two [V368I + M647 V], and two no polymorphisms. In 12 patients with acute encephalopathy (Cases 1–12), six (Cases 1–3 and 5–7) had a thermolabile F352C CPT II variant (1 F352C only and five [F352C + V368I]), and five (Cases 8–12) had the V368I CPT II variant (4 V368I only and one [V368I + M647 V]) and one (Case 4) showed no CPT II variant. Two patients with acute encephalopathy who died (Cases 1 and 2) had a thermolabile F352C CPT II variant (1 F352C only and the other [F352C + V368I]). In three patients with febrile delirium associated with influenza infection (cases 13–15), only case 13 (brief febrile seizure and unusually long febrile delirium) had the [F352C + V368I] CPT II variant. No other reported CPT II mutations or polymorphisms were detected.

There was no significant difference in the age at onset (41.0 ± 23.3 vs. 24.3 ± 12.7 months of age, $p = 0.18$), duration of high fever (52.0 ± 35.3 vs. 63.0 ± 44.9 h, $p = 0.28$), and duration of seizures (40.5 ± 40.1 vs. 56.7 ± 23.4 h, $p = 0.12$) between the six patients with acute encephalopathy with a thermolabile F352C CPT II variant (Cases 1–3,5–7) and six patients with acute encephalopathy without this thermolabile variant (Cases 4,8–12) (Mann–Whitney U-test).

5.2. Lymphocyte CPT II activity in the patients

As shown in Fig. 2(b), CPT II activity using peripheral lymphocytes of a patient with a thermolabile F352C CPT II variant was significantly reduced to about 50% during incubation for 120 min at 41 °C as compared to those at 30 and 37 °C. All patients with a thermolabile F352C CPT II variant showed a significant reduction of CPT II activity at 41 °C.

Fig. 2(a) shows CPT II activity in a patient with the V368I CPT II variant without reduction even at 41 °C.

5.3. Blood ATP levels in patients with acute encephalopathy

As shown in Fig. 3, ATP levels in the extracts of whole blood in the acute phase of encephalopathy during high fever were significantly low (0.58 ± 0.16 mM, $n = 10$) compared with those in the convalescent phase (1.08 ± 0.27 mM, $n = 5$) and with those of patients with febrile seizure status (1.01 ± 0.36 mM, $n = 9$). The blood ATP levels in the acute phase of encephalopathy revealed no significant difference when compared to those of patients with mitochondrial disease exhibiting several symptoms (0.79 ± 0.39 mM, $n = 6$).

6. Discussion

Although the precise pathomechanisms of acute encephalopathy have yet to be clarified, it is postulated that some genetically-determined factors might be involved, because some types of acute encephalopathy are more frequent in Japanese than in Caucasians. Chen et al. [12] demonstrated that the thermolabile phenotype of CPT II variations such as the F352C CPT II variant or complex [F352C + V368I] CPT II variant might be a principal genetic background of IAE in Japanese. On the basis of the analysis of fatty acid oxidation and cellular ATP production in COS-7 cells transfected with wild-type and variant *CPT2* cDNAs at 37 and 41 °C, Yao et al. [14] suggested that the compound *CPT2* variants with thermolabile phenotypes are the main cause of multiple-organ failure, particularly in high ATP-consuming organs as well as endothelial cells and play a major role in the etiology of IAE.

In the 12 patients with acute encephalopathy studied, six patients (Cases 1–3 and 5–7) had thermolabile F352C CPT II variants (F352C CPT II variant alone in one case and complex [F352C + V368I] CPT II variants in five cases), which were reported to be frequently noted in severe IAE patients [12,14]. Of the six patients, two patients (Case 1, IAE and Case 2, *Hemophilus influenzae*-associated septic encephalopathy) died despite intensive care. Case 2, who died of fatal septic encephalopathy [16], showed a high acylcarnitine ratio ((C16 + C18:1)/C2:0.203) on admission. This value corresponded to the ratio (>0.09) of the high-risk group of patients with IAE showing a fatal outcome, thus reflecting the disorder of mitochondrial β-oxidation. [12]. The remaining six patients (Cases 4 and 8–12) with acute encephalopathy without a thermolabile F352C CPT II variant followed a relatively mild clinical course (Table 1). Out of the six patients, five had a V368I CPT II variant.

As shown in Fig. 2, the CPT II activities of lymphocyte in patients with the F352C CPT II variant showed thermal instability, that is, a marked activity reduction at 41 °C, while those in patients with the V368I CPT II variant did not. There was no significant difference in the age at onset, duration of high fever, and duration of seizures between the six patients with the F352C CPT II variant (Cases 1–3 and 5–7) and six patients without this variant (Cases 4 and 8–12). Therefore, taken together, it seems likely that a thermolabile F352C CPT II variant might be related to the severity of disease, that is, the rapidity of progression of brain edema. In Caucasians, two polymorphisms of CPT II, p.V368I and p.M647 V, occur with a frequency of 0.5 and 0.25, respectively, exhibiting a Hardy–Weinberg equilibrium. A third polymorphism, p.F352C, occurs with a frequency of 0.21 exclusively in the Japanese population [17]. Therefore, this thermolabile F352C CPT II variant might be one of the predisposing factors to trigger the pathomechanism of acute encephalopathy in Japanese.

The CPT system regulates the entry of long-chain fatty acids into the mitochondrial matrix for β-oxidation. Fatty acid oxidation is an important source of acetyl-CoA for maintaining the tricarboxylic acid cycle.

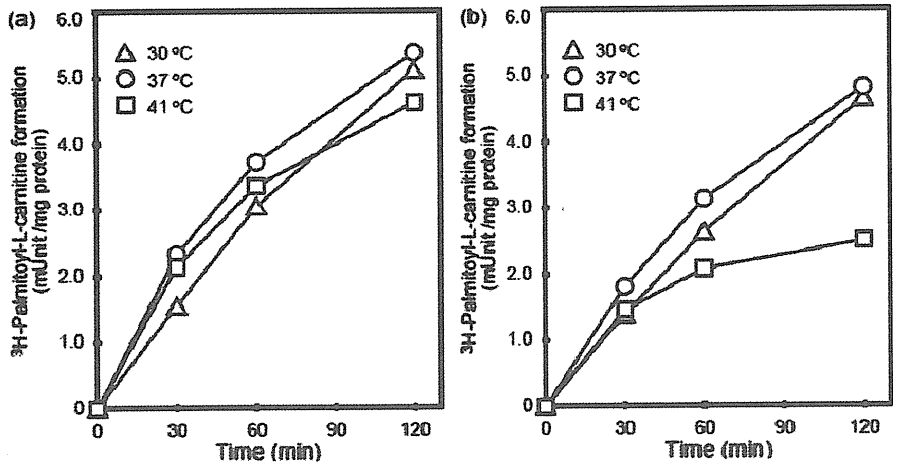

Fig. 2. (a) Lymphocyte CPT II activity in case 12 (influenza-associated encephalopathy) with V368I CPT II variant at 30, 37 and 41 °C. No definite reduction of CPT II activity was observed at 41 °C. (b) Lymphocyte CPT II activity in Case 1 (influenza-associated encephalopathy) with a thermolabile F352C CPT II variant at 30, 37 and 41 °C. At 41 °C, the CPT II activity decreased to about 50% of that at 37 °C after 2-h-incubation.

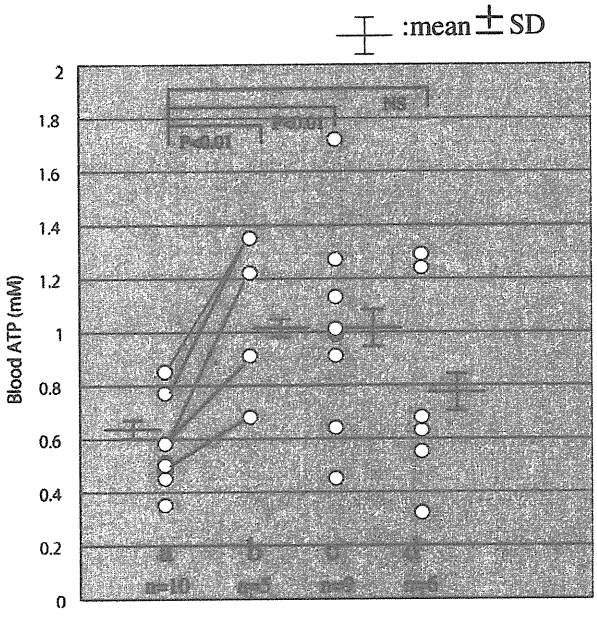

Fig. 3. ATP levels in whole blood in patients with acute encephalopathy (acute (a) and convalescent phase (b)), febrile seizure status (c) and mitochondrial disease (d). In five patients with acute encephalopathy, blood ATP level recovered at convalescent phase.

