Table 1. Summary of the relationship between retinoic acid receptor (RAR) expression and clinicopathological findings in endometrial cancer

Clinicopathological characteristic (number of patients)	RAR			*P*-value
	α	β	γ	
Stage				
I (66)	18.11 ± 10.06	36.62 ± 29.80	3.45 ± 3.17	NS
II (12)	22.33 ± 19.05	25.92 ± 30.19	3.25 ± 3.93	
III (22)	16.91 ± 11.18	33.50 ± 26.44	5.77 ± 8.69	
IV (3)	17.67 ± 5.51	35.33 ± 30.62	3.67 ± 2.08	
Grade				
Well-differentiated (49)	17.59 ± 9.62	36.27 ± 28.78	3.88 ± 3.42	NS
Moderate-differentiated (32)	22.19 ± 14.97	37.22 ± 30.36	3.28 ± 3.08	
Poorly differentiated (22)	14.36 ± 7.72	27.41 ± 27.33	5.00 ± 8.78	
Myometrial invasion				
<1/2 (62)	18.34 ± 11.74	34.08 ± 30.49	3.25 ± 2.98	NS
≥1/2 (38)	17.76 ± 11.42	33.05 ± 27.30	3.92 ± 3.35	
Vessel involvement				
+ (29)	17.62 ± 12.32	31.55 ± 29.74	3.83 ± 3.14	NS
− (34)	16.47 ± 6.72	35.38 ± 30.72	3.03 ± 2.94	
Recurrence				
+ (16)	15.19 ± 8.48	26.31 ± 26.27	3.06 ± 2.82	NS
− (87)	18.91 ± 11.93	36.21 ± 29.30	4.09 ± 5.26	
Prognosis				
Alive (95)	18.52 ± 11.57	34.14 ± 29.31	9.00 ± 14.04	NS
Dead (8)	16.13 ± 11.15	41.00 ± 25.07	3.51 ± 3.11	

For evaluation of RARs' expression, we determined the labeling index. Results are expressed as the mean ± SE. NS, not significant.

expression of each histological type. There was no significant correlation between the expression of each of the RAR subtypes in endometrial carcinoma (data not shown).

The relationships between the expression of the RAR subtypes and the clinicopathological findings in endometrial carcinoma are summarized in Table 1. There was no statistically significant correlation between LI for any of the other RAR subtypes and the clinicopathological parameters, including clinical stage, histological grade, myometrial invasion, vascular involvement, recurrence rate, and overall survival.

Discussion

Retinoic acids exhibit diverse biological properties that may potentially contribute to their antitumor effect. They inhibit cell proliferation and angiogenesis, and can induce cell differentiation and apoptosis.[16,17] RARβ repression has been reported in preneoplastic oral-cavity lesions,[18] non-small-cell lung cancer,[19–21] breast cancer,[22] and esophageal cancer.[23] Although other retinoid receptors were expressed in these tissues, only RARβ levels were significantly lower in the premalignant and tumor tissues. RARβ expression was selectively lost in premalignant oral lesions, and was able to be restored by retinoic acid treatment.[18] The restoration of RARβ expression was associated with a clinical response, suggesting a role for RARβ, both as a mediator of the retinoic acid response and as a biological marker in chemoprevention trials.[18] This was confirmed in renal cancer, in which upregulation of RARβ correlated with a response to 13-*cis*-retinoic acid and interferon α-2a.[24] Thus, the correlation with RARβ repression led to the hypothesis that RARβ could act as a tumor suppressor. In addition, introduction of RAR-β protein into retinoic acid-insensitive breast cancer cell lines has been shown to restore retinoic acid responsiveness.[25] In our study, RARβ was detected predominantly in endometrial hyperplasia, compared with endometrial carcinoma. These results suggest that suppression of RAR-β expression may inhibit the differentiation of endometrial epithelium in endometrial carcinoma.

In recent studies, the retinoid isotretinoin was not effective for chemoprevention in stage I non-small-cell lung cancer or early stage head and neck squamous-cell carcinoma.[26,27] The retinoid-signaling pathway was studied in normal and neoplastic tissues to determine why preclinical retinoid activity did not readily translate into clinical success. It was discovered that expression of RARβ is frequently silenced in epithelial carcinogenesis, which led to the hypothesis that RARβ acts as a tumor suppressor that is partially responsible for the limited clinical activity of classical retinoids.[28,29] To examine the effect of the RAR-specific ligand AM580 on RARβ expression, we carried out MTT assay and real-time RT-PCR analysis using the Ishikawa cell line. Although AM580 inhibited cell growth and induced RARβ mRNA expression in Ishikawa cells, no statistically significant correlation was obtained between the expression of RARβ and clinicopathological parameters in human endometrial carcinoma. RARβ has four isoforms that are generated differentially by means of the promoters P1 and P2 and alternative splicing.[30] Our studies evaluated RARβ expression as a monolithic entity and did not distinguish between the various RARβ isoforms that have been identified in humans. Differential expression of different RARβ isoforms, at least in part, might underlie the contradictory associations of RARβ expression. However, it awaits further investigations for clarification.

Retinoids are useful tools for identifying critical target genes and pathways that can reduce carcinogenesis.[31,32] Accumulating evidence suggests that retinoids play a role in regulating the function of the endometrium.[33,34] Retinoids have also been reported to affect the expression of a number of genes in the endometrium, such as matrix metalloproteinases and interleukin-6.[35] Although the profile of retinoid receptors of epithelial cells has been elucidated,[11,12,36] the effect of retinoids on the proliferation of normal epithelial cells remains unknown. In our study, AM580 inhibited cell growth and induced RARβ mRNA expression in Ishikawa cells, and the expression level of RARβ in endometrial carcinoma was significantly lower than that in endometrial hyperplasia. AM580 might possibly be used as a treatment for

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endometrial carcinoma. However, it awaits further investigations for clarification.

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Progesterone receptor isoforms as a prognostic marker in human endometrial carcinoma

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The possible role of specific progesterone receptor (PR) isoforms (PRA and PRB) as predictive factors in endometrial carcinoma is unclear. The present study was undertaken to evaluate the clinical significance of intratumoral PR isoform status in patients with endometrioid endometrial carcinoma. We studied 103 cases of endometrioid endometrial carcinoma using immunohistochemistry. We correlated the findings with various clinicopathological parameters of the patients. PRA and PRB immunoreactivity was detected in 51/103 (48.5%) and 79/103 (76.7%) of carcinoma cases, respectively. A significant positive correlation was detected between the status of PRB immunoreactivity and the amount of PRB mRNA by real-time reverse transcription–polymerase chain reaction ($P = 0.012$). PR isoform expression was significantly lower in the cases with higher histological grade ($P = 0.0001$ and $P = 0.002$, for PRA and PRB, respectively). Cases that were negative for either one or both PR isoforms were significantly associated with shorter disease-free and overall survival of the patients. The absence of either one or both of these two PR isoforms was detected in all nine patients who died (100.0%), whereas the absence of these immunoreactivities was detected only in 43 of 94 (45.7%) patients who had lived during the same period. In addition, multivariate analysis demonstrated that an absence of PRA immunoreactivity was an independent risk factor in disease-free survival of the patients ($P = 0.0258$). The results of our study demonstrated that loss or absence of PR isoform expression determined by immunohistochemistry could become an important prognostic indicator in patients with endometrioid endometrial carcinoma. (*Cancer Sci* 2006; 97: 1308–1314)

Endometrial carcinoma is one of the most common malignancies of the female genital tract and its incidence, especially that of endometrioid endometrial carcinoma, has increased recently.[1] It is well known that uterine endometrial proliferation is under the control of both estrogen and progesterone. One of the physiological roles of progesterone in the regulation of glandular epithelium of the endometrium is to induce cellular differentiation and to antagonize estrogen-mediated cell proliferation.[2] Endometrial carcinogenesis is strongly associated with continued estrogen exposure without progesterone influence.[3,4] Progesterone has clinically been demonstrated to provide some protection against stimulatory effects of estrogenic agents. In addition, hormone replacement therapy using combinations of estrogens and progesterones yields a lower risk of endometrial carcinoma, despite increasing the incidence of breast carcinoma.[5,6] A number of the patients who wished to preserve their fertility were treated with progestin as a primary endocrine therapy for atypical hyperplasia and well-differentiated adenocarcinoma, although the effects of this treatment on the clinical outcome of patients have not always been satisfactory.[7–9]

Both estrogen and progesterone act through intranuclear receptors, estrogen receptors (ER) and progesterone receptors (PR), which belong to the superfamily of steroid hormone receptors.[10] 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 progesterones in the great majority of estrogen-responsive cells.[11] In normal cycling human endometrium, PR is expressed abundantly in glandular epithelium during the proliferative phase of the cycle.[12] PR is present in two isoforms, termed PRA and PRB.[13] PRA is the truncated form of PRB, lacking 164 amino acids at the NH_2 terminus. These isoforms are translated from the same gene, but transcription is initiated from different promoters.[14] Studies addressing the individual effects of PR isoforms have been reported. Vegeto *et al.* reported that PRA could repress PRB activity in cells in which PRA was not transcriptionally active, and that PRA might be associated with a cell- and promotor-specific repressor of PRB.[15] Giangrande *et al.* also reported that differential cofactor binding resulted in the opposing transcriptional activities of PRA and PRB.[16] In addition, microarray analyses of human breast cancer cells expressing either PRA or PRB have confirmed that each PR isoform has a unique set of target genes, with little overlap.[17] 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. We previously reported that loss of PRB was a significant prognostic factor in epithelial ovarian cancer.[18,19] In addition, breast carcinoma patients with PRA-rich tumors are in general associated with poorer disease-free survival rates.[20] In endometrial carcinoma, several studies demonstrated the PR isoform status of carcinoma cells.[21–23] Arnett-Mansfield *et al.* reported a reduced expression of either one or both of the PR isoforms in the great majority of endometrial tumors, compared with hyperplastic or normal endometrium.[21] De Vivo *et al.* demonstrated a polymorphism in the PRB promoter, which results in increased transcription of the PRB isotype. In a population-based study, this polymorphism was reported to be associated with increased risk for endometrial carcinoma.[22] In addition, hypermethylation of PRB alleles was detected in endometrial carcinoma.[23]

Results of previous studies demonstrated that high levels of ER and PR were directly correlated with a lower tumor grade, less myometrial invasion, and a lower incidence of lymph node metastases in the patients with endometrioid endometrial carcinoma.[24–27] In addition, the status of ER and PR in these carcinomas has been reported as an independent prognostic factor of the patients.[28] However, it is also true that there are many controversies regarding the possible roles of specific PR isoforms as predictive factors in endometrial carcinoma.[21,29–32] Fujimoto *et al.* reported that PRA could not be detected in advanced endometrial tumors.[29] In accordance with this, they later reported that PRB was expressed predominantly in distant metastases of endometrial carcinoma.[30] In contrast, Kumar *et al.* reported that downregulation of PRB may be associated with poorly differentiated endometrial carcinoma.[31] Sakaguchi *et al.*

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also proposed that the drastic decrement of PRB but not of PRA resulted in poor prognosis in endometrial carcinoma, although histological type was not described in their study.[32]

Therefore, in the present study, we carried out immunohistochemical analysis of 103 cases of endometrioid endometrial carcinoma, and correlated the findings with the clinicopathological features of the patients, including their clinical outcome, in order to study the possible roles and correlation between PR isoforms and prognosis of the patients.

