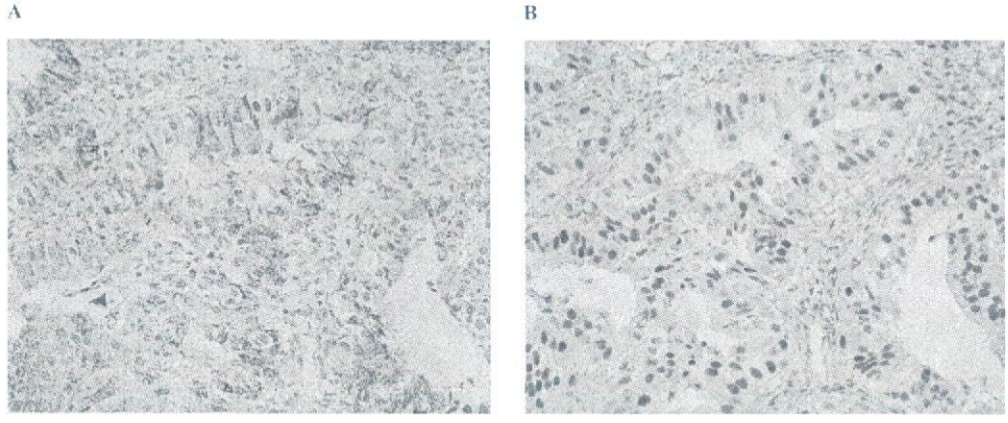

Fig. 3. Immunohistochemical staining of endometrioid endometrial carcinoma in serial tissue sections. A, Polyclonal antibody against SLC22A16. B, Mouse monoclonal antibody against PR. The vast majority of SLC22A16-immunoreactive cancer cells were also positive for PR. Original magnification, ×400. (Ref. 62. Reproduced with permission from Sato *et al.*: *Int J Gynecol Pathol* 26: 53–60. 2007)

that its level was significantly reduced during the proliferative phase. SLC22A16 protein was detected in 59 of 124 (48%) endometrial cancer specimens and 3 of 7 (43%) endometrial carcinoma cell lines. There was also a significant positive correlation between SLC22A16 and progesterone receptor expression (Fig. 3). Furthermore, SLC22A16 mRNA levels were increased in endometrial cancer cell lines in the presence of progesterone. These results suggest that it may be possible to use progestins to increase the response of endometrioid endometrial carcinoma to adriamycin-based chemotherapeutic regimens through SLC22A16 expression.

3) Aromatase inhibitor

Aromatase inhibitors are considered the most effective endocrine treatments in the postmenopausal patients with estrogen-dependent breast carcinoma [4]. The large multi-center trials all demonstrated that aromatase inhibitors contributed significantly to improved disease-free survival and good tolerability in breast carcinoma patients [4, 5]. There remain some controversies as to whether or not aromatase inhibitors are effective in patients with endometrial carcinoma, although intratumoral aromatase activity is more frequently detected in endometrial endometrioid carcinoma than in breast carcinoma [22, 63, 64]. We previously examined the biological changes in endometrial carcinoma tissues before and after aromatase inhibitor treatment. Five of 15 human endometrial carcinoma demonstrated decreased [^{3}H] thymidine uptake or Ki-67 labeling following aromatase inhibitor treatment [22]. Berstein *et al.* reported similar results [63]. A Gynecologic Oncology Group Study (GOG) was not able to demonstrate distinct clinical efficacy with aromatase inhibitor treatment. Partial responses were detected in 9% of 23 unselected patients with recurrent or persistent endometrial carcinoma, most of which had poorly differentiated tumors [64]. Recently, a multi-center phase II trial for letrozol was conducted in 32 recurrent or advanced endometrial carcinomas in postmenopausal women, and one of 28 (4%) case had a complete response, two (7%) were associated with partial responses, and 11 out of 28 (39%) patients had a stable disease for a median duration of 7 months [65]. Therefore, the roles of aromatase inhibitors in well-differentiated hormone-receptor-positive or hormone-sensitive endometrial carcinoma remain in dispute. Further studies, including the possibility of application and indication of aromatase inhibitors, are required to establish an aromatase inhibitor therapy as one form of endocrine treatment of endometrial carcinoma in post-menopausal patients.