The CPT II is ubiquitously expressed in all tissues that require fatty acid oxidation as an energy-producing pathway [18]. CPT II deficiency is a disorder of long-chain fatty acid oxidation. It is classified into three clinical types based on the age at onset and disease severity: lethal neonatal form, severe infantile hepatocardiomuscular form, and myopathic form. It is clear that our patients' clinical manifestations did not correspond to any of these three types. The thermolabile instability of the F352C CPT II variant in our cases explains the situation whereby impaired energy metabolism could occur during high fever due to a secondary CPT II deficiency in spite of the absence of symptomatic manifestations of CPT II disorder in daily life at a normal temperature [12,14].

Olpin et al. [19] reported based on mutation analysis that when CPT II activities are above 20% of controls, fatty acid oxidation in fibroblasts is usually within the normal range (>70% of controls). However, under heat stress, fasting, acidosis, and seizures, moderately lowered CPT II activity due to the thermolabile F352C CPT II variant may accelerate the disease process of acute encephalopathy.

Blood ATP levels in the acute phase of encephalopathy during high fever were significantly lower than those in the convalescent phase and also with those of patients with febrile seizure status. This suggests that mitochondrial energetic failure may be more severe in patients with acute encephalopathy, and the pathological process of acute encephalopathy should differ from the febrile seizure status. The low levels of ATP in the acute phase of encephalopathy were normalized in the convalescent phase in line with clinical recovery. Interestingly, blood ATP levels in the acute phase of encephalopathy corresponded to those of mitochondrial disease with several symptoms. Yao et al. [14] showed that COS-7 cells transfected with thermolabile [F352C + V368I] CPT II variants exhibited significantly decreased fatty acid oxidation and subsequent intracellular ATP reduction at 41 °C. The decreased ATP levels seemed to reflect systemic mitochondrial dysfunction including the blood brain barrier (BBB) at the acute phase of encephalopathy in our cases. The ATP demand per body weight is so high in infants that a thermolabile CPT II variant induced-ATP reduction might lead to a greater susceptibility to the pathophysiology of encephalopathy in children than in adults.

The brain capillary endothelium is characterized by a greater density of mitochondria than that of peripheral capillaries [20]. This greater mitochondrial density is required to maintain the significant active transport mechanisms, electrochemical gradients, autoregulatory adjustments, and regulation of tight junctional complexes. As such, the requirement of a constant ATP supply may make the BBB particularly susceptible to acute hypoxic insult [21]. From a similar perspective, BBB breakdown may occur at an initial stage of encephalopathy under the condition of ATP reduction, thus leading to subsequent brain edema due to complex cascade of hypercytokinemia, excitotoxicity, and oxidative stress. Although there is one hypothesis that cytokine storm due to virus–glial cell interaction might cause endothelial cell damage (BBB breakdown) leading to brain edema and neuronal injury [11], we consider that endothelial cell damage might induce in turn cytokine production resulting in neuronal damage in patients with thermolabile F352C CPT II variant irrespective of encephalopathy type .

In three patients with febrile delirium associated with influenza virus infection (Cases 13–15), Case 13 with a thermolabile F352C CPT II variant developed a short seizure and an intermittent confused state with visual hallucinations and agitation lasting 6 h. Cases 14 and 15 without F352C CPT II variant showed short-term consciousness alteration and abnormal behavior without seizures. All patients' brain MRIs were normal, and they fully recovered. Although more extensive study is needed, the grade of febrile delirium associated with influenza virus was more severe in a case with a thermolabile F352C CPT II variant when compared with that in cases without F352C CPT II variant.

Given that a thermolabile CPT II variant might be one of the predisposing factors for acute encephalopathy, we should revise the therapeutic strategy from the acute phase. Considering the rapid progression of encephalopathy and associated low CPT II activity during high fever, immediate hypothermia, sufficient glucose infusion, and L-carnitine supplementation should be adopted as treatment options. We speculate that the immediate hypothermia led to the recovery of the lowered CPT II activity and, thus, mitochondrial energy failure became minimal in many tissues including the brain capillary endothelium, leading to less severe damage to the central nervous system.

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PAPS papers

Wnt signaling and telomerase activation of hepatoblastoma: correlation with chemosensitivity and surgical resectability

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Chemosensitivity

Abstract

Purpose: Recently, it became apparent that telomerase directly modulated Wnt signaling as a cofactor in a β-catenin transcriptional complex. In this study, we investigated Wnt/β-catenin signaling and telomerase activation in hepatoblastoma (HBL).

Methods: Tumors derived from 56 HBL cases treated with the Japanese Study Group for Pediatric Liver Tumors (JPLT) Protocol-2 were analyzed for oncogenic mutations (missense mutations and interstitial deletions in the third exon) of the *CTNNB1* gene-encoding β-catenin and for the expression levels of telomerase reverse transcriptase (*TERT*).

Results: Oncogenic mutations of *CTNNB1* were detected in 42 cases (75%). The expression levels of *TERT* were significantly higher in 14 cases without mutation ($P < .05$) and in 8 cases with metastasis ($P < .01$). Interestingly, Wnt/β-catenin target genes were significantly activated in the tumors without mutations ($P = .013$). In cases with mutations, preoperative chemotherapy was more effective ($P = .008$), and complete resection rate was higher ($P = .034$). Consequently, 2 patients with mutations and 4 patients without mutations died of disease ($P = .013$). High expression of *TERT* was detected in all tumors of these dead patients.

Conclusions: Wnt/β-catenin signaling in the HBLs without *CTNNB1* mutations was activated by high expression of *TERT*. The clinical courses in HBLs without *CTNNB1* mutations seemed to be unfavorable because of chemoresistance and low rates of resectability.

Hepatoblastoma (HBL) is one of the most common malignant liver tumors in children, and more than 70% of these tumors are diagnosed in patients younger than 2 years [1]. Hepatoblastoma tumors, which are derived from hepatic precursor cells, tend to be morphologically similar to immature hepatocytes, but the molecular abnormalities in

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his tumor have not been elucidated [2]. The incidence of HBL is significantly elevated in patients with familial adenomatous polyposis [3-5] who carry germ-line mutations in the *APC* tumor suppressor gene [6], which encodes a large cytoplasmic protein that plays a role in the Wnt signaling pathway [7]. Mutation or deletion of exon 3 in *CTNNB1* gene-encoding β-catenin has been frequently detected in HBL, thus suggesting an overactivation of the Wnt signal pathway [8,9]. Moreover, a germ-line mutation of the *AXIN1* gene, which encodes a protein forming a multiprotein complex involved in Wnt signaling, has been reported in cases with HBL [10]. As a result, the genesis or progression of HBL involves abnormal Wnt signaling and stimulating transcription of target genes, which include E-cadherin [11] and cyclin D1 [12].

Highly proliferative cell types such as embryonic cells and cancer cells require telomere maintenance strategies to protect the integrity of their genomes [13,14]. This enzyme uses its own RNA as a template to synthesize telomeric DNA and is mainly regulated by the expression levels of telomerase reverse transcriptase (TERT), the catalytic component of this enzyme. The relative levels of telomerase activity and TERT carry prognostic information in a variety of pediatric tumors, and this correlation seems to be particularly emphasized in neuroblastoma [15]. Thus, we already measured the levels of telomerase activity and *TERT* messenger RNA (mRNA) expression in HBL. Then we determined that the levels of *TERT* and telomerase activities were correlated with outcomes of the patients with HBL [16]. However, the correlation between telomerase activation and abnormal Wnt signaling in HBL had remained unclear.

A recent report revealed that TERT interacts with BRG1 (Brahma-related gene 1, also called *SMARCA4*), a SWI/SNF (SWItch and Sucrose NonFermentable)-related chromatin remodeling protein, and directly activates Wnt-dependent signaling pathway [17,18]. Therefore, TERT expression might be correlated to activate Wnt-signal pathway in HBL. To elucidate a significant level of integration between the 2 pathways—Wnt signaling and *TERT* activation—we investigated alterations of *CTNNB1* and *BRG1* expression and *TERT* activation and examined the activation of Wnt/β-catenin–dependent genes in HBL. Then we compared the activation of these 2 pathways with the clinicopathologic features and outcome of the patients in HBL.

1. Materials and methods

1.1. Patients

Fifty-six patients with HBL (36 boys and 20 girls, with a median age of 13 months) underwent tumor resection and partial hepatectomy between December 2000 and November 2008 at the institutions of the Japanese Study Group for Pediatric Liver Tumors (JPLT). All patients were treated in the JPLT-2 study [19], which consisted of 2 different protocols: cisplatin and pirarubicin as a first-line protocol and ifosfamide, pirarubicin, etoposide, and carboplatin as a second-line protocol. The Human Ethics Review Committee of our university approved the study protocol, and a signed informed consent was obtained from each parent (Approval of Ethics Committee No. 20).