Materials and Methods

Endometrial carcinoma patients and tissue preparation. One hundred and three endometrioid endometrial carcinomas (49 well differentiated, 32 moderately differentiated, 22 poorly differentiated; 66 stage I, 12 stage II, 22 stage III, 3 stage IV) were retrieved from the surgical pathology files of Tohoku University Hospital, Sendai, Japan. The protocol for this study was approved by the Ethics Committee at Tohoku University School of Medicine (Sendai, Japan). None of the patients examined had received irradiation, hormonal therapy or chemotherapy prior to surgery. The median follow-up time of the patients examined in this study was 60 months (range, 2–148 months). The disease-free and overall survival times of the patients were calculated from the time of initial surgery to recurrence or death, or the date of last contact. The survival times of patients still alive or lost to follow-up were censored in December 2004. The clinicopathological findings of the patients, including age, histology, stage, grade and preoperative therapy, were retrieved by extensive review of the charts. A standard primary treatment for endometrial carcinoma at Tohoku University Hospital was surgery consisting of total abdominal hysterectomy, salpingo-oopholectomy, pelvic and/or para-aortic lymphadenectomy and peritoneal washing cytology. Eighty-five out of 103 patients (83%) in this study underwent complete surgery as above. Six out of 85 patients had lymph node metastasis. The remaining 18 patients (17%) underwent total abdominal hysterectomy and salpingo-oopholectomy without lymphadenectomy because of obesity or their poor performance status. The lesions were classified according to the Histological Typing of Female Genital Tract Tumors by WHO and staged according to the International Federation of Gynecology and Obstetrics system.[33,34] Sixty-eight out of 103 patients received pelvic radiation therapy (50 Gy) or three to six courses of chemotherapy, consisting of the cisplatin-based combination regimen CAP (60–70 mg/m^2 cisplatin, 40 mg/m^2 doxorubicin and 500 mg/body cyclophosphamide) after operation. Patients who had early stage and low-grade disease (stage IA, G1, stage IA, G2 and stage IB, G1) and patients who were associated with poor performance status did not receive any adjuvant therapy. None of the patients received hormone therapy after operation. All specimens were processed routinely (i.e. 10% formalin fixed for 24–48 h, paraffin embedded, and thin sectioned [3 μM]).

Antibodies. Monoclonal antibodies for PRA (hPRa7) and PRB (hPRa2) were purchased from NeoMarkers (Fremont, CA, USA). The PRA (hPRa7) antibody used in this study recognized both PRA and PRB in immunoblot analysis.[35] However, Mote *et al.* reported that hPRa7 did not recognize PRB on immunohistochemistry in fixed tissues even after antigen retrieval, as evidenced by the absence of immunostaining by this antibody of the PRB-expressing MDA-MB-231/PRB cell line.[36] This was considered to be due to the inaccessibility of the epitope on PRB recognized by hPRa7 in 10% formalin-fixed and paraffin-embedded tissue specimens, possibly due to alteration of the conformation of the molecule in which the hPRa7 epitope is located in such a way to reduce its accessibility in immunohistochemistry. hPRa2 recognizes PRB exclusively.[35,37] Monoclonal antibodies for ERα, ERβ and Ki67 were purchased from Novocastra (Benton, NC, UK), Genetex (San Antonio, TX, USA) and DAKO Cytomation (Carpinteria, CA, USA), respectively.

Immunohistochemistry. Immunostaining was carried out by the streptavidin–biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan). Antigen retrieval was carried out using an autoclave treatment for 5 min in citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate, pH 6.0). The dilutions of the primary antibodies used in our study were as follows: PRA, 1/100; PRB, 1/100; ERα, 1/50; ERβ, 1/1500; and Ki67, 1/50. The antigen–antibody complex was visualized with 3,3′-diaminobenzidine (DAB) solution (1 mM DAB, 50 mM Tris-HCl buffer [pH 7.6] and 0.006% H_2O_2), and counterstained with hematoxylin. Proliferative-phase endometrial glands were used as positive controls for immunohistochemistry of PR isoforms[26] and breast cancers were used as positive controls for ERα and ERβ. As a negative immunostaining control, normal rabbit or mouse IgG was used instead of the primary antibodies. No specific immunoreactivity was detected in these tissue sections.

Scoring of immunoreactivity. Evaluation of PRA, PRB, ERα, ERβ and Ki-67 was carried out in high-power fields (×400) using a standard light microscope. Two of the authors (SS and KI) searched all of the tissue sections simultaneously and determined the most representative areas using a double-headed light microscope. In all of the cases examined, a total of more than 500 tumor cells from three different representative fields were counted independently by the two authors, and the percentage of immunoreactivity (i.e. the labeling index [LI]) was determined. After completely reviewing the immunostained sections of each lesion, two of the authors (SS and KI) independently divided the cases into the following two groups: +, >10% positive cells; and –, <10% positive cells. Layfield *et al.* proposed the separation of ER- and PR-positive cases using LI cut-off points of 10% in the immunohistochemical analysis of human breast cancer.[38] The eighth St Gallen meeting also recommended that approximately 10% positive staining of cells for either ER or PR might be considered as a reasonable threshold for definite endocrine responsiveness.[39] Therefore, in the present study, we used the same cut-off point of 10% between positive and negative PR isoforms, based on the results of the studies above. Cases with discordant results (interobserver differences of >5%) were reevaluated simultaneously the two authors above using a double-headed light microscope. Consequently, the interobserver differences were less than 5% in this study.

Reverse transcription–polymerase chain reaction. Thirty-three specimens of fresh frozen tissues of endometrial carcinoma (i.e. specimens frozen immediately in liquid nitrogen and stored at −80°C) were available for the present study. Total RNA was extracted by homogenizing frozen tissue samples in 1 mL TRIzol reagent (Life Technologies, Gaithersburg, Grand Island, NY, USA), followed by phenol–chloroform extraction and isopropanol precipitation. All RNA samples were quantified by spectrophotometry and stored at −80°C until processing for reverse transcription (RT). Total RNA (4 μg) was denatured at 70°C for 10 min and was reverse transcribed in the presence of 50 ng/μL Oligo (deoxythymidine) primer (Invitrogen, Carlsbad, CA, USA), 2.5 mmol/L $MgCl_2$, 0.5 mmol/L deoxy-NTPs, 10 mmol/L dithiothreitol and 10 IU ribonuclease H-reversed transcriptase (Superscript II RT, Invitrogen) for 60 min at 42°C and 15 min at 70°C on a PTC-200 Peltier Thermal Cycler DNA Engine (MJ Research, Watertown, MA, USA). RT–polymerase chain reaction (PCR) analysis was carried out in order to examine the presence or absence of genomic DNA contamination. The RT step was performed in the absence of Superscript II RNase H-reverse transcriptase, followed by PCR. RT-PCR products lacking reverse transcriptase in the initial RT step were run on an ethidium-bromide-stained 2% agarose gel. No bands were detected in these samples (data not shown). After an initial

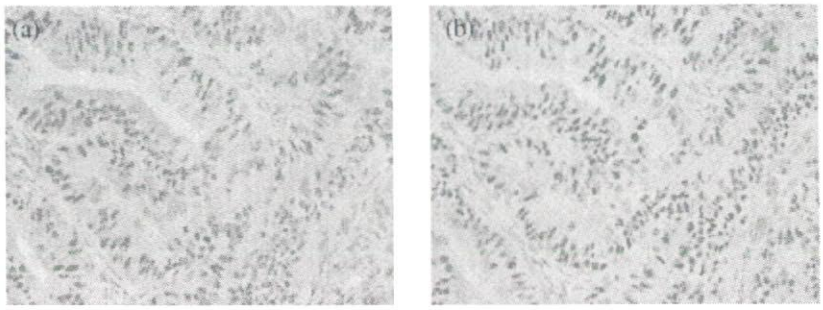

Fig. 1. Immunohistochemical staining for (a) progesterone receptor A (PRA) and (b) progesterone receptor B (PRB) in endometrioid endometrial carcinoma. PRA and PRB immunoreactive proteins were detected in the nuclei of carcinoma cells of G1 adenocarcinoma. Original magnification, ×400.

1 min denaturation step at 96°C, 35 cycles of PCR were carried out on thermal cycle under the following conditions: 45 s denaturation at 94°C, 30 s annealing at 58°C, and a 1.5 min extension at 72°C. In addition, cDNA was used as a template for real-time PCR. Real-time PCR was carried out with the Light Cycler System (Roche Diagnostics, Mannheim, Germany) using the DNA-binding dye SYBER Green I (Roche Diagnostics). The 20-μL reaction mixture contained 3 mM $MgCl_2$ for PRB and β-actin primer, 10 pmol/L of each primer and DNA-binding dye LightCycler-Fast Start DNA Master SYBR Green I. β-Actin expression was used to verify the integrity of RNA from each specimen. Human gene-specific primers used to amplify PRB and β-actin were as follows: PRB 5′ sense, ACACCTTGCC-TGAAGTTTCG and PRB 3′ antisense, CTGTCCTTTTCTGG-GGGACT (196 bp); β-actin 5′ sense, CCAACCGCGAGAA-GATGAC and β-actin 3′ antisense, GGAAGGAAGGCTGG-AAGAGT (459 bp). An initial denaturing step at 95°C for 10 min was followed by 35 cycles of 95°C for 15 s, 10 s annealing at 58°C (PRB) and 63°C (β-actin), and extension for 13 s at 72°C. The fluorescence intensity of the double-strand-specific SYBER Green I, which reflects the amount of specific PCR products formed, was read by the LightCycler at 85°C after the end of each extension step.[40] Using automated programs of the LightCycler software, the amount of PRB and β-actin template in each sample was calculated so as to dilute the standard cDNA equally. The actual values of PRB were corrected by the value of the β-actin template. Although conventional quantitative PCR requires the use of purified plasma cDNA in the construction of a standard curve, it was possible to semiquantify the PCR products with the LightCycler using purified cDNA of known concentrations.[41,42] In initial experiments, PCR products were purified and subjected to direct sequencing (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI PRISM 310 Genetic Analyzer; Perkin-Elmer PE Applied Biosystems, Foster City, CA, USA) to verify amplification of the correct sequences. Frozen breast cancer tissue was used as a positive control. Negative control experiments did not contain cDNA substrate to study the presence of exogenous contamination of DNA. No amplified products were detected under these conditions.