4) Retinoids

Retinoids, metabolites of vitamin A, have been demonstrated to play an important role in *in situ* estrogen metabolism through the regulation of steroid hormone receptors and 17-HSDs. In a breast carcinoma cell line, retinoids increased the level of 17-HSD type

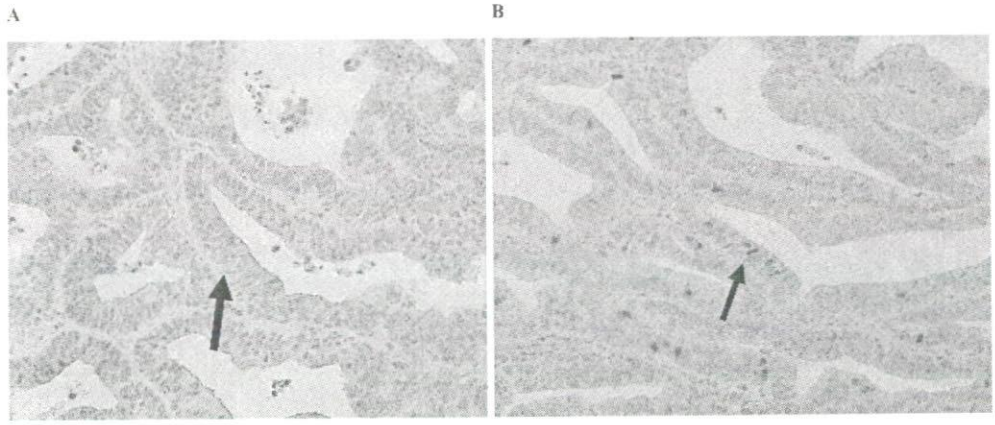

Fig. 4. Immunohistochemistry for PPARγ and p21. (×200) Arrows: immunoreactive cells. A, PPARγ immunoreactivity was detected in the nuclei of the endometrial carcinoma cells. B, p21 immunoreactivity was also detected in the nuclei of the endometrial carcinoma cells. (Ref. 79. Reproduced with permission from Ota *et al.*: *Clin Cancer Res* 12: 4200–4208. 2006)

1 mRNA [66]. Suzuki *et al.* revealed a significant correlation between retinoic acid receptor α and 17-HSD type 1 expression in breast carcinoma [67]. In addition, retinoids are considered to be effective chemopreventive and chemotherapeutic agents in a variety of human epithelial and hematopoietic neoplasms [68, 69]. Kudelka *et al.* reported clinically favorable results following retinoid-based treatment of patients with cisplatin-resistant metastatic endometrial carcinoma [69]. We previously reported that retinoids markedly increased the level of 17-HSD type 2 mRNA in a time- and dose- dependent manner in an endometrial carcinoma cell line [33]. We also detected a significant correlation between retinoid X receptor γ and 17beta-HSD type 2 expression in endometrial carcinoma. Our results suggest that retinoid is involved in the modulation of *in situ* estrogen metabolism by stimulating the expression of 17-HSD type 2 and may be one of the important candidates as a new endocrine-related agent in endometrial carcinoma. However, further clarification remains necessary.

5) Peroxisome proliferator-activated receptor (PPAR) ligand

Peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear hormone receptor superfamily of transcription factors. PPARs function as transactivation factors following heterodimerization with retinoid X receptors (RXRs), and bind to specific response elements of various target genes [70]. PPARs have a subfamily of three different isoforms: PPARα, PPARβ/δ, and PPARγ.

PPARγ plays important roles in the regulation of lipid homeostasis, adipogenesis, insulin resistance, and in the development of various organs [71, 72]. The naturally occurring PPARγ ligand, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), activates PPARγ at micromolar concentrations in humans *in vivo* [73]. Synthetic PPARγ ligands, known as thiazolidinediones (TZDs), have been used for the treatment of insulin resistance in type II diabetes mellitus. In addition, TZDs have been proposed in differentiation-mediated therapy of various human carcinomas associated with high levels of PPARγ [74]. Various *in vitro* studies have demonstrated that PPARγ ligands exhibit a potent antiproliferative activity for a wide variety of neoplastic cells [75]. A PPARγ agonist was reported to inhibit the proliferation of carcinoma cells, and phase II clinical trials using PPARγ ligands have recently been performed as a novel therapeutics for patients with advanced breast carcinoma, histologically-confirmed prostate carcinoma, liposarcoma, and metastatic colon carcinoma [76].