The clinicopathologic parameters and outcomes for these patients were analyzed. The clinical stages of disease were determined at the time of initial biopsy or resection according to the classification of the pretreatment extent of disease (PRETEXT) staging system, which was based on the number of liver segments involved, the extent of local invasion, the extent of regional lymph node involvement, and the presence of distant metastases [20]. The PRETEXT system is based on the hepatic surgical anatomy, which is divided into 4 sectors, namely, an anterior and a posterior sector on the right and a medial and a lateral sector on the left [21]. Histologically, all tumors were HBLs. The pathologic classification of HBLs by Haas et al [22] and the Japanese Society of Pathology [20] divide HBLs into 2 major subtypes, namely, a well-differentiated (fetal) type and a poorly differentiated (embryonal) type. Complete responses (CRs) and partial responses (PRs) of primary tumors by preoperative chemotherapy were evaluated using Response Evaluation Criteria in Solid Tumors criteria (http://www.recist.com/).

1.2. Tissue samples

Tumor tissue specimens and their corresponding normal liver tissue specimens were obtained at surgery or biopsy from 56 patients with HBL before chemotherapy, immediately frozen, and stored at −80°C until use. The preparation of DNA was performed by standard proteinase K–phenol–chloroform extraction, as reported previously [23]. Total cellular RNA was extracted from tumor tissues by the acid guanidinium–phenol–chloroform method [24].

1.3. Detection of mutations and deletions of *CTNNB1* gene

For the detection of mutations and deletions in the *CTNNB1*gene, genomic DNA derived from each tumor and the corresponding liver tissue was amplified by polymerase chain reaction (PCR) using a primer pair specific for exon 3 (Fig. 1) as described previously [8]. To detect a large deletion including exon 3, the identities of PCR products were analyzed by 2% agarose gel electrophoresis. To detect the point mutation in exon 3, the PCR products were reamplified with internal primers: 5′-AAAATCCAGCGTGGACAATGG-3 (BCAT-3) and 5′-TGTGGCAAGTTCTGCATCATC-3′ (BCAT-4). Their nucleotide sequences were determined with the DNA sequencer ABI 3100 (Applied Biosystems, Life Technologies, Carlsbad, CA). Mutation

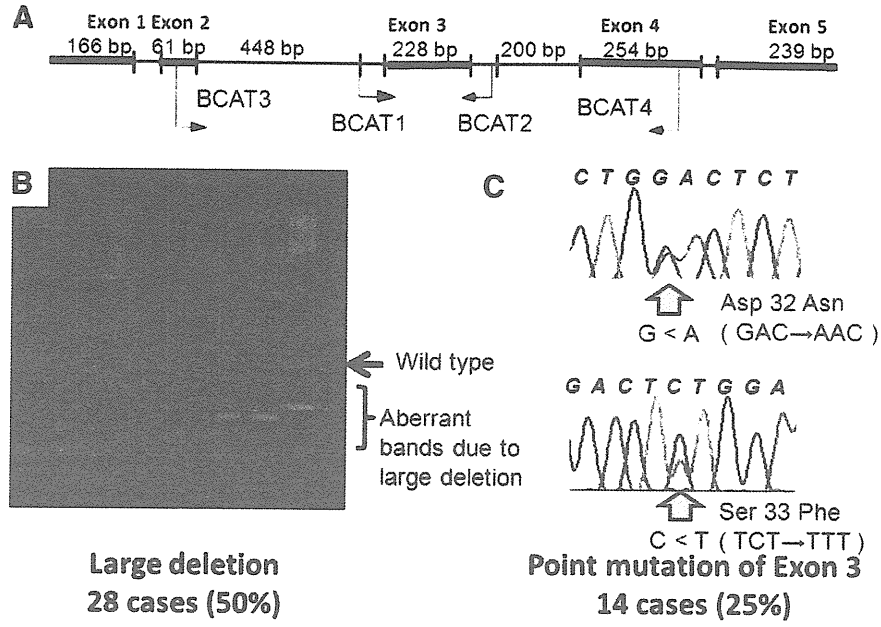

Fig. 1 Schematic illustration of the location of *CTNNB1* gene deletion and mutations in HBLs. A. Locations of each primer set for detection of point mutations (BCAT1 and BCAT2) and deletion (BCAT3 and BCAT4). B. Representative electrophoresis for detection of large deletion and mutations. In the present study, large deletions involving exon 3 were detected in 28 cases (50%). C. Representative sequencing data for detection of point mutation of exon 3. In the present study, point mutations of exon 3 were detected in 14 cases (25%).

identification was confirmed with at least 2 amplification reactions from the original DNA.

1.4. Quantitative reverse transcription–PCR for *TERT*, *BRG1*, and Wnt/-catenin target genes

The expression levels of *TERT*, *BRG1*, and Wnt/β-catenin target genes including *MYC*, *CCND1*, *ABCB1*, *MMP7*, *AXIN2*, and *BIRC5* in each tumor and the corresponding liver tissue were estimated by the quantitative reverse transcription–PCR as described previously [25]. For *BRG1* mRNA expression analysis, wild type and splicing variant types are usually detectable in human somatic cells. The expression levels of these 2 mRNAs were equivalent in HBL tumors (data not shown). Therefore, we used the expression levels of the wild type as *BRG1* mRNA expression. Results of 3 or more independent measurements were averaged, and the relative gene expression levels were calculated as ratios to *GAPDH* expression levels for each sample. In this study, we divided the tumors into low-*TERT*-expression tumors (<0.01) and high-*TERT*-expression tumors (≥0.01) because *TERT* expression levels of noncancerous liver tissues were less than 0.01.

1.5. Statistical analysis

Correlations between the *CTNNB1* oncogenic mutations and *TERT* mRNA expression levels or each of the other factors were analyzed using the Wilcoxon *t* test, χ^2 test, or Fisher exact test where appropriate. Differences were considered significant at $P < .05$.

2. Results

2.1. Oncogenic mutations in the *CTNNB1* gene

Analyses of oncogenic mutations (missense mutations and interstitial deletions in exon 3) were performed by focusing on exon 3 of *CTNNB1* in 56 HBL tumor samples, using standard PCR followed by subsequent DNA sequencing. Forty-two (75%) of 56 malignant tumors had deletions or mutations (Table 1). Deletions (n = 28) were more frequent than the missense mutations (n = 14) in HBLs, as reported previously [8,26,27]. All these interstitial deletions included the whole or a part of exon 3. These mutants were in-frame deletions and could cause the generation of mutant CTNNB1 (β-catenin) proteins with a reduced molecular mass that lacked all or a part of the NH_2-terminal phosphorylation sites required for their own degradation. On the other hand, *CTNNB1* missense mutations were found in 14 tumors. They were Asp (GGA)32Tyr(GAC) (n = 4), Asp(GGA)32Ala(GGC) (n = 3), Ser(TCT)33Ile(CCT) (n = 1), Gly(GGA)34Arg(CGA) (n = 2), Gly(GGA)34Val(GTA) (n = 1), Gly(GGA)34Glu (GAA) (n = 1), Thr(ACC)41Ala(GCC) (n = 1), and Pro (CCT)44His(CAT) (Table 1). To examine whether these alterations in the *CTNNB1* gene were somatic or germ-line

Table 1 Clinicopathologic factors and *CTNNB1* mutation or *TERT* expression

Clinicopathologic factors		n	*CTNNB1*			*TERT*[a]	
			Wild	Mutation	Deletion	Low	High
Sex	Male	36	12	9	15	14	22
	Female	20	2	5	13	7	13
Age at diagnosis (y)	<1	19	4	7	8	6	13
	1-2	20	8	2	10	8	12
	2<	17	2	5	10	7	12
PRETEXT	1	6	1	2	3	2	4
	2	13	5	1	7	5	8
	3	25	4	8	13	11	14
	4	11	4	3	4	3	8
Extrahepatic factors	−	43	12	9	22	19	27
	+	13	2	5	6	6	8
Distant metastasis	−	48	12	12	24	19	29
	+	8	2	2	4	2	6
Pathology	Fetal	22	4	6	12	12	10
	Embryonal	20	8	4	8	5	15
	Unknown	14	2	4	8	3	11
Response to chemotherapy	CR, PR	37	6	9	22	17	20
	SD, PD	13	7	3	3	2	11
Resection	Complete	37	6	9	22	17	20
	Incomplete	19	8	5	6	4	15
Outcome	Alive	50	10	12	28	21	29
	Dead	6	4	2	0	0	6

SD indicates stable disease; PD, progressive disease.