Statistical analyses. Statistical analysis was carried out using SAS software (StatView, Version 5.0; SAS, Cary, NC, USA). The statistical significance of the association between PRA and PRB immunoreactivity and other parameters (grade, stage, age, ERα LI, ERβ LI and Ki-67 LI) was evaluated using the Mann–Whitney *U*-test and the χ^2-test. The statistical significance between PRA and PRB immunoreactivity was calculated using a correlation coefficient (r) and regression equation. The statistical significance between PRB immunoreactivity determined by immunohistochemistry and the status of mRNA determined by RT-PCR was evaluated using Fisher's exact probability test, and the statistical significance between PRB immunoreactivity and amounts of PRB mRNA determined by real time RT-PCR was evaluated using the Mann–Whitney *U*-test. The Kaplan–Meier method and statistical significance was calculated using a log-rank test. Univariate and multivariate analyses were evaluated using Cox's proportional hazards model. P-values less than 0.05 were considered significant.

Results

Immunohistochemistry and RT-PCR. Immunoreactivity for PRA and PRB was detected in the nuclei of carcinoma cells (Fig. 1). ERα, ERβ and Ki-67 were also confined exclusively to the nuclei of epithelial cells (data not shown). RT-PCR was carried out to confirm the expression of PRB using 33 cases in this study (Figs 2,3), because PRA has no specific sequence to distinguish it from PRB mRNA by RT-PCR. Twenty-five of these 33 cases were PRB positive and eight cases were PRB negative, as determined by immunohistochemistry. PRB mRNA was detected in 21 out of these 25 PR-positive cases (84%) and was not detected in five out of eight PRB-negative cases (Fig. 2). There was a statistically significant positive correlation between PRB immunoreactivity and mRNA expression examined by RT-PCR analysis ($P = 0.02$). In addition, amounts of PRB mRNA determined by real time RT-PCR were 8.89 (median values) in these PRB-positive and 0.41 (median values) in PRB-negative cases. A significant positive correlation was detected between PRB immunoreactivity and the amounts of PRB mRNA ($P = 0.012$) (Fig. 3). Eighty out of 103 cases (77.7%) demonstrated either or both PR isoforms in immunohistochemistry. Fifty-one out of 103 cases (48.5%) were PRA positive. Among these 51 PRA-positive cases only one case (1.9%) was PRA positive and PRB negative. However, PRB-positive cases were 76.7% (79/103), and 29 of these 79 PRB-positive cases (36.7%) were both PRB positive and PRA negative. The proportion of cases positive for both PRA and PRB was 48.5% (50/103), whereas the proportion of cases negative for both PRA and PRB was 22.3% (23/103). There was a significant positive correlation between PRA and PRB expression in endometrial carcinoma ($P = 0.004$). Results of the associations between clinicopathological parameters and immunoreactivity of PRA and PRB are summarized in Table 1. The status of PRA in G1, G2 and G3 endometrial carcinoma was 67.3% (33/49), 46.6% (15/32) and 13.6% (3/22), respectively, and the status of PRB was 87.8% (43/49), 78.1% (25/32) and 50.0% (11/22), respectively. PR immunoreactivity was significantly lower for carcinoma with higher histological grade ($P = 0.0001$ and $P = 0.002$, for PRA and PRB, respectively), whereas there were no correlation among the clinical stages of the cases. PRA and PRB expression was significantly positively correlated with ERα LI, and inversely with Ki-67 LI.

Relationship between PR isoform expression and prognosis. Progesterone receptor isoform status was evaluated as a prognostic variable in the patients with endometrioid endometrial carcinoma using univariate analysis. Results of univariate analysis are summarized in Table 2. The following variables were significantly associated with poorer disease-free survival and overall survival of the patients at the $P < 0.05$ levels: absence of PRA immunoreactivity; absence of PRB immunoreactivity;

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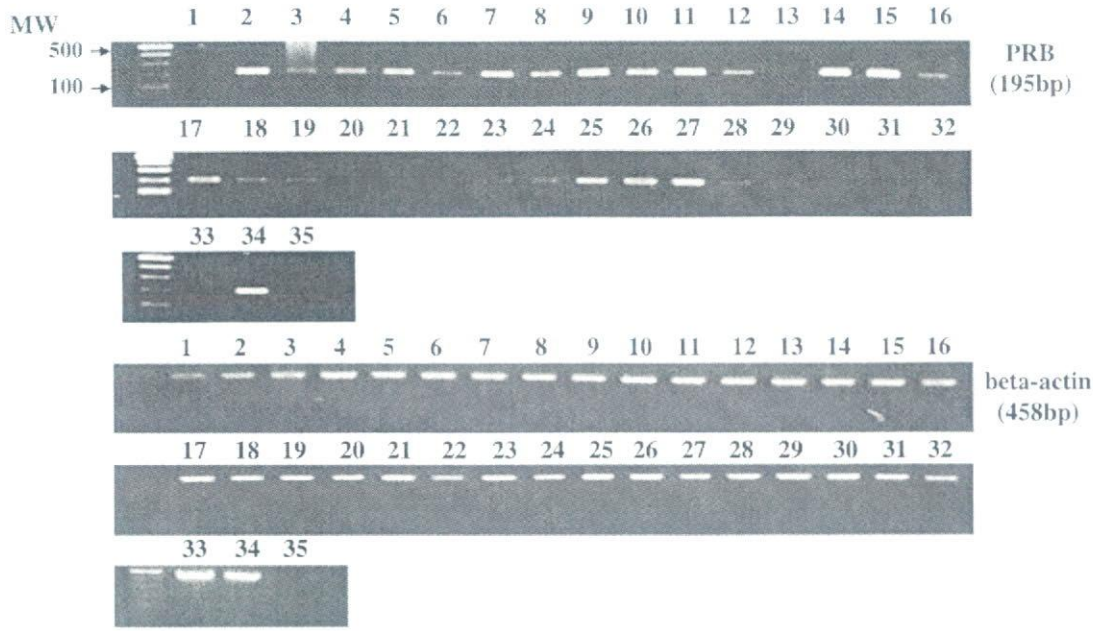

Fig. 2. Reverse transcription–polymerase chain reaction (RT-PCR) analysis of total RNA extracted from endometrioid endometrial carcinoma. Nos 4, 10, 13, 21, 29, 30, 31, 32 and 33 are progesterone receptor B (PRB)-negative cases, as determined by immunohistochemistry. No. 34 is a positive control. No. 35 is a negative control. PRB mRNA was detected in 21 out of these 25 PR-positive cases (84%) and not detected in five out of eight PR-negative cases. There was a statistically significant positive correlation between PRB immunoreactivity and mRNA expression examined by RT-PCR analysis ($P = 0.02$, Fisher's exact probability test).

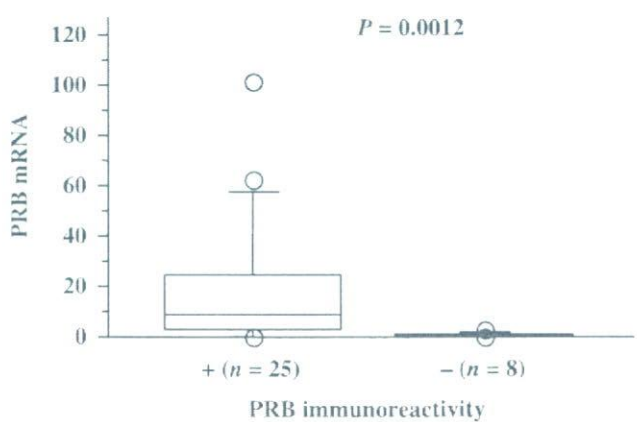

Fig. 3. Correlation between progesterone receptor B (PRB) immunoreactivity and its mRNA level determined by quantitative reverse transcriptin–polymerase chain reaction analyses in human endometrial carcinoma. There was a statistically significant positive correlation between PRB immunoreactivity and the amount of PRB mRNA ($P = 0.012$, Mann–Whitney U-test).

and histological grades. The disease-free and overall survival curves of the patients according to the Kaplan–Meier method are demonstrated in Fig. 2. The 5-year disease-free and overall survival rates were 95.6% and 96.4%, respectively, for PRA-positive cases and 71.1% and 84.3%, respectively, for PRA-negative cases. Patients with negative PRA in these carcinoma tissues were associated with a significantly poorer prognosis than those of PRA-positive cases at both disease-free ($P = 0.0009$) and overall survival ($P = 0.0098$) (Fig. 2A,B). Fig. 2 also demonstrates the greater disease-free and overall survival of the PRB-positive cases compared to PRB-negative cases ($P = 0.0007$ and $P = 0.0116$, respectively). The 5-year disease-free and overall survival times were 90.5% and 94.1%, respectively, for PRB-positive cases and 61.3% and 75.9%, respectively, for PRB-negative cases. In addition, the absence of either one or both of these two PR isoforms was associated with a significantly poorer prognosis at disease-free survival ($P = 0.0005$) (Fig. 2C). In addition, the absence of either one or both of these two PR isoforms was detected in all nine patients who died (100.0%), whereas the absence of these immunoreactivities was detected only in 43 of 94 (45.7%) patients who lived during the same period.

In order to determine whether the prognostic value of PRA or PRB expression was independent of other risk factors associated with clinical outcome of the patients with endometrioid endometrial carcinoma, we examined the results using multivariate analysis. The prognostic factors examined were the status of PRA or PRB, ER, stages and histological grades. As shown in Table 3, absence of PRA in carcinoma tissue was statistically significant as an independent risk factor only in disease-free survival of the patients ($P = 0.0258$), although PRB status was not a significant factor in disease-free or overall survival. Histological grade turned out to be an independent risk factor only in overall survival of the patients.

Discussion

This is the first study demonstrating that the absence of not only PRA but also PRB expression determined by immunohistochemistry is an important prognostic indicator of patients with endometrioid endometrial carcinoma. Progesterone is known to be one of the very important endocrine factors regulating cellular proliferation of the endometrium and its effects are mediated through PR.[10] PR has two isoforms, PRA and PRB, but the exact biological or clinical differences between the roles

Table 1. Correlation between progesterone receptor isoform A and B (PRA and PRB) immunoreactivity and clinicopathological parameters in endometrial carcinoma

Parameter	Total (n = 103)	PRA			PRB		
		+ (n = 51)	− (n = 52)	P-value	+ (n = 79)	− (n = 24)	P-value
Age (years)							
50	22	15	7		19	3	
>50	81	36	45	**0.048**	60	21	0.27
Grade							
1	49 (47.6%)	33	16		43	6	
2	32 (31.0%)	15	17		25	7	
3	22 (21.4%)	3	19	**0.0001**	11	11	**0.002**
Stage							
I, II	78 (75.7%)	40	38		63	15	
III, IV	25 (24.3%)	11	14	0.526	16	9	0.08
ERα LI (median)	23	34	11	**0.003**	34	4.5	**<0.0001**
ERβ LI (median)	5	5	8	0.3	11	2	0.089
Ki67 LI (median)	32	27	40	**0.003**	30	46	**0.002**

ER, estrogen receptor; LI, labeling index.