The expression and effectiveness of PPARγ has been extensively studied in various human carcinomas but little is known about PPARγ in uterine endometrial carcinoma. In addition, obesity, excess estrogen, type II diabetes, and hypertension are important risk factors for endometrial carcinoma [77, 78], but the effects of PPARγ agonists on endometrial carcinoma are largely unknown. Very recently, we examined the expression

of PPARγ mRNA and protein in normal endometria and its disorders [79]. PPARγ immunoreactivity was detected in 11/23 (48%) cases of proliferative phase endometrium, 14/19 (74%) cases of secretory phase endometrium, 27/32 (84%) cases of endometrial hyperplasia, and 67/103 (65%) cases of carcinoma. PPARγ immunoreactivity was significantly lower in endometrial carcinoma than in secretory phase endometrium and in endometrial hyperplasia. There was a significant positive association between the status of PPARγ and p21 expression in endometrial carcinoma (Fig. 4). In addition, the PPARγ agonist, 15d-PGJ_2 inhibited cell proliferation and induced p21 mRNA in endometrial carcinoma cell lines. Our findings suggest that the PPARγ ligand, 15d-PGJ_2, exhibits antiproliferative activity against endometrial carcinoma. These results strongly suggest that synthetic PPARγ ligands should be important drug candidates, not only for prevention but also for endocrine treatment of endometrial carcinoma.

5. Hormone replacement therapy (HRT) and the risk of malignancies

Hormone replacement therapy (HRT) has been available for many years. HRT is the most effective intervention to date for the relief of estrogen-deficiency symptoms after menopause. The use of HRT has increased among postmenopausal women worldwide [80]. However, the risk of malignancies associated with HRT remains controversial.

The possible increased risks of endometrial carcinoma associated with exogenous estrogen in postmenopausal women were postulated in the 1970s. This risk of endometrial carcinoma increases in a dose- and time-dependent manner in women receiving estrogen alone (ERT; estrogen replacement therapy). Estrogens at higher levels and over the long-term increased the risk of endometrial carcinoma 5-fold and beyond [81]. Several studies have reported that the addition of progestin to estrogen (HRT; hormone replacement therapy) reduced the increased incidence of endometrial carcinoma associated with unopposed estrogen [81–83]. The Women's Health Initiative (WHI) study randomized 16608 women; 8506 were treated with HRT and 8102 received placebo [84]. Endometrial carcinomas were observed in 22 patients of the HRT group (0.05%) and in 25 patients of the placebo group (0.06%), corresponding with a hazard ratio of 0.83 (95% CI 0.47–1.47) [85]. Similar results were found in the Heart and Estrogen/Progestin Replacement Study (HERS) and the Million Women Study (MWS) [86, 87]. Considering the recent results of the WHI-, HERS-, and MWS-studies, the available data have clearly demonstrated that combined HRT reduced the risk of development of human endometrioid endometrial carcinoma.

However, the WHI study recently demonstrated the possible increasing risks of breast cancer associated with HRT. The WHI study showed that women receiving estrogen plus progestin (HRT) exhibited an increased risk of invasive breast carcinoma (hazard ratio (HR), 1.24; 95% CI, 1.01–1.54), although women receiving estrogen alone (ERT) demonstrated no increased risk of occurrence of invasive breast carcinoma (HR, 0.77; 95% CI, 0.59–1.01) [84, 88, 89].

These clinical data suggest that the biological roles of estrogen and progestin in tumorgenesis are different between the endometrium and breast, although both are considered "estrogen-dependent tissues".

6. Conclusion

The enzymes responsible for intratumoral estrogen metabolism and biosynthesis are markedly different between human breast and endometrial carcinoma, although both are considered "estrogen-dependent malignancies". 17-HSD type 1 plays an important role in the regulation of high E2 levels in breast carcinoma tissues, while 17-HSD type 1 was not detected and 17-HSD types 2 and 5 are essential for the maintenance of E2 concentrations in endometrial carcinoma tissues (Fig. 1). In addition, the biological significance of the PR isoforms differs between endometrial and breast carcinomas. Clinical data concerning HRT and estrogen-dependent cancer risk also support these findings. These basic and clinical findings help to understand the biology and provide the new knowledge for the prevention, diagnosis and treatment of human endometrial carcinoma. Although aromatase inhibitors are the most effective endocrine treatments for estrogen-responsive breast carcinoma, specific endocrine treatment of endometrial carcinoma should be considered in the future. However, this awaits further investigations for clarification.

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