[a] Tumors were divided into low-*TERT*-expression tumors (<0.01) and high-*TERT*-expression tumors (≥0.01) because *TERT* expression levels of noncancerous liver tissues were less than 0.01.

alterations, an analysis using the matched tumor and noncancerous liver specimens revealed that all of the detected mutations and deletions were somatic (data not shown).

2.2. Levels of mRNA expression of *TERT* and *BRG1* and correlation with the oncologic mutations of the *CTNNB1* gene

TERT mRNA expression was detected in 46 of 56 tumor specimens, and the levels ranged from 0.002 to 0.364 (mean, 0.021). Expression of *TERT* was detected in only 4 of 56 noncancerous liver specimens and ranged from 0.002 to 0.006. The expression levels were significantly higher in tumor specimens ($P < .001$). The levels of *BRG1*mRNA expression ranged from 0.358 to 4.933 (mean, 0.907) in tissue samples. Expression levels of *TERT* and *BRG1* were compared with oncologic mutations in the *CTNNB1* gene (Fig. 2). *TERT* expression levels in the tumors without oncologic mutations in the *CTNNB1* gene were significantly higher than in those with oncogenic mutations in the *CTNNB1* gene ($P < .05$). *BRG1* expression levels in the tumors without abnormalities of the *CTNNB1* gene were also higher but not significantly. However, the expression levels of these 2 genes showed a positive correlation in the tumors without oncologic mutation of the *CTNNB1* gene (Fig. 2).

2.3. Levels of mRNA expression of Wnt/-catenin target genes and correlation with the oncologic mutations of the *CTNNB1* gene or *TERT* mRNA expression

Expression levels of *MYC*, *CCND1*, *ABCB1*, *MMP7*, *AXIN2*, and *BIRC5* were compared with oncologic mutations in the *CTNNB1* gene (Fig. 3A) and with expression levels of *TERT* mRNA (Fig. 3B). Expression levels of *MYC*, *CCND1*, and *MMP7* in the tumors without oncologic mutations in the *CTNNB1* gene were significantly higher than in those with oncogenic mutations in the *CTNNB1* gene ($P < .05$). Expression levels of *MYC*, *CCND1*, and *ABCB1* in the tumors with high TERT expression were significantly higher than in those with low TERT expression ($P < .05$). These results indicated that Wnt/β-catenin target genes were significantly activated in the tumors without oncologic mutations in the *CTNNB1* gene and in those with high TERT expression.

2.4. Genetic abnormalities in the *CTNNB1* gene, levels of *TERT* mRNA expression, and clinicopathologic features of the patients

Table 1 shows the correlation between oncogenic mutation in the *CTNNB1* gene or *TERT* mRNA expression and clinicopathologic features of the patients. Regarding age

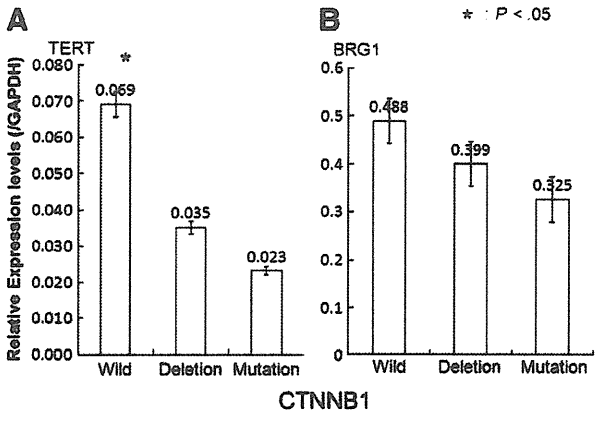

Fig. 2 *CTNNB1* mutation and *TERT* or *BRG1* expression. A, The expression levels of *TERT* in tumors without *CTNNB1* oncologic mutation were significantly higher than those with *CTNNB1* mutations ($P < .05$). B, The expression levels of *BRG1* in tumors without *CTNNB1* oncologic mutation were higher than those with *CTNNB1* mutation but not significantly.

at diagnosis, histologic type, PRETEXT classification, extrahepatic factors, and metastasis, there were no significant correlations with *CTNNB1* oncologic mutations or *TERT* expression. However, 4 of 6 deceased patients did not have *CTNNB1* gene abnormalities ($P = .013$). The levels of *TERT* mRNA expression were high in the older patients but not significantly. Histologic types in 22 fetal (well-differentiated)-type tumors and 20 embryonal (poorly differentiated)-type tumors did not significantly correlate with oncogenic *CTNNB1* mutations but showed significantly high *TERT* expression in embryonal-type tumors. In PRETEXT classification, the levels of *TERT* mRNA expression were not correlated with PRETEXT classification (Fig. 4). In tumors

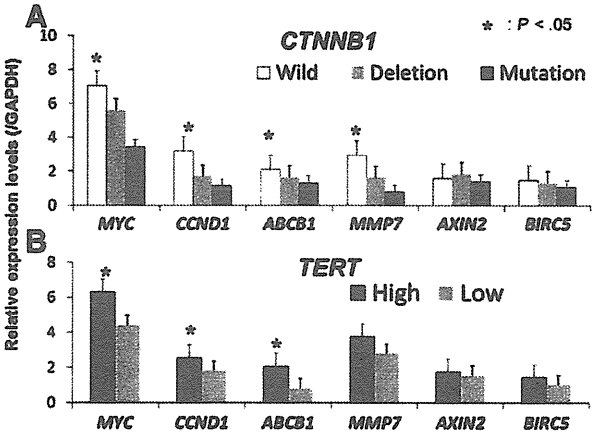

Fig. 3 Expression levels of Wnt/β-catenin target genes and *CTNNB1* mutation or *TERT* expression. A, The expression levels of *MYC*, *CCND1*, *ABCB1*, and *MMP7* in tumors without *CTNNB1* oncogenic mutations were significantly higher than others ($P < .05$). B, The expression levels of *MYC*, *CCND1*, and *ABCB1* in tumors with high *TERT* expression were significantly higher than others ($P < .05$).

with distant metastasis, *TERT* mRNA expression levels were high but not significantly. The levels of *TERT* mRNA expression were significantly high in metastatic HBLs and in embryonal (poorly differentiated) HBL ($P = .0146$ and $P = .0234$, respectively).

2.5. Oncologic mutations of the *CTNNB1* gene, levels of *TERT* mRNA expression, and therapeutic efficiency and patient outcome

Complete responses and PRs of primary tumors were analyzed in 50 patients who underwent neoadjuvant chemotherapy. Complete response or PR was obtained in 31 of 37 tumors with the *CTNNB1* oncogenic mutation and in 6 of 13 tumors without mutation ($P = .008$). Complete response or PR was also obtained in 17 of 19 tumors with low *TERT* expression and in 19 of 31 tumors without high *TERT* expression ($P = .031$). Surgical resectability including metastatic tumors was obtained in 31 of 42 tumors with *CTNNB1* oncogenic mutations and in 6 of 14 tumors without mutation ($P = .034$). Moreover, surgical resectability was obtained in 17 of 21 tumors with low *TERT* expression and in 15 of 35 tumors with high *TERT* expression ($P = .068$). Therefore, HBL tumors with *CTNNB1* oncogenic mutation and HBL tumors with low *TERT* expression showed high rates of therapeutic response including chemosensitivity. In 6 dead patients, 4 did not have *CTNNB1* oncogenic mutations and all showed high *TERT* expression.

3. Discussion

In the present study, we surveyed the oncogenic mutations of the *CTNNB1* gene and *TERT* expression in 56 HBL cases treated with the JPLT-2 protocol [28]. The frequency of *CTNNB1* deletions or mutations including exon 3 was substantial, 75% (42/56) in the present study, and this percentage was similar to previous reports, including Japanese articles [8,27,29,30]. Because the Wnt signaling pathway plays an important role in embryonic development, this pathway appears to have an important role in the tumorigenesis of HBL. On the other hand, we identified high levels of *TERT* mRNA expression as well as telomerase activity as independent prognostic factors in patients with HBL [16,18].