Table 2. Univariate analyses (P-values) of predictors of disease-free and overall survival for 103 patients with endometrial carcinoma

Variable	Disease-free survival	Overall survival
PRA (positive vs negative)	**0.0055**	**0.0354**
PRB (positive vs negative)	**0.0022**	**0.0225**
Age (≤50 years vs 50 years)	0.1159	0.0854
Stage (I/II vs III/IV)	0.2029	0.1163
Histological grade (1–3)	**0.0276**	**0.0067**
ERα (positive vs negative)	**0.0426**	0.2667
ERβ (positive vs negative)	0.4832	0.3965
Ki67 (positive vs negative)	0.4722	0.3487

ER, estrogen receptor; PR, progesterone receptor.

of these two PR isoforms in endometrial carcinoma remains largely unknown. The results of our present study demonstrated that PRB was more common than PRA in endometrioid endometrial carcinoma, which is consistent with a recent report by Miyamoto *et al.*[43] They reported PRB LI of 30.4%, whereas those of PRA were 11.3% in endometrial carcinoma. Sakaguchi *et al.* also reported that PRB expression was more common than PRA expression in endometrial carcinoma.[32] However, Arnett-Mansfield *et al.* reported that PRA, not PRB, was dominant in endometrial carcinoma.[21] This discrepancy of results may be explained by the number of cases examined, because Arnett-Mansfield *et al.* examined a relatively small number of cases (46 cases), whereas our present study as well as others examined PR expression in more than 100 patients with endometrial carcinoma. We demonstrated previously that PRB was expressed dominantly in all types of epithelial ovarian cancer.[18,19] In human breast cancer, however, PRA was dominant in invasive ductal carcinoma.[20,44] Therefore, the biological significance of PR isoforms may differ depending on tumors, even among human estrogen-dependent carcinomas.

Progesterone receptor and ER are known to be among the most extensively studied biological prognostic markers in endometrial carcinoma. However, the status of PR isoforms and their possible roles in conjunction with clinical outcome in patients with endometrial carcinoma have not been fully

Table 3. Multivariate analyses of predictors of disease-free and overall survival for 103 patients with endometrial carcinoma

Preditor	Disease-free survival		Overall survival	
	HR (95% CI)	P-value	HR (95% CI)	P-value
PRA (positive vs negative)	0.171 (0.036–0.808)	**0.0258**	0.196 (0.022–1.764)	0.1522
Histological grade (1–3)	1.333 (0.728–2.440)	0.3514	2.371 (0.948–5.931)	0.065
ERα (positive vs negative)	0.509 (0.186–1.394)	0.1888	0.748 (0.187–2.992)	0.6818
Stage (I–IV)	0.374 (0.231–2.287)	0.1053	1.451 (0.785–2.685)	0.2352
PRB (positive vs negative)	0.37 (0.121–1.125)	0.0798	0.445 (0.102–1.932)	0.2797
Histological grade (1–3)	1.569 (0.852–2.888)	0.1481	2.838 (1.116–7.217)	**0.0285**
ERα (positive vs negative)	0.557 (0.192–1.610)	0.2798	0.794 (0.188–3.360)	0.9184
Stage (I–IV)	0.2191 (0.837–2.171)	0.2192	1.387 (0.730–2.635)	0.3174

CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; PR, progesterone receptor.

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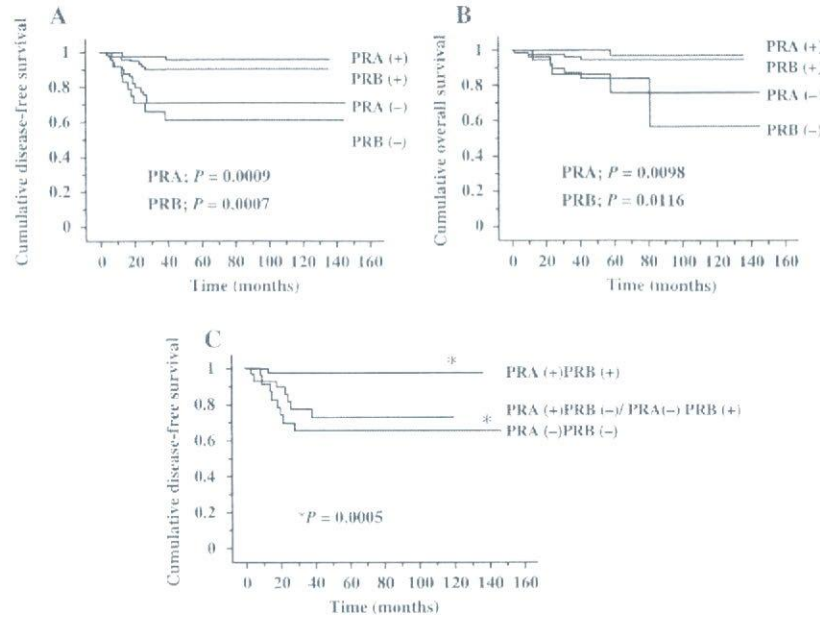

Fig. 4. Correlation between progesterone receptor A (PRA) or B (PRB) isoform immunoreactivity and (A) recurrence, and (B) survival for patients with endometrioid endometrial carcinoma. (C) Correlation between immunoreactivity for both isoforms and recurrence for patients with endometrioid endometrial carcinoma.

characterized. There have been some reports demonstrating the status of PR isoforms and clinical prognosis in endometrial carcinoma.[32,43] Miyamoto *et al.* carried out immunohistochemical analysis and demonstrated that PRB expression occurred significantly more frequently in grade 1 and was inversely correlated with poor prognosis on clinical outcome of patients, whereas PRA expression was also significantly higher in grade 1 and was inversely correlated with Ki-67 expression, but not with prognosis of the patients. They concluded that PRA and PRB expression was significantly correlated with biologically malignant potential.[43] Sakaguchi *et al.* examined mRNA levels of the PR isoforms and reported a significant positive correlation between PRA and PRB mRNA expression in endometrial carcinoma.[32] They quantified the mRNA levels of PRAB (PRA + PRB) using real-time RT-PCR, and they also calculated the mRNA levels of PRA from these data. There were no significant differences in the level of PRA mRNA between normal endometrium and each histological grade, although PRB expression was significantly higher in G1. In addition, PRB mRNA, but not PRA mRNA, status was significantly correlated with survival in endometrial carcinoma.[32] However, in these previous studies, the combined results for loss of expression of both of the PR isoforms and their prognostic correlations were not examined in endometrioid endometrial carcinoma. In the present study, both PRA and PRB were significantly lower for the higher histological-grade carcinoma cases, which is consistent with the results of previously reported studies.[30,32,43] Loss of both PRB and PRA expression in carcinoma tissue was significantly associated with an adverse clinical outcome in the patients. The absence of either one or both of these PR isoforms was associated with a significantly poorer prognosis at disease-free survival. In addition, multivariate analysis demonstrated that an absence of PRA immunoreactivity was an independent risk factor in disease-free survival of the patients (Table 3). Furthermore, only one case was PRB negative among these 51 PRA-positive cases. The number and disease-free survival curve was similar between the groups of PRA^-PRB^- and PRA^+PRB^-/PRA^-PRB^+ (Fig. 4C). These results all indicate that the status of PRA in endometrial cancer is quite important in determining the postoperative course of the patients.

Each PR status is considered to strongly influence the abnormal proliferative, invasive and metastatic potential of endometrial carcinoma cells. Microarray analysis of human endometrial carcinoma cells expressing either PRA or PRB confirmed that each PR isoform has distinctly different target genes, with little overlap.[45] Several investigators demonstrated that progesterone acts principally through PRB to inhibit endometrial carcinoma cell invasiveness modulated by adhesion molecules, including integrin and matrix metalloproteinases.[46,47] However, Hanekamp *et al.* demonstrated recently that endometrial carcinoma cell lines, which expressed only PRA, expressed higher levels of cadherins and demonstrated a lower level of invasive properties compared to the cell lines that expressed PRB.[48] They also demonstrated that the loss of expression of both PR isoforms was associated with increased expression of CD44 and CSPG/versican, invasion-related proteins. They further suggested that these results may represent an early and possibly initializing event in the development of a more invasive phenotype in endometrial carcinoma.[49] Results of these studies in cell lines also suggest that a decrease or loss of PRA and/or PRB expression should become an important factor that contributes to invasive and metastatic potential and eventually poor prognosis in human endometrial carcinoma. Dai *et al.* studied the effectiveness of adenovirus-mediated PR gene transduction in combination with progestin therapy in mouse xenograft models, and demonstrated that the presence of both PRA and PRB provided a substantial benefit to animal survival compared with PRB alone.[50] Results of an inverse correlation between both PR isoforms and Ki-67 expression in our study also suggest the important roles of each PR isoforms for protecting against aggressive proliferation and development. In summary, the results of the present study indicate that the loss of PR isoform expression, especially PRA, in human endometrioid endometrial carcinoma may result in aggressive biological characteristics that play important roles in prognosis and recurrence.

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Coffee consumption and the risk of primary liver cancer: Pooled analysis of two prospective studies in Japan

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Although case-control studies suggested that coffee consumption is associated with a decreased risk of liver cancer, no prospective cohort study has been carried out. To examine the association between coffee consumption and the risk of liver cancer, we conducted a pooled analysis of data available from 2 cohort studies in Japan. A self-administered questionnaire about the frequency of coffee consumption and other health habits was distributed to 22,404 subjects (10,588 men and 11,816 women) in Cohort 1 and 38,703 subjects (18,869 men and 19,834 women) in Cohort 2, aged 40 years or more, with no previous history of cancer. We identified 70 and 47 cases of liver cancer among the subjects in Cohort 1 (9 years of follow-up with 170,640 person-years) and Cohort 2 (7 years of follow-up with 284,948 person-years), respectively. We used Cox proportional hazards regression analysis to estimate the relative risk (RR) and 95% confidence interval (CI) of liver cancer incidence. After adjustment for potential confounders, the pooled RR (95% CI) of drinking coffee never, occasionally and 1 or more cups/day were 1.00 (Reference), 0.71 (0.46–1.09) and 0.58 (0.36–0.96), respectively (p for trend = 0.024). In the subgroup of subjects with a history of liver disease, we found a significant inverse association between coffee consumption and the risk of liver cancer. Our findings support the hypothesis that coffee consumption decreases the risk of liver cancer. Further studies to investigate the role of coffee in prevention of liver cancer among the high-risk population are needed.

Key words: coffee; liver neoplasms; incidence; prospective studies; Japan

Primary liver cancer is the third most common cause of death from cancer worldwide.[1] The incidence of liver cancer is highest in Eastern Asia, including Japan.[2] Although its incidence is lower in Europe[3,4] and the United States,[5] it has been increasing over the last few decades.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are established causes of liver cancer,[6] and 59.6% and 23.6% of liver cancers worldwide are considered attributable to HBV and HCV, respectively.[7] Epidemiologic studies have indicated that alcohol drinking[8,9] and tobacco smoking[10] are also associated with an increased risk of liver cancer.

There are several sets of data supporting the possibility that coffee consumption has a preventive effect against liver cancer. Animal experiments have indicated that coffee has inhibitory effects against chemical carcinogenesis in liver tissue.[11] Furthermore, epidemiologic studies have demonstrated that coffee consumption is inversely related to serum liver enzyme activity,[12–15] and has an inverse association with the incidence of liver cirrhosis.[16,17] Recent case-control studies in Italy and Greece have suggested that coffee consumption is associated with a decreased risk of liver cancer.[18–20]

All the existing epidemiologic evidence related to coffee consumption and liver cancer has been derived only from case-control studies.[18–20] To further clarify the association between coffee consumption and the risk of liver cancer, a prospective cohort study is essential. Our present study was conducted to examine the association between coffee consumption and the risk of primary liver cancer based on population-based prospective cohort studies in Japan.

Material and methods

Study cohorts

Our present study was based on a pooled analysis of 2 prospective cohort studies in Japan. The study designs for the 2 studies have been described in detail elsewhere.[21–23] Briefly, for Cohort 1, we delivered a self-administered questionnaire in January 1984 to 33,453 residents, 40 years of age or older, in 3 municipalities of Miyagi Prefecture. Usable questionnaires were returned from 31,345 (93.7%) of the subjects. For Cohort 2, we delivered a self-administered questionnaire between June–August 1990 to 51,921 residents, 40–64 years of age, in 14 municipalities of Miyagi Prefecture. Usable questionnaires were returned from 47,605 (91.7%) of the subjects. Study protocols for the 2 cohorts were approved by the institutional review board of Tohoku University Graduate School of Medicine. We considered that the return of the self-administered questionnaires signed by the subjects implied their consent to participate in the study.

Exposure data

In both cohorts, the questionnaire included items inquiring about the frequency of recent consumption of 3 kinds of beverages (coffee, green tea, black tea) and food items, as well as questions on smoking status and history of disease. In the question about history of liver disease, the subjects were simply asked, "Have you had any liver disease?" Thus, we did not ascertain the name of the liver disease. Alcohol consumption was assessed by asking if the subject had never drunk, or was a former, or current, drinker. Current drinkers were also asked about their frequency of drinking and the amount of alcohol consumed on one occasion.

We asked the subjects about their frequency of coffee consumption according to 5 categories: never, occasionally, 1–2 cups per day, 3–4 cups per day and 5 or more cups per day. No question about the method used to brew the coffee was asked. The volume of a typical cup of coffee was 150 ml in the study region. The validation study of beverage consumption indicated that the self-reported frequency of coffee consumption among the subjects was satisfactorily valid and reliable. One hundred thirteen subjects in the study population responded to the questionnaire twice, 1 year apart, and provided four 3-day diet records within the year. Spearman's coefficient for the correlation between the amounts of coffee consumed according to the questionnaire and the amounts

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TABLE I – CHARACTERISTICS OF THE SUBJECTS ACCORDING TO COFFEE CONSUMPTION[1]

	Coffee consumption (cups/day)					
	Cohort 1			Cohort 2		
	Never	Occasionally	≥1	Never	Occasionally	≥1
No. of subjects	4938	9507	7959	6954	14130	17619
Age (years), mean ± SD[2]	60.4 ± 11.8	56.0 ± 10.5	52.4 ± 9.7	54.2 ± 7.0	52.8 ± 7.2	48.9 ± 7.1
History of liver disease (%)	5.4	4.7	4.5	6.6	4.8	4.4
Male (%)	41.0	46.1	52.5	47.1	46.8	51.0
Alcohol drinking (%)						
Never	48.9	38.3	33.4	45.9	43.7	39.0
Formerly	7.7	5.0	5.1	6.6	5.1	5.4
Occasionally	21.0	32.6	34.6	18.4	24.8	28.9
Daily, <45.6 g/d	7.0	8.5	9.4	8.0	8.3	9.2
Daily, ≥45.6 g/d	15.4	15.7	17.4	21.1	18.2	17.6
Smoking (%)						
Never	62.4	60.6	47.2	56.9	55.8	47.3
Formerly	13.8	12.4	13.4	14.0	12.7	10.2
Daily, <19 cigarettes	10.6	10.7	12.7	10.5	10.7	11.0
Daily, ≥20 cigarettes	13.2	16.3	27.7	18.6	20.8	31.7
Daily consumption (%)						
Green tea (≥3 cups/day)	57.0	68.6	60.3	47.9	52.2	39.6
Black tea (≥3 cups/day)	0.7	0.9	2.7	0.3	0.4	0.8

[1]$n = 61, 107$.–[2]SD denotes standard deviation.

consumed according to the diet records was 0.70, and the correlation between consumption measured by the 2 questionnaires over 1 year was 0.72.

Follow-up

The end point in our analysis was incidence of primary liver cancer defined as the topography code C22.0 and fifth digit behavior code for neoplasms/3 according to the International Classification of Diseases for Oncology (2nd Ed.; ICD-O-2).[24]

For both cohorts, we followed the vital and residential status of each subject using a population registry for each municipality. We ascertained the incidence of cancer using the Miyagi Prefectural Cancer Registry, one of the earliest and most accurate population-based cancer registries in Japan.[25] In this registry, the relevant cases were abstracted from medical records of hospitals by a medical doctor or trained medical record reviewer, except for the cases reported from an institution to the registry. A follow-up was conducted from 1 January 1984–31 December 1992 for Cohort 1, and from 1 August 1990–31 March 1997 for Cohort 2.

We excluded cancer cases prevalent at the baseline (541 cases in Cohort 1 and 1,110 cases in Cohort 2). Then, we excluded subjects who did not answer the question about coffee consumption (8,400 subjects in Cohort 1 and 7,792 subjects in Cohort 2). Consequently, our analysis included 22,404 subjects (10,588 men and 11,816 women) including a total of 70 cases of liver cancer (50 men and 20 women) in Cohort 1, and 38,703 subjects (18,869 men and 19,834 women) including a total of 47 cases of liver cancer (41 men and 6 women) in Cohort 2.

Diagnosis of the 117 primary liver cancer cases was confirmed by medical records ($n = 90$, 76.9%) or death certificates alone ($n = 27$, 23.1%). In the 90 cases of primary liver cancer reviewed from medical records, the diagnosis was confirmed by histologic or cytologic examination in 43 cases, and by imaging (ultrasonography, computed tomography, magnetic resonance imaging, or angiography) alone in 35 cases. Although medical records had been reviewed, no further information on the basis of diagnosis was obtainable from the registered data in 12 cases. Among the 43 cases of primary liver cancer established by histologic or cytologic examination, the histological types were hepatocellular carcinoma (ICD-O-2 morphology code M-8170/3, $n = 35$), unspecified cancer (M-8000/3, $n = 6$), adenocarcinoma (M-8140/3, $n = 1$) and hemangiosarcoma (M-9120/3, $n = 1$).

Statistical analysis

We counted the number of person-years of follow-up for each subject from the beginning of follow-up until the date of diagnosis of liver cancer, the date of emigration from the study districts, the date of death or the end of follow-up, whichever occurred first. Total person-years accrued were 170,640 for Cohort 1 and 284,948 for Cohort 2. We combined the upper 3 categories of coffee consumption into the single category "1 or more cups/day" because of the small number of subjects in each category. In fact, the numbers of patients with liver cancer who reported drinking coffee 1–2, 3–4 or 5 or more cups/day were 19, 11 and 0, respectively. Relative risk was computed as the incidence rate among subjects in each category of coffee consumption divided by the rate among those who had never drunk coffee. We considered subjects who had never drunk coffee as the reference group.

We used Cox proportional hazards regression analysis to estimate the relative risk (RR) and 95% confidence interval (CI) of liver cancer incidence according to categories of coffee consumption and to adjust for potentially confounding variables, using SAS version 8.2 statistical software (SAS Inc., Cary, NC).

As the primary outcome, we examined the association between coffee consumption and the risk of incidence of primary liver cancer. We considered the following variables to be potential confounders: age (in years), gender, history of liver disease (yes or no), alcohol consumption (never drinker, former drinker, occasional drinker [current drinker less often than daily], daily drinker who consumed <45.6 g alcohol/day, or 45.6 g or more alcohol/day), and smoking status (never smoker, former smoker, currently smoking 1–19 cigarettes/day, currently smoking at least 20 cigarettes/day).

To obtain a summary measure of the results from Cohort 1 and Cohort 2, we used the general variance-based method.[26] The p-values for the analysis of linear trends were calculated by treating the coffee consumption category as an ordinal variable. All reported p-values are 2-tailed, and differences at $p < 0.05$ were considered statistically significant.

Results

Table I compares the characteristics of subjects according to coffee consumption. The subjects with higher coffee consumption tended to be younger and male, and were more likely to be drinkers and heavy smokers (20 cigarettes or more/day) and were less likely to have a history of liver disease. The consumption of

TABLE II – RELATIVE RISK (RR) AND 95% CONFIDENCE INTERVAL (CI) OF LIVER CANCER ACCORDING TO COFFEE CONSUMPTION

Variable	Coffee consumption (cups/day)			*p* for Trend
	Never	Occasionally	≥1	
No. of cases of liver cancer/person-years				
Cohort 1	29/36,988	25/74,226	16/59,427	
Cohort 2	12/51,017	21/104,459	14/129,471	
Age, gender-adjusted RR (95% CI)				
Cohort 1	1.00	0.48 (0.28–0.82)	0.44 (0.24–0.83)	0.0076
Cohort 2	1.00	0.92 (0.45–1.87)	0.61 (0.28–1.33)	0.19
Pooled	1.00	0.61 (0.40–0.94)	0.50 (0.31–0.82)	0.0041
Multivariate RR[1] (95% CI)				
Cohort 1	1.00	0.56 (0.33–0.97)	0.53 (0.28–1.00)	0.038
Cohort 2	1.00	1.05 (0.52–2.16)	0.68 (0.31–1.51)	0.30
Pooled	1.00	0.71 (0.46–1.09)	0.58 (0.36–0.96)	0.024

[1]Multivariate RR was adjusted for age (in years), gender, history of liver disease (yes or no), alcohol consumption (never drinker, former drinker, occasional drinker [current drinker less often than daily], drinker who consumed less than 45.6 g alcohol/day, 45.6 g or more alcohol/day), and smoking status (never smoker, former smoker, currently smoking 1–19 cigarettes/day, currently smoking at least 20 cigarettes/day).

green tea did not vary according to the consumption of coffee. We observed a similar tendency in Cohort 2.

Table II shows the association between coffee consumption and the risk of primary liver cancer. We found that higher coffee consumption was significantly associated with a lower risk of incidence of liver cancer. The pooled multivariate RR (95% CI) of liver cancer in subjects who drank coffee never, occasionally, and 1 or more cups/day were 1.00, 0.71 (0.46–1.09) and 0.58 (0.36–0.96), respectively (p for trend = 0.024). In the analysis of each cohort, a similar trend was observed. Results remained essentially the same when we excluded the 41 cases (22 cases [13 men and 9 women] in Cohort 1, 19 cases [17 men and 2 women] in Cohort 2) with liver cancer diagnosed in the first 3 years of follow-up (data not shown).

When we did not count 27 cases confirmed by death certificate only (DCO) as primary liver cancer, the point estimate of the RR of liver cancer had a similar trend. The pooled multivariate RR of liver cancer in subjects who drank coffee never, occasionally, and 1 or more cups/day were 1.00, 0.87 (0.52–1.45) and 0.73 (0.41–1.29), respectively (p for trend = 0.25).