Telomerase activation has been reported in many kinds of malignant tumors including childhood and adult cancers, and approximately 80% to 90% of these malignant tumors showed telomerase activity [31-33]. In some kinds of tumors, high telomerase activity has been reported as a marker of tumor aggressiveness and poor prognosis [31,32]. In childhood tumors, telomerase activity and *TERT* mRNA expression were also detected in most cases of neuroblastoma, retinoblastoma, and nephroblastoma [18]. In neuroblastoma and retinoblastoma, a significant correlation between high telomerase activities and poor patient outcomes was previously

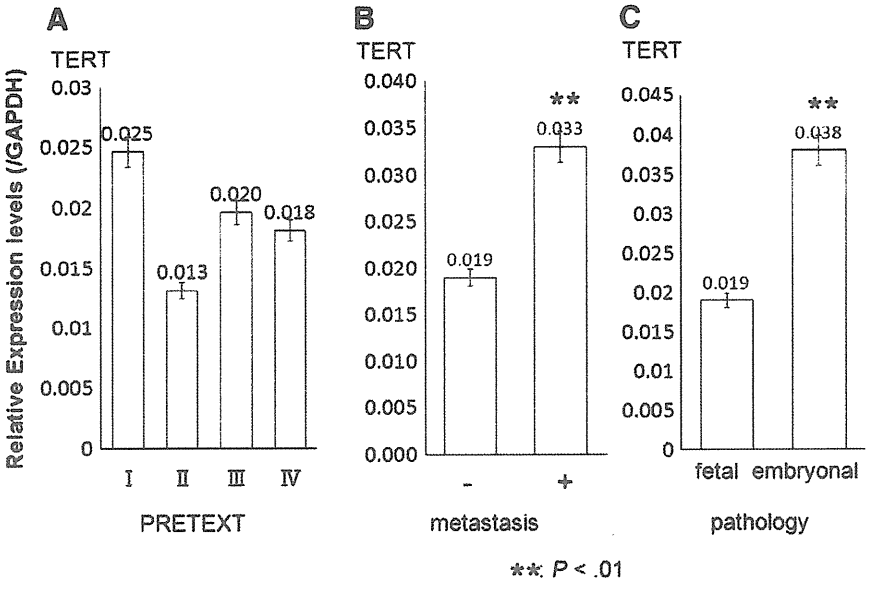

Fig. 4 Pretreatment extent of disease classification, metastasis, pathology, and *TERT*. A, The expression levels of *TERT* in tumors were not different from those in the tumors classified by PRETEXT. B, The expression levels of *TERT* in tumors with distant metastasis were significantly higher than those without metastasis ($P < .01$). C, The expression levels of *TERT* by embryonal (poorly differentiated) tumors were significantly higher than those of fetal (well-differentiated) tumors ($P < .01$).

reported [15,34]. Therefore, telomerase activity was correlated with poor prognosis of patients with HBL, indicating that activation of telomerase may correlate with malignant potential in most childhood malignant tumors.

Recently, telomerase was identified as a direct modulator of Wnt/β-catenin signaling by serving as a cofactor in a β-catenin transcriptional complex. TERT interacts with BRG1 (also called *SMARCA4*), a SWI/SNF-related chromatin remodeling protein, and activates Wnt-dependent reporters as a β-catenin-interacting protein [17]. Thus, Wnt/β-catenin signaling could be activated by telomerase as well as the accumulation of β-catenin caused by oncogenic mutations of *CTNNB1* gene exon 3. The present study provides supportive evidence for this interpretation. As shown in Fig. 3, *TERT* expression was significantly increased in the tumors without *CTNNB1* oncogenic mutation. Moreover, Wnt signaling target genes such as *MYC*, *CCND1*, *ABCB1*, and *MMP7* were activated in HBL tissue samples with high expression of *TERT* as well as those with *CTNNB1* oncogenic mutations using real-time reverse transcription–PCR. Therefore, the tumorigenesis of some HBLs might be correlated with telomerase activation instead of *CTNNB1* oncogenic mutation in a manner similar to the activation of Wnt/β-catenin signaling such as *APC* gene mutation [35] and *AXIN1* and *AXIN2* [10,36].

Interestingly, the analysis of the clinicopathologic features of our series of patients revealed that HBL with high expression of *TERT* showed poor responsiveness to neoadjuvant chemotherapy and low rates of resectability of tumors. Therefore, such HBL tumors showed poor outcome, indicating that *TERT* expression might be correlated with malignant potential in HBL. The tumors without *CTNNB1* oncogenic mutations also showed poor responsiveness to neoadjuvant chemotherapy, low rates of resectability of tumors, and poor outcomes, indicating that almost all these tumors acquired telomerase activation. These data are in accord with our previous report [37] and telomere-telomerase biology of pediatric tumors [18,32]. Our recent analysis of JPLT-2 indicated that exclusion of low-risk patients from postoperative chemotherapy could spare some of its serious adverse effects, but in tumors with high malignancy, complete resection and chemotherapy might be insufficient and new aggressive strategies should be implemented. The observations in our study suggest that telomerase inhibition is an effective strategy for high-risk HBL [18]. On the other hand, HBL with oncogenic mutations of the *CTNNB1* gene showed high response rates to neoadjuvant chemotherapy and high rates of resectability of tumors when telomerase was not activated. These molecular markers might be useful for predicting the chemosensitivity and resectability of HBL tumors.

In this study, we investigated the oncogenic mutations of the *CTNNB1* gene and expression of telomerase (*TERT*) in HBL tissue samples. These 2 alterations were found in most HBLs and may play important roles in liver carcinogenesis in children. Most interestingly, the activation of Wnt/β-catenin signaling including telomerase activation may play a critical role in the carcinogenesis and progression of HBLs.

Acknowledgments

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栄養環境と代謝のエピジェネティクス

江原達弥，亀井康富，高橋真由美，袁　勲梅，小川佳宏

これまで遺伝素因によるところが大きいと考えられてきた「太りやすい」「生活習慣病になりやすい」体質の獲得に，栄養環境による代謝関連遺伝子のエピジェネティクス制御が影響を与えていることが明らかとなりつつある．特に胎児期～新生児期は臓器が形成・成熟する可塑性の高い時期であり，この時期の栄養環境が肝臓，骨格筋，脂肪組織など代謝に重要な臓器のエピゲノムに変化を引き起こし，成人期の疾患罹患性に影響を与える可能性がある．本稿では，栄養環境の観点から，代謝疾患とエピジェネティクスに関する最近の研究の動向と今後の展望を概説したい．

キーワード● 栄養環境，生活習慣病，肥満，DNAメチル化，メタボリックメモリー

はじめに

代謝関連疾患の多くは多因子疾患であり，遺伝素因と栄養環境などの環境因子が複雑に相互作用することにより発症する．その分子基盤の1つとしてエピジェネティックな遺伝子発現制御が注目されている（図1）．例えば，疫学調査や動物モデルを用いた研究により，われわれの生体内では胎児期や新生児期の栄養環境が何らかの形で記憶され（メタボリックメモリー※1），その後の肥満症や生活習慣病※2など代謝関連疾患の罹患性に影響を与えるという概念が提唱されている（developmental origins of health and disease：DOHaD）が，この記憶のしくみとしてエピジェネティクスの関与が想定されている．すなわち，胎児期～新生児期に曝された栄養環境により代謝関連遺伝子のDNAメチル化，ヒストン修飾などが個体ごとに調節され，その後維持されることで遺伝子発現量に個体差が生じた結果，成人期の肥満や生活習慣病の罹患性に影響を与えると考えられる（図2）．本稿では，栄養環境が関連遺伝子のエピジェネティクス制御を介して代謝疾患の罹患性に影響を与える可能性について概説したい．

※1 メタボリックメモリー
過去の一定時期に曝された環境により代謝機能に生じた変化が，細胞内に記憶されたかのように長期的に持続する状態を表したもので，過去の血糖コントロールの良し悪しが糖尿病治療の良否に影響を与えるという疫学研究などから提唱された．エピジェネティクスの関与が指摘されている．

※2 生活習慣病
食習慣，運動習慣，休養，喫煙，飲酒などの生活習慣が，その発症・進行に関与する疾患群であり，1996年に旧厚生省が導入した言葉である．具体的な疾患には，癌，心臓病，糖尿病，脳卒中，高血圧などがあげられる．

Epigenetics of nutritional environment and metabolism
Tatsuya Ehara[1)4)] /Yasutomi Kamei[1)2)] /Mayumi Takahashi[1)] /Xunmei Yuan[1)] /Yoshihiro Ogawa[1)3)] : Department of Molecular Medicine and Metabolism, Medical Research Institute, Tokyo Medical and Dental University[1)] /Department of Organ Network and Metabolism, Medical Research Institute, Tokyo Medical and Dental University[2)] /Global Center of Excellence Program, Medical Research Institute, Tokyo Medical and Dental University[3)] /Functional Food Research Department, Food Science & Technology Institute, Morinaga Milk Industry Co., Ltd.[4)]（東京医科歯科大学難治疾患研究所分子代謝医学分野[1)]／東京医科歯科大学難治疾患研究所臓器代謝ネットワーク研究部門[2)]／東京医科歯科大学グローバルCOEプログラム[3)]／森永乳業株式会社食品基盤研究所素材機能研究部[4)]）