Table III shows the association between coffee consumption and the risk of liver cancer in subgroup analyses. The RR of liver cancer were below unity irrespective of whether the subjects were younger or older, male or female, current drinkers or not, current smokers or not and had had liver disease or not. A significant inverse association between coffee consumption and the risk of liver cancer was observed in the subjects with a history of liver disease (p for trend = 0.047), whereas the association was not significant in the subjects without a history of liver disease.

We also examined the relationship between green tea consumption and the risk of primary liver cancer, but the relationship was null. After adjustment for the same covariates as those used for analysis of coffee consumption, the pooled RR (95% CI) of primary liver cancer in subjects who drank 2 or less, 3–4, and 5 or more cups of green tea/day were 1.00, 1.20 (0.75–1.94) and 0.90 (0.56–1.44), respectively (p for trend = 0.70). We were unable to estimate the relationship between consumption of black tea and liver cancer incidence because the proportion of subjects who drank 1 or more cups of black tea/day was only 7.8% in Cohort 1 and 2.8% in Cohort 2.

Discussion

In this pooled analysis of 2 prospective cohorts, we found a statistically significant inverse association between coffee consumption and the incidence risk of primary liver cancer. This result is consistent with recent case-control studies in Italy and Greece.[20] Consumption of coffee in our subjects was not particularly low in comparison with the Western population. The proportion of subjects who reported drinking 1 or more cups of coffee/day was 41.9% in our study and 58.5% in the United States.[16] Almost half of the liver cancer cases occurred in the 2,985 subjects who reported a history of liver disease at the baseline. Although we had no specific information on liver diseases, this result was consistent with the strong association between chronic liver diseases such as chronic hepatitis or liver cirrhosis and the risk of liver cancer.[27]

Our study had several strengths. We recruited our subjects from the general population and identified a large number of cases of liver cancer among them. The information on coffee consumption and other variables was obtained before the cases of liver cancer were diagnosed, thus avoiding any effect of recall bias. The questionnaire used for measuring coffee consumption had a reasonably high level of validity and reproducibility. In addition, the inverse association between coffee consumption and the risk of liver cancer was unchanged after adjustment for, and stratification by, potential confounders. Moreover, to avoid any potential bias from subclinical conditions, we excluded subjects in whom liver cancer was diagnosed in the first 3 years of follow-up. The inverse association was unchanged after this exclusion.

Our study also had some limitations. First, we had no information about history of HBV or HCV infection. The prevalence of hepatitis B surface antigen (HBsAg) and antibodies against HCV (anti-HCV) among subjects 40 years of age or older in this area was 1.87% and 2.19%, respectively.[28] In Japan, 28% and 43% of liver cancers are estimated to be attributable to HBV and HCV, respectively.[7] If these viral infections were related to change in coffee consumption, the association between coffee consumption and the risk of liver cancer would be confounded. In our study, the RR of liver cancer were below unity, irrespective of whether the subjects had liver disease or not. In a previous study,[17] the inverse relationship between coffee consumption and the odds ratio of liver cirrhosis was independent of HCV and HBV infection. Because of the strong association between these virus infections and the risk of liver cancer, however, even a weak inverse association between these viral infections and coffee consumption could introduce negative confounding, which would lead to overestimation of the effect of coffee consumption on the decreased liver cancer risk. Measurement of HBV and HCV infections would be needed in further prospective studies.

Second, primary liver cancer cases identified on the basis of death certificates alone without confirmation by medical records might have a possibility of misclassifying secondary metastasis to the liver as primary liver cancer. We carried out an additional analysis not considering the DCO cases as primary liver cancer. The inverse association between coffee consumption and the risk of primary liver cancer was not materially changed. We believe it

TABLE III – POOLED MULTIVARIATE RELATIVE RISK (RR) AND 95% CONFIDENCE INTERVAL (CI) OF LIVER CANCER ACCORDING TO COFFEE CONSUMPTION BY VARIOUS SUBGROUPS

	Coffee consumption (cups/day)			p for Trend[1]
	Never	Occasionally	≥1	
Age				
40-59 (n = 46,718)				
No. of cases	14	23	20	
Multivariate RR[1] (95% CI)	1.00	0.88 (0.44–1.74)	0.81 (0.40–1.64)	0.59
60- (n = 14,389)				
No. of cases	27	23	10	
Multivariate RR[1] (95% CI)	1.00	0.58 (0.32–1.09)	0.44 (0.21–0.93)	0.015
Gender				
Male (n = 29,457)				
No. of cases	28	36	27	
Multivariate RR[1] (95% CI)	1.00	0.73 (0.44–1.21)	0.64 (0.37–1.12)	0.11
Female (n = 31,650)				
No. of cases	13	10	3	
Multivariate RR[1] (95% CI)	1.00	0.66 (0.28–1.57)	0.54 (0.14–2.07)	0.12
Alcohol drinking				
Never (n = 21,914)				
No. of cases	14	10	4	
Multivariate RR[1] (95% CI)	1.00	0.69 (0.29–1.65)	0.46 (0.14–1.52)	0.095
Former (n = 2,974)				
No. of cases	8	6	6	
Multivariate RR[1] (95% CI)	1.00	0.60 (0.20–1.78)	0.74 (0.23–2.39)	0.58
Current (n = 28,750)				
No. of cases	15	28	14	
Multivariate RR[1] (95% CI)	1.00	0.90 (0.47–1.71)	0.56 (0.24–1.29)	0.097
Smoking				
Never (n = 27,233)				
No. of cases	12	14	2	
Multivariate RR[1] (95% CI)	1.00	0.90 (0.38–2.11)	0.27 (0.06–1.32)	0.10
Former (n = 6,164)				
No. of cases	14	9	2	
Multivariate RR[1] (95% CI)	1.00	0.53 (0.21–1.34)	0.18 (0.04–0.84)	0.012
Current (n = 18,334)				
No. of cases	11	16	19	
Multivariate RR[1] (95% CI)	1.00	0.80 (0.36–1.75)	0.90 (0.41–1.97)	0.84
History of liver disease				
yes (n = 2,985)				
No. of cases	23	17	13	
Multivariate RR[1] (95% CI)	1.00	0.51 (0.27–0.97)	0.52 (0.25–1.07)	0.047
no (n = 58,122)				
No. of cases	18	29	17	
Multivariate RR[1] (95% CI)	1.00	0.98 (0.53–1.80)	0.75 (0.37–1.50)	0.33

[1]Multivariate RR was adjusted for age (in years), gender, history of liver disease (yes or no), alcohol consumption (never drinker, former drinker, occasional drinker [current drinker less often than daily], daily drinker who consumed less than 45.6 g alcohol/day, 45.6 g or more alcohol/day), and smoking status (never smoker, former smoker, currently smoking 1–19 cigarettes/day, currently smoking at least 20 cigarettes/day). Each model stratified by gender, alcohol consumption, smoking status and history of liver disease did not include variables for each stratum, respectively.

is unlikely that the DCO cases distorted the inverse association substantially.

Third, we excluded 16,192 subjects because they did not answer the question on coffee consumption. Fifty-one cases of liver cancer were diagnosed in this group. We considered that the characteristics of subjects who did not report their coffee consumption were essentially similar to those of subjects who did. The 2 groups were similar with respect to the prevalence of current smokers (35.5% and 35.4% of the groups, respectively), current alcohol drinkers (51.4% and 54.2%, respectively), and a history of liver disease (4.2% and 4.9%, respectively), apart from the distribution of age classes (subjects 40–59 years of age made up 53.6% and 76.5% of the groups, respectively) and gender (men made up 41.3% and 48.2%, respectively). The pooled multivariate RR (95% CI) of liver cancer in the subjects who did not answer the question about their coffee consumption, as compared to those who did, was 1.23 (0.87–1.74). Thus, our result might not have been substantially biased by exclusion of the subjects who did not answer the question on coffee consumption.

Fourth, we were unable to distinguish between never and former coffee drinkers, as this information was not collected at the baseline. Such information would allow more precise estimation of the effects of coffee on liver cancer in further studies. Finally, we did not investigate the method used for brewing coffee. For practical purposes, however, we can consider that most of the subjects would have consumed instant or filtered coffee because unfiltered coffee is rarely consumed in Japan.[29]

Among the subjects with a history of liver disease, we observed a significant inverse relationship between coffee consumption and liver cancer. We speculate that coffee may prevent liver cancer more effectively among subjects with liver disease than among those without liver disease. If the subjects with a history of liver disease had reduced their coffee consumption at the time of baseline data collection because of ill health, an inverse association between coffee consumption and liver cancer would have been observed. Among the subjects with a history of liver disease, we did not observe a decreasing trend in the proportion of former alcohol drinkers and former smokers, according to the frequency of coffee consumption, who might have quit drinking and smoking due to ill health. In our data, among the subjects with a history of liver disease, the proportions of former alcohol drinkers who drank coffee never, occasionally or 1 or more cups/day were

13.5%, 11.5% and 13.1% respectively, and the corresponding proportions of former smokers were 17.7%, 19.3% and 17.6%, respectively. Kuper *et al.*[19] failed to estimate the odds ratio of liver cancer for coffee drinkers among a subgroup of subjects with HBsAg or anti-HCV because there were no controls who did not drink coffee among these subjects. Further studies to elucidate the preventive effects of coffee consumption against liver cancer among subjects with chronic hepatitis or liver cirrhosis are needed.

Meanwhile, among the subjects without a history of liver disease, the inverse association between coffee consumption and the risk of liver cancer was not significant, but the RR of liver cancer was below unity. Among the subjects without a history of liver disease, we could not conclude from our data whether we might fail to detect a significant inverse association between coffee consumption and the risk of liver cancer due to insufficient statistical power or whether there might be no association.

It remains unclear which ingredient(s) of coffee is protective against liver cancer. Mutagenic and antimutagenic effects of coffee and caffeine on cultured cells of bacterial and mammalian origin have been demonstrated, but mutagenic effects would be almost non-existent at the usual levels of coffee consumption in humans.[30] The caffeine concentration in coffee and green tea is 0.06% and 0.02%, respectively.[31] Caffeine might not have a protective effect against liver cancer because our study indicated that consumption of green tea was not associated with the risk of liver cancer. Coffee also contains chlorogenic acid, a phenolic compound, whose inhibitory effects on chemical carcinogenesis in the liver have been demonstrated in an animal model.[11] The diterpenes cafestol and kahweol, both present in coffee, have been implicated in anticarcinogenic activity,[32] but it seems unlikely that they would have had a protective effect against liver cancer in this study group because their quantity is almost negligible in instant and filtered coffee.[33]

In conclusion, we have found that coffee consumption is significantly associated with a decreased incidence of liver cancer. In addition, subgroup analysis among our subjects with a history of liver disease showed an inverse association between coffee consumption and the risk of liver cancer. Further studies to clarify the role of coffee in prevention of liver cancer among the population at high risk are needed.

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環境由来化学物質の胎児期曝露の影響

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要旨

ダイオキシン類，PCBs，メチル水銀など環境由来の化学物質による周産期曝露に起因した健康影響が危惧されている。健康影響が最も危惧される集団は胎児と新生児であり，その健康リスクを評価するため，周産期における化学物質曝露をモニタリングするとともに，出生児の成長，特に認知行動面の発達を追跡する前向きコホート調査を計画し，599組の新生児－母親の登録を得て疫学調査を進めている。まだ児の発達と化学物質曝露の関係について解析途中であるが，母親毛髪総水銀，臍帯血および母体血甲状腺ホルモン関連指標の分析を終えるとともに，臍帯血ダイオキシン類およびPCBsについて高分解能ガスクロマトグラフィー質量分析装置（GC/MS）を用いた解析を実施中である。本コホート調査の概要を紹介するとともに，化学分析の状況についてまとめ，PCB曝露のレベルについて海外で行われたコホート調査の結果との比較を試みた。

はじめに

ダイオキシン類，PCBsおよびメチル水銀といった化学物質は，難分解性および脂溶性の特徴を有しており，そのため環境中に蓄積し食物連鎖による生物濃縮を受け，ヒトは主に魚介類を介して取り込むと考えられる。その曝露レベルは低いものの，発生，成長過程にある胎児や新生児は中枢神経系の成長過程にあり，成人に比較して，これら化学物質の曝露に対する感受性が高いと考えられる。

PCBもしくはメチル水銀に関しては，1980年代から1990年代にかけて海外でいくつかの出生コホート調査が行われている。調査が行われた実施地点を図1に示すとともに，PCBに関する報告について表1にその主な報告内容を整理した。

PCBの影響については，多くの報告で児の心理行動，認知面に対して何らかの影響があることを示唆する結果となっている[1]。全体的な傾向としては，母乳を介した曝露よりは，胎児期曝露の影響が大きいことが示唆される。例外はドイツで行われた疫学であり，臍帯血中PCBではなく母乳中PCBが児の認知面の発達の遅れと関連したことが報告されている[2]。授乳については，授乳そのものが児の発達を促す要因となっていることも調査から示されており，母乳を介した曝露のリスク評価は今後の課題となっている。

いずれにしても，胎児または新生児の時期は脳の発生，発達時期に相当し，環境の変化に対する感受性が高い。さらに，成人におけるこのような化学物質の主な摂取経路は食事であり，ダイオキシン類耐容１日摂取量（TDI）についてみれば多くの成人が基準以下とされている。しかしながら，児は母体に長年にわたって蓄積した化学物質を胎盤または母乳を通して短期間に受け取ることとな

図1　PCBもしくはメチル水銀による健康影響が調べられた海外の主な出生コホート調査

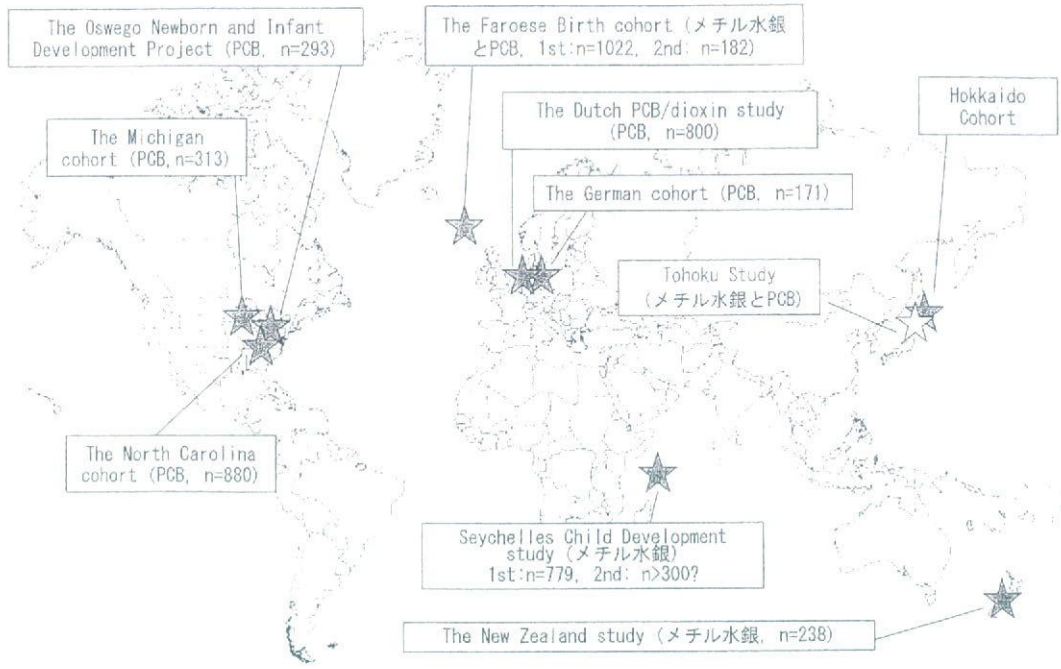

表1　海外におけるコホート調査の結果

Test	Major finding			Reference
	Fish intake	Prenatal exposure[1]	Postnatal exposure[1]	
Michigan 1980-1981				
NBAS (60 hr)	Motor immaturity, Poorer lability of states, Hypoactive reflexes	No relation		16
BSID (5, 7 mo)	No relation	No relation		17
FTII (5, 7 mo)	Less performance	Less performance	No relation	18
MS (4 yr)		Poorer scores in verbal and numerical memory	Weak relation	19
IQ test (11 yr)		Intellectual impairment	No relation	15
North Carolina, 1978-1982				
NBAS (72 hr)		Less muscle tone, Lower activity levels, Hyporeflexive[2]		20
BSID (2 yr)		Lower psychomotor scores[2]		21
MDS (2 yr)		No relation[2]		22
MS (3-5 yr)		No relation[2]		23
Oswego, NY, 1991-1994				
NBAS (48 hr)	Lower scores in habituation, autonomic and reflex	Lower scores in habituation, autonomic and reflex	No relation	24
FTII (6 and 12 mo)		Less performance	No relation	13
Performance test (4.5 yr)		Increase in errors of commission	No relation	25
Netherlands, 1990-1992				
PNE (10-21 d)		No relation	Less muscle tone, Reduced neurological optimality	26
BSID (3 mo)		Lower psychomotor scores	No relation	27
BSID (7 mo)		No relation	Lower psychomotor scores	27
Neurological (18 mo)		Lower optimality	No relation	28
Neurological (42 mo)		No relation	No relation	29
K-ABC (42 mo)		Intellectual impairment[3]	No relation	30
Neuropsychological (9 yr)		Longer respone time	Week relation	31
Auditory P300 (9 yr)		Longer P300 latencies	No relation	32
Dusseldorf, 1993-1995				
BSID (7 mo)		No relation	Lower mental scores	33
FTII (7 mo)		No relation	No relation	33
BSID (30 mo)		No relation	Lower mental and psychomotor scores	2
K-ABC (42 mo)		No relation	Intellectual impairment	2
Faroe Islands, 1994-1995				
PNE (2 wk)		No relation[3]	No relation	34

1 Cord blood PCB level for prenatal exposure and maternal milk PCB level for postnatal exposure. 2 Prenatal PCB exposure was estimated based on the maternal milk PCB level obtained at birth. 3 Maternal blood PCB level.
Neurological and cognitive tests are abbreviated as follows. Neonatal Behavioral Assessment Scale (NBAS), Bayley Scales of Infant Development (BSID), Fagan Test of Infant Intelligence (FTII), McCarthy Scales (MS), Mental Development Scales (MDS), the Prechtl Neurological Examination (PNE), Kaufman Assessment Battery for Children (K-ABC)

り，例えば新生児が母乳を通して摂取する量はTDIの40～100倍にも達するとも試算されている。周産期，特に胎児期における化学物質曝露の健康リスクの評価が求められている。

わが国では，ダイオキシン類，PCB，メチル水銀などの化学物質は主に魚介類の摂取によって取り込まれると考えられているが，一方で魚介類は栄養学的に優れた栄養素を含んでいる。特に不飽和脂肪酸は新生児の中枢神経系の発達に必須と考えられれている。例えば，海外の疫学調査の中でSeychelles共和国で行われたコホート調査では，化学物質曝露の負の影響は見出されていないが[3]，このSeychelles共和国は多様な魚を摂取する食習慣を有しており，日本における魚摂取の状況に近い。Seychelles共和国ではPCBsによる魚の汚染はきわめて低いとされているため，わが国の状況との単純な比較は難しいものの，多様な魚を多食する食習慣を有する集団では化学物質の健康リスクも異なる可能性がある。疫学調査を進めるうえでは，化学物質曝露の健康リスクのみならず，魚摂取の意義を総合的に評価する研究が必要となっている[4]。

コホート調査の概要

1 登録

我々が東北地方で進めているコホート調査（Tohoku Study of Child Development：TSCD）の概要を図2に示した。プロトコールの詳細は文献を参照されたい[5]。2001年1月から2003年9月にわたり，仙台市内の複数の医療機関にて事前に調査の概要を説明し，インフォームドコンセントを実施し文書による同意を得た。低体重，早期産，除外疾患などを考慮し最終的に599名の新生児－母親のペアを登録した。出生した児の発達を追跡するため，東北大学医学系研究科内のコラボスペースに調査のための部屋を設置，音，温湿度環境に加え，児の安全性や居住性に配慮した環境にて発達検査を進めている。なお，調査に先立ち東北大学医学系研究科倫理委員会に研究計画の申請を行い許可を得ている。

2 児の成長の追跡

児の成長を追跡するための神経行動学的な手法に関して，生後3日目にブラゼルトン新生児行動評価（NBAS）を，生後7カ月で新版K式発達検査，

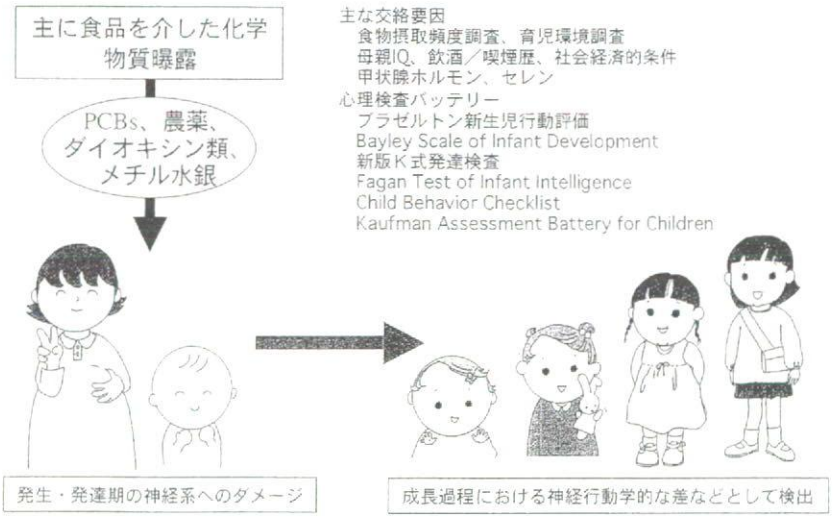

図2　コホート調査の概要
周産期における化学物質曝露と把握しつつ，出生児の成長を，認知行動面の発達を中心に追跡する。周産期に受けた神経系へのダメージが，児の成長過程で表れると危惧される。