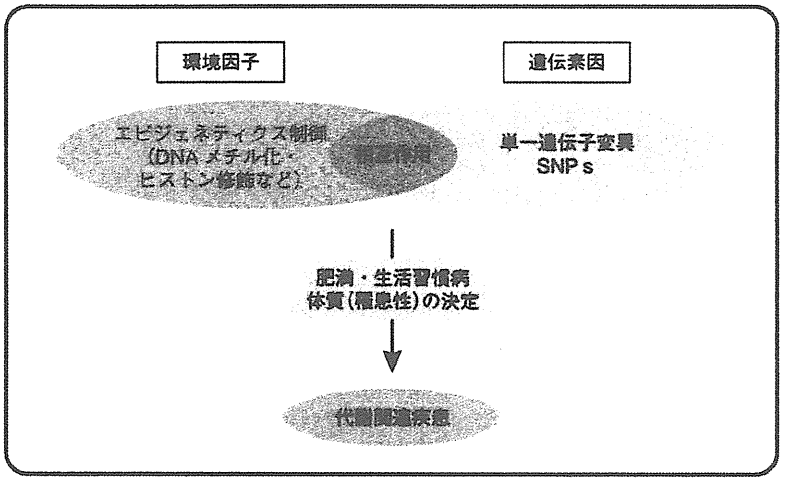

図1　代謝関連疾患発症における環境因子としてのエピジェネティクス修飾
代謝関連疾患は環境因子と遺伝素因の複雑な相互作用により発症する代表的な多因子疾患である．肥満や糖尿病には，単一遺伝子変異により発症する頻度が低いものと疾患感受性を付与する一塩基多型（SNPs）が関連する比較的頻度の高いものがある．一方，塩基配列の変化を伴わないものとして，栄養環境など種々の外的要因（環境因子）によりもたらされる後天的な遺伝子修飾（DNAメチル化，ヒストン修飾など）による遺伝子発現制御（エピジェネティクス）の機序が注目されている

1 胎児期の栄養環境とエピジェネティクス

● 母親の低栄養とエピジェネティクス

多くの疫学研究により，母胎内で低栄養に曝された低出生体重児は将来代謝関連疾患の発症リスクが高まることが指摘されている（Barker仮説）[1]．例えば，第二次大戦末期の「オランダ飢饉」を経験した母親の出生児は成人後に肥満や耐糖能障害，高血圧を発症しやすい[2]．前述の胎児期の低栄養を再現する動物モデルとして，妊娠期の母体のカロリー制限，子宮動脈結紮などにより得られる子宮内発育遅延（IUGR）モデルがある．カロリー制限により得られたIUGRマウスは出生直後こそ低体重を示すが，その後急激な体重増加により対照群と体重差が認められなくなる（catch-up growth）．このマウスの成獣期に高脂肪食を負荷すると，対照群と比較して体重・体脂肪量の増加，耐糖能とインスリン抵抗性の増悪を示す[3]．また，収縮期圧が上昇し，心臓冠動脈周囲血管の線維形成，心筋の肥大が生じることが報告されている[4]．一方，IUGRラットでは腎臓のネフロン数の減少，骨格筋のミトコンドリア機能の低下が報告されている[5]．

これらのメカニズムの大部分は不明だが，いくつかの遺伝子のエピジェネティックな変化が報告されている．雌性IUGRラット骨格筋において，糖取り込みを担う*Glut4*遺伝子のプロモーター領域のヒストンH3は新生仔期から転写抑制性の修飾に変化しており，成獣になってもこれが維持されるという[6]．また，子宮動脈結紮により作製されたIUGRラットは2型糖尿病を呈するが，これには膵β細胞機能に重要な転写因子*Pdx1*遺伝子プロモーター領域のDNAメチル化増加やヒストン修飾の変化による発現減少が伴うことが報告されている[7]．その他，ヒトIUGR新生児の臍帯血由来の造血幹細胞において，糖代謝に重要な転写因子*HNF4A*をはじめとする，複数の遺伝子座のDNAメチル化に対照群との差が検出されている[8]．

● 母親の過栄養とエピジェネティクス

母親の肥満もまた，胎児期の低栄養と同様に出生児の肥満リスクを増加させることが指摘されており[9][10]，マウスなどの動物実験においても，高脂肪食を与え過栄養にした母獣の産仔が肥満や糖尿病などの生活習慣

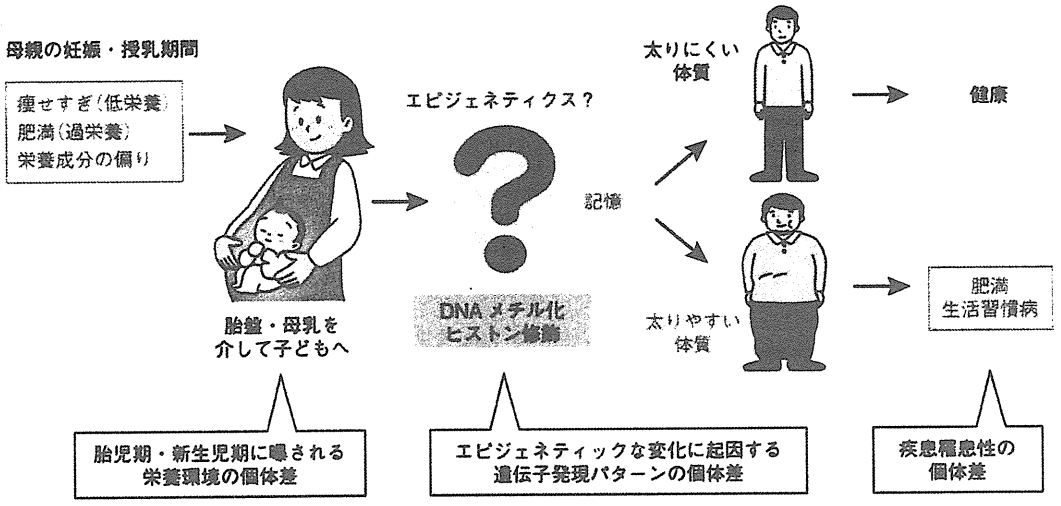

図2　胎児期・新生児期の栄養環境と代謝関連疾患
胎児期～新生児期に，胎盤や母乳を介して曝された栄養環境に応じてDNAメチル化やヒストン修飾など，エピジェネティックな変化が生じ，これが記憶として維持され，遺伝子発現パターンの個体差を生むことにより，成人期の肥満症や生活習慣病の罹患性に影響を与える可能性が注目されている

病病態を示すことが報告されている[11)12)]．エピジェネティックな制御の関与が想像されるが，メカニズムの詳細な報告はなされていない．肥満状態の母体の胎内環境は，低栄養状態のそれとは大きく異なるはずで，それにもかかわらず両者が同様の表現型を呈することは興味深い．このことは，胎児期の栄養環境が代謝機能に作用するメカニズムが単純ではなく，それぞれの栄養環境に応じて対象臓器・遺伝子が異なるなど，非常に複雑であることを意味するものであろう．さらに最近，交配前の雄ラットに高脂肪食を負荷すると，その雌性の仔において糖代謝が変化することが報告されており，父親の過栄養も子孫の代謝機能に影響を与える可能性がある[13)]．この場合，生殖系列に生じたエピジェネティックな変化が子孫に受け継がれる可能性が示唆される．

● 母親の栄養素摂取とエピジェネティクス

DNAやヒストンのメチル化は，メチオニン・葉酸代謝経路において，メチル基転移酵素により*S*-アデノシルメチオニン（SAM）からメチル基が供与されることにより生じるが，このSAMの合成には，メチオニン，葉酸，コリン，ビタミンB_{12}のような食事由来の栄養素がメチル基ドナーとして重要である（図3A）．したがって，妊娠期の母親にこれら栄養素摂取の過不足が生じると，胎児のDNAやヒストンのメチル化状態が変化し，遺伝子発現パターンの変化を介して表現型に影響を与える可能性が指摘されている．例えばA^{vy}マウスの体毛色に関する研究では，メチル基ドナーを豊富に含む飼料を妊娠期に摂取した母マウスの産仔において，体毛色の決定にかかわる*agouti*遺伝子プロモーター領域のDNAメチル化と体毛色が変化することが報告されている[14)]．さらに，妊娠期のメチル基ドナー摂取を制限された母ヒツジの産仔のDNAメチル化がゲノムワイドに変化し，またインスリン抵抗性や高血圧といった生活習慣病病態を呈するという報告もなされている[15)]．

2 肝臓の脂質代謝と栄養環境，エピジェネティクス

肝臓の脂質代謝機能の異常はインスリン抵抗性や脂肪肝などの生活習慣病病態を引き起こす．最近，肝臓の脂質代謝機能が胎仔期～新生仔期の栄養環境の影響

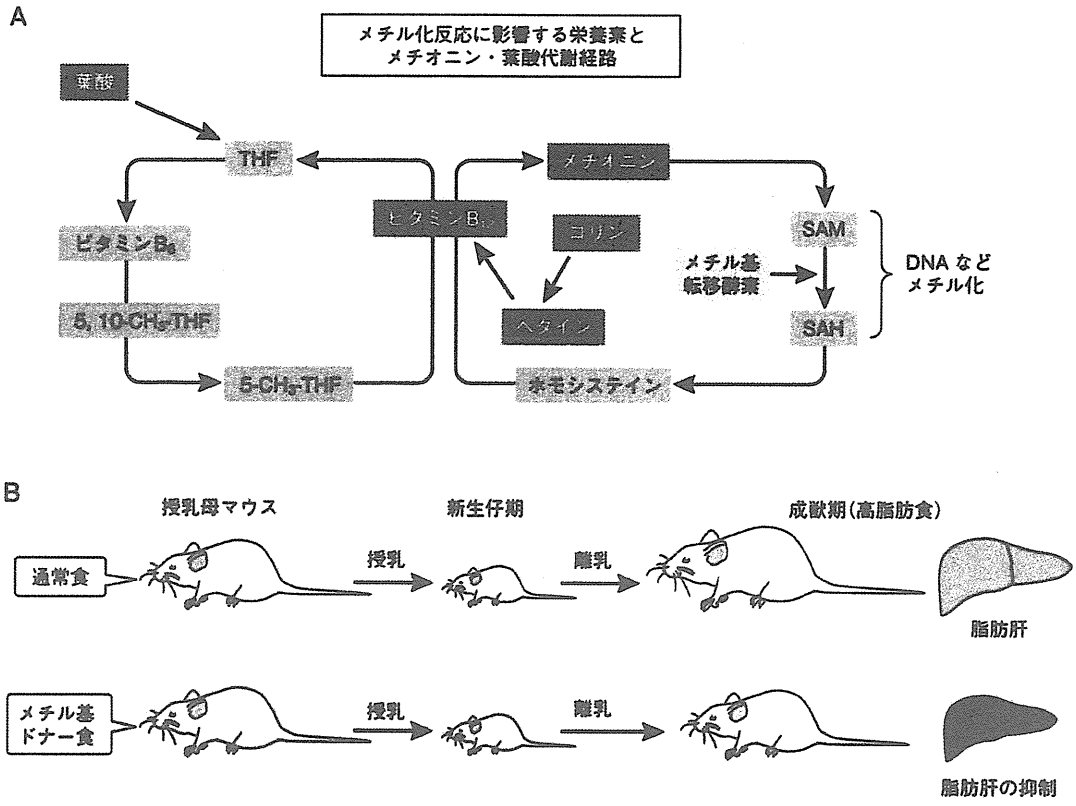

図3　栄養素とメチル化

A）DNAやヒストンなどのメチル化反応のメチオニン・葉酸代謝経路における位置づけを示す．食事由来の栄養素がDNAやヒストンなどのメチル化反応に重要な役割を担う．栄養環境の悪化によるこれら栄養素の過不足がエピジェネティックな変化を引き起こし，肥満症・生活習慣病の素因を形成する可能性が考えられる．THF：tetrahydrofolate，SAM：*S*-adenosylmethionine，SAH：*S*-adenosylhomocysteine．B）授乳期にメチル基ドナー食を与えた母マウスが育てた仔マウスは，離乳後の高脂肪食摂取による脂肪肝形成が抑制された．「メチル基ドナー食」中にはメチオニン，葉酸，ビタミンB_{12}など（A図中■で示した栄養素）が豊富に含まれる[19]

を受けることを示唆する報告がなされている．妊娠期～授乳期に高脂肪食を与えた母獣が生み育てた仔の肝臓の中性脂肪蓄積量が増加し，脂肪肝や非アルコール性脂肪性肝疾患を生じやすいことがサル，マウスなどの動物実験により示されている[16)17)]．一方，われわれは最近，メチル基ドナーを豊富に含む飼料により授乳期の母マウスを飼育することにより，経母乳的にメチル基ドナーを豊富に摂取した仔マウスでは，離乳後の高脂肪食負荷による脂肪肝の形成が著しく抑制されることを見出している（図3B）．これらのことから，肝臓の脂質代謝機能は胎仔期～新生仔期に曝された栄養環境に従って，DNAメチル化を含むエピジェネティクス制御を受けて調節されることが想像される．

われわれは脂質代謝にかかわる遺伝子のうち，グリセロール3リン酸にアシル基を導入する脂肪合成の律速酵素GPAT1がDNAメチル化による制御を受け，栄養環境によってDNAメチル化状態が変動しうることを見出している（図4）．すなわち，GPAT1の遺伝子プロモーターにおけるDNAメチル化の程度は遺伝子発現と逆相関し，DNAメチル化の変動はGPAT1特異的で他の脂肪合成遺伝子には認められなかった．また，培養細胞を用いた検討では，DNAメチル化により転写

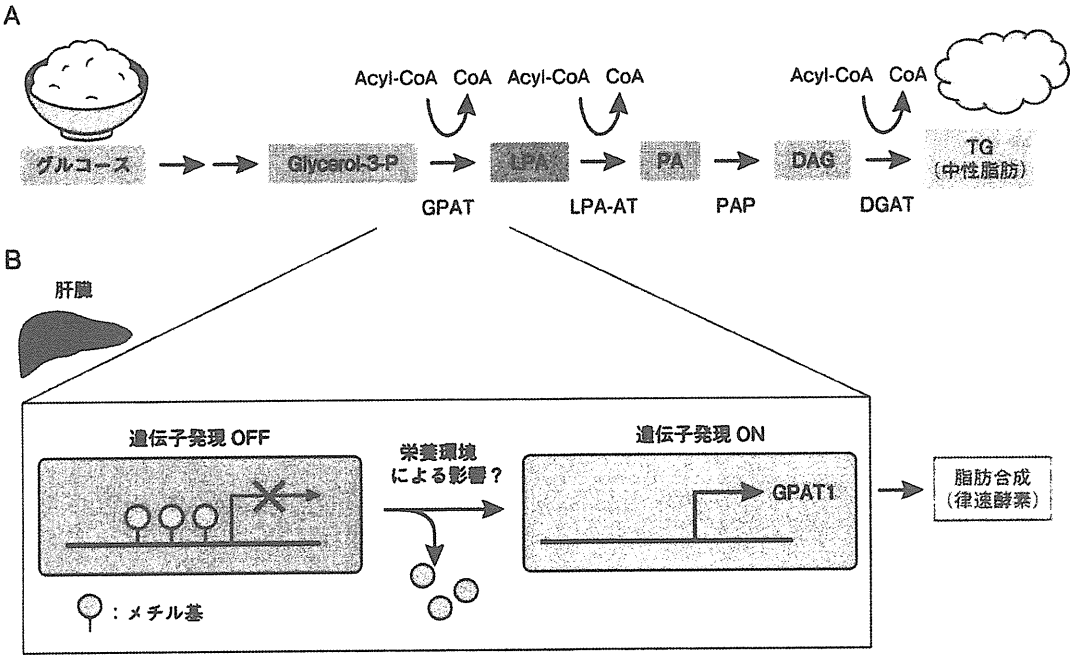

図4　中性脂肪合成とGPAT1のDNAメチル化制御
A）食事中の炭水化物由来のグルコースは数段階の酵素反応を経て中性脂肪に変換される．GPATはグリセロール3リン酸(glycerol-3-P)にアシル基を転移する律速酵素である．LPA：lysophosphatidic acid，PA：phosphatidic acid，DAG：diacylglycerol，TG：triacylglycerol，GPAT：glycerol-3-phosphate acyltransferase，LPA-AT：lysophosphatidic acid acyltransferase，PAP：phosphatidic acid phosphatase，DGAT：diacylglycerol acyltransferase．B）GPAT1はDNAメチル化による転写制御を受け，プロモーター領域のDNAメチル化により発現抑制される．栄養環境によるDNAメチル化変化の可能性が示唆される

活性が直接抑制された．肝臓の脂質代謝関連遺伝子のDNAメチル化による制御機構を解明することで，栄養環境が疾患罹患性に影響を与えるしくみの理解につながると期待される．