Bayley Scales of Infant Development（BSID）およびFagan Test of Infant Intelligence（FTII）を，生後18カ月で新版K式発達検査およびBSIDを，生後30カ月でChild Behavior Checklist for 2-3 years（CBCL）を，生後42カ月でKaufman Assessment Battery for Children（K-ABC）を進めている。これらの追跡調査への出席率はおおむね82～88%で推移している。

検査バッテリーについては，新版K式発達検査はわが国における標準的な発達検査であるが，海外では表1に示すようにBSIDがよく用いられており[6]，TSCDの研究成果の国際比較を想定して新版K式発達検査とBSIDの併用による方法を採用した。BSIDは国内で標準化されておらず，1993年に第2版に改定された後は国内での使用例も見当たらない。そのため，Rochester大学の小児発達研究グループ（Davidson教授）との共同作業によりプロトコールの和訳と信頼性評価を実施した[7]。また，FTIIは乳幼児のもつ新奇選好を応用した視覚認知検査であり，将来の知的能力と高い相関をもつとされている[8]。海外の調査でもよく用いられている検査項目であり（表1），我々の調査でも日本の乳幼児で新奇選好が認められている[9]。なお，生後42カ月では児の神経運動機能の評価を試みるため，デンマークで開発されたCATSYS2000[10]の中から，身体重心動揺およびふるえ検査を試みている。

a. 交絡要因

児の成長と化学物質曝露を関連づけるうえで，母親の食事調査（半定量式食物摂取頻度調査），社会経済的要因（Hollingshed four facors version），育児環境調査，母親IQ（Raven's Standard Matrices）により実施している。

b. 化学分析

生体試料の化学分析について，母親毛髪総水銀ならびに臍帯血および母体血甲状腺ホルモン関連指標（TSH，総および遊離T3/T4）については全例で分析を終了した。総水銀分析は還元気化法により，甲状腺ホルモン関連指標は電気化学発光免疫測定法により分析を行った。

表2　文献比較の対象としたコホート調査とその化学分析の方法

Study	Method				No of	Lipid	Comment	Reference
	Extraction	Clean-up	GC	Detection	congeners identified	determination		
North Carolina, 1978-1982	Liquiud	Florisil	Packed	ECD	-	Not identified	Webb-McCall method	35
Michigan 1980-1981	Liquiud	Florisil	Packed	ECD	-	Not identified	Webb-McCall method	36
Netherlands, 1990-1992	Liquiud	Florisil	High Resolution	ECD	4	Gravimetric (milk)	Milk: 17 PCDD/F congeners, 3 planer and 23 non-planar PCBs. Plasma: Sum of 118, 138, 153, and 180	28
Oswego, NY, 1991-1994	Liquiud	Florisil	High Resolution	ECD	68	Gravimetric	a) Sum of 68 congeners b) Sum of highly chlorinated congeners	13
Dusseldorf, 1993-1995	Solid -Liquiud	Florisil	High Resolution	ECD	3	Photometric	Sum of 138, 153, and 180	2
Faroe Islands, 1994-1995	Solid -Liquiud	Florisil	High Resolution	ECD	6	Photometric (milk)	1.65 x Sum of 138, 153 and 180	34
Nonavik, Quebec, 1996-2000	Liquiud	Florisil	High Resolution	ECD	14	Gravimetric (milk) Enzymatic (serum)		37
Osaka, 1998	Liquiud	Florisil	Packed	ECD	-	Gravimetric	Japanese official procedure	11
Chiba and Yamanashi, 2002-2003	Liquiud	Silica gel	High Resolution	MS	All	Enzymatic (serum)		12
Tohoku, 2001-2003	Liquiud	Silica gel	High Resolution	MS	All	Gravimetric	Whole blood was used.	5

文献37，11および12は児の発達を追跡するコホート調査ではないが，比較のため記載した。
ダイオキシン類を分析したコホート調査は，オランダの疫学調査で母乳での分析が行われたのみであり，PCBについてまとめた。

有機塩素系化学物質のうち，ダイオキシン類はレポータージーンアッセイであるCALUX AssayおよびGC/MSによる方法とし，またPCBs全異性体分析もGC/MSによる方法とした。海外におけるコホート調査では，表2にそれぞれの調査で用いられた分析法を整理したが，生体試料中のPCBの分析はいずれもECDによる検出であり全異性体分析は行われていない。また，ダイオキシン類の分析に関しては，オランダの疫学調査にて母乳中濃度が測定されているのみである。本調査では，臍帯血を用いたダイオキシン類およびPCB全異性体分析を実施しているが，このような精密分析は初めての試みとなる。

まだ分析途中であるものの，高感度解析法の採用により，臍帯血でもほとんどの試料で2,3,7,8-TCDDを筆頭に多様なダイオキシン類が検出されている。中間報告になるが，臍帯血全血中のダイオキシン類濃度の中央値は，0.022 pg-TEQ/g-wet（0.005～0.13）であり，総PCBは115 pg/g-wet（36～670），脂肪含量は0.27%（0.18～0.72）となっている。過去のコホート調査では血清もしくは血漿の値が示されているが，血球画分にはダイオキシン類およびPCBはほとんどど含まれないともされており，臍帯血のヘマトクリットを50%と仮定すると，血漿中の化学物質の濃度は全血での値の約2倍となる。

海外のコホート調査との比較 —PCB曝露に着目して

過去の海外のコホート調査では，PCBの分析結果が報告されているため，PCBについて文献的な比較を試みた。表3に臍帯血の分析結果を，さらに我々はまだ母乳の分析結果を得ていないものの，表4に母乳の分析の比較の結果を示した。国内における曝露レベルの参考値とするため，国内分についてはコホート調査以外からも引用した[11,12]。

数値は中央値での比較を優先し，臍帯血では表記単位はng/mlとした。文献上で脂肪重量当たりの数値が記載されている場合には，我々の調査で得られた脂肪含量0.27%を用いて換算した数値も記載した。臍帯血PCBについて異性体情報が記載されていた場合には，生体試料中の存在比率が最も高いIUPAC#153の値を記載するとともに，New

表3　臍帯血中PCBレベルに関する文献比較
ΣPCBに加え，#153および高度塩素化PCB（塩素数7-9）についても算出可能なものは記した

Study	No.	Chemical	Geometric mean		Comment
North Carolina, 1978-1982	744	Σ PCB	<4.27	ng/ml	
Michigan 1980-1981	293	Σ PCB	2.7	ng/ml	
Netherlands, 1990-1992	373	Σ PCB	0.38	ng/ml	
	373	153	0.15	ng/ml	
Oswego, NY, 1991-1994	293	Σ PCB	0.52	ng/g-wet	
	293	Σ 7-9 Cl PCB	0.05	ng/g-wet	
Dusseldorf, 1993-1995	141	Σ PCB	0.39	ng/ml	
Nonavik, Quebec, 1996-2000	98	Σ PCB	0.76[a]	ng/ml	279.9 ng/g-lipid (70.8-1420.1)
	98	153	0.23[a]	ng/ml	86.9 ng/g-lipid (13.4-550.9)
Chiba and Yamanashi, 2002-2003	20	Σ PCB	0.14[b]	ng/g-wet	63.8b ng/g-lipid (31-110)
Tohoku, 2001-2003	42	Σ PCB	0.23[b,c]	ng/ml	全血で 0.115ng/ml (0.035-0.67)
	42	153	0.05[b,c]	ng/ml	全血で 0.026 ng/ml (0.007-0.140)
	42	Σ 7-9 Cl PCB	0.06[b,c]	ng/ml	全血で 0.031 ng/ml (0.008-0.211)

[a]脂肪率027%と仮定して計算した。[b]Median。[c]全血での濃度をHt50と仮定して血漿値に換算した。

York州Oswegoでの調査からは塩素数7～9個の高度塩素化PCBが児の発達との関連性が高いと報告されていることから[13]，高度塩素化PCBについても並記した。なお，母体血PCBの比較はすでに論文でも報告されており[14]，今回は記載しなかった。

臍帯血中の総PCBについてみると，我々の結果を含め国内の曝露レベルは海外に比較して低値となっている。総PCBについては各調査で積算の方法が若干異なるものの，IUPAC#153のみに着目しても同様な結果であった。しかしながら，高度塩素化PCBに着目すると，我々の結果はOswegoの曝露レベルに匹敵した。すなわち，Oswegoの総PCB値が高いのは，塩素数1-3の低度塩素化PCBの割合が多いためであり[13]，これは分析方法論上のクリーンアップや検出装置の特性に起因するものとも考えられた。

次に，母乳中PCBのレベルについて比較すると，国内の曝露レベルはOswego調査に匹敵するか，もしくは全体として低くなる傾向にあった。その一方で，Faroe諸島における曝露が高いことが明らかであり，1980～1981年に実施されたMichiganにおける調査とほぼ同じレベルの曝露であることが示唆された。Faroe諸島においてはメチル水銀による健康影響についても調査が進められているが，PCBsとメチル水銀の複合曝露による健康影響が強く懸念され，Faroe諸島におけるPCBの胎児期曝露のリスク評価が課題とも考えられた。なお，Michiganにおける調査では，量的なPCB曝露は母乳を介した寄与が大きいものの，出生後の曝露の影響は見出せなかった[15]。理由として，児の脳の感受性が胎児期に高いこと，また授乳行為そのものが児の発達を促す効果が期待されるため，と述べられている。

おわりに

化学物質による周産期曝露の健康リスクの解析を進めるうえで，児の発達を追跡すること，交絡要因を的確に把握すること，そして適切な曝露指標を得ることが重要と考えられる。TSCDはその解析途上であり，結論を得るにはまだ時間がかかるものと思われるが，近い将来，ダイオキシン類，PCB，メチル水銀曝露と児の健康リス

表4　母乳中PCBレベルに関する文献比較

Study	No.	Chemical	Geometric mean		Range	Comment
North Carolina, 1978-1982	617	ΣPCB	1530	ng/g-lipid		Milk at 6 weeks postpartum
Michigan 1980-1981	124	ΣPCB	829.7	ng/g-lipid		Milk at 0.5-4.5 months postpartum
Netherlands, 1990-1992	194	ΣPCB	404.8	ng/g-lipid		Milk at 2 weeks postpartum
	194	#153	174.7	ng/g-lipid		
Oswego, NY, 1991-1994	86	ΣPCB	153	ng/g-lipid		Milk at 1-3 months postpartum
Dusseldorf, 1993-1995	126	ΣPCB	404	ng/g-lipid		Milk at 2-4 weeks postpartum
Faroe Islands, 1994-1995	168	ΣPCB	1520	ng/g-lipid	70-18500	Milk at 3-4 days postpartum
Nonavik, Quebec, 1996-2000	116	ΣPCB	385.6	ng/g-lipid	75.7-1915.8	Milk at 1 month postpartum
	116	#153	131.6	ng/g-lipid	21.7-727.9	
Osaka, 1998	49	ΣPCB	200*	ng/g-lipid		Milk at 2-4 weeks postpartum

オランダの疫学調査では母乳中ダイオキシン類の分析が行われており，総TEQ（PCDD/Fs + co-PCBs) 62 pg-TEQ/g-lipid.
*Arithmetic mean.