おわりに

「太りやすい」「生活習慣病になりやすい」体質の獲得に，胎児期～新生児期の栄養環境による代謝関連遺伝子のエピジェネティクス制御が影響を与えることが明らかとなりつつある．また，われわれは成獣肥満マウスの脂肪組織でDNAメチル化酵素Dnmt3aの発現量が著しく増加することを見出しており，成獣期においても栄養環境によるDNAメチル化制御が行われている可能性がある[18]．栄養環境によるエピジェネティクス制御の分子機構が明らかになることにより，その知見に即した栄養環境の提案・栄養成分の補充などによる，疾患になりにくい体質づくりなど，新たな疾患予防法の確立につながることを期待したい．

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Profile　筆頭著者プロフィール

江原康弥：2001年，上智大学理工学部卒業．'03年，上智大学理工学研究科修了．同年，森永乳業株式会社に入社し，'07年より同社食品基盤研究所に配属．'08年より東京医科歯科大学難治疾患研究所分子代謝医学分野，小川佳宏教授のもとで生活習慣病とエピジェネティクスに関する研究に参加，社会人大学院生として現在に至る．

シリーズ［ちょっとした疑問］

日本人単胎妊娠女性のフリーT_4, TSHの基準参考値について

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Key words | 妊娠（pregnancy）, 甲状腺ホルモン（thyroid hormone）, 基準値（reference intervals）

前号とも関連しますが、妊婦さんの甲状腺ホルモンコントロールには悩まされます。動態がダイナミックに変化する上に測定自体が妊娠により影響されやすいからです。

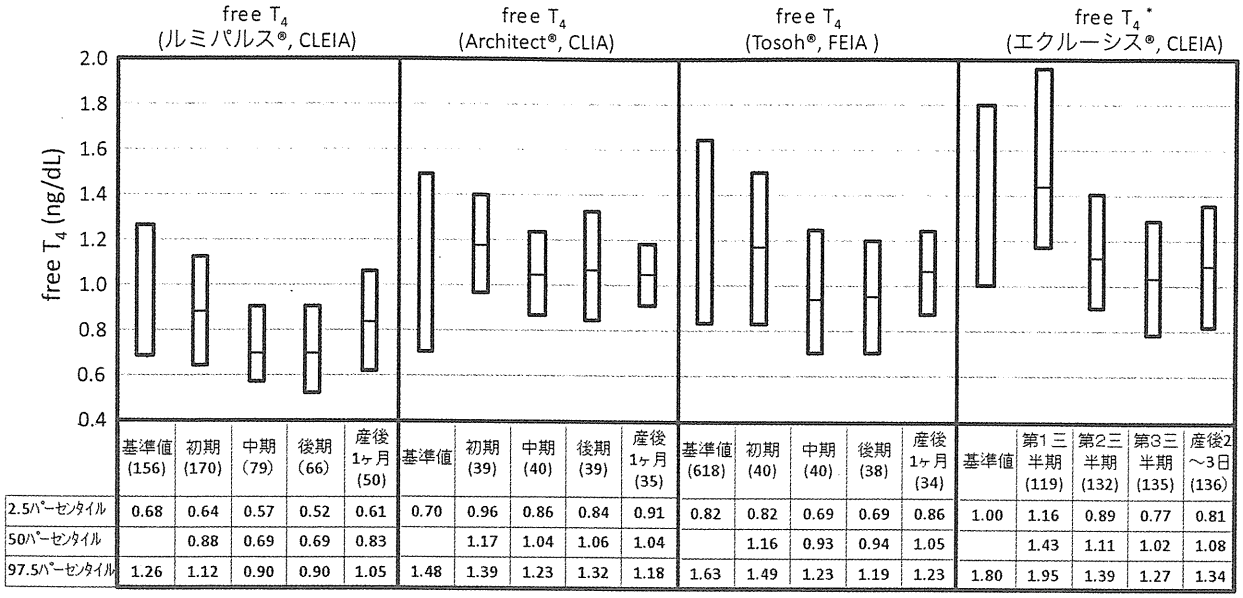
今回は妊婦さんのFT_4・TSH測定値につき、この領域で現在、最もアクティブに御活躍の荒田尚子先生にお答えいただきました（担当：田中 祐司）。

妊娠中の母体の明らかな甲状腺機能異常は，流早産，死産，妊娠高血圧症候群，心不全，胎盤早期剥離，低出生体重児などの原因になることが明らかである。海外からの報告では，潜在性甲状腺機能低下症でさえ，流早産や児の精神運動発達遅延の原因になりうるという報告もある。こうしたことから，甲状腺疾患の既往や家族歴を有するもの，甲状腺腫や甲状腺機能異常の症状や兆候があるもの，甲状腺自己抗体陽性やそのほかの自己免疫疾患合併，不妊治療歴や流早産の既往，頭部または頚部放射線治療既往を有するなど，いわゆるハイリスク妊娠においては妊娠初期に甲状腺機能のスクリーニングを行うことが複数のガイドラインで推奨されている[1),2)]。一方で，妊娠初期のヒト絨毛性ゴナドトロピンの甲状腺刺激作用や妊娠中の甲状腺ホルモン需要の増大，胎盤での甲状腺ホルモンの代謝などにより妊娠各期におけるフリーT_4値やTSH値は非妊娠時とは異なった動きを示し，測定系の相異によりさらにその程度は異なることが報告されている[1)]。わが国において，妊娠中の甲状腺ホルモン値の基準値としてエクルーシス®（ロシュ・ダイアグノスティックス社）を用いた栗岡らの2005年の報告があるが[3)]，近年の院内検査室で頻用されるキットでの基準値はない。そこで，わが国の甲状腺ホルモン値，TSH値測定系のシェアの大部分を占めているルミパルス®（富士レビオ社），Architect®（アボット社），Tosho®（東ソー）を用いたフリーT_4，TSH値の基準参考値を甲状腺疾患既往のない単胎妊娠女性の各妊娠時期における血清を用いて作成した。

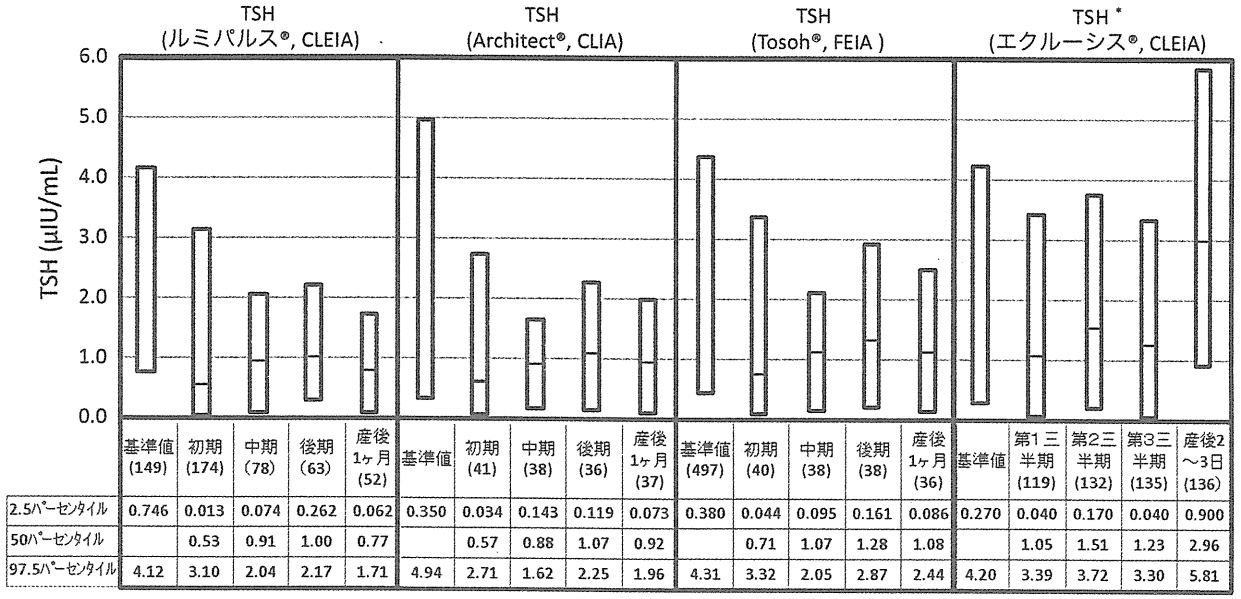
「子宮内発育に関連する母体の病態を診断・予防するための妊婦の基準値の設定に関する研究」より得られた糖尿病，甲状腺疾患，高血圧などの内科疾患のない単胎妊娠女性の残血清を使用した。同研究は，国立成育医療研究センター，国立病院機構福島病院において実施され，妊娠初期，妊娠中期，妊娠後期，産後１ヵ月時の健診時に採血が行われた。同血清を利用して，ルミパルス®（富士レビオ社），Architect®（アボット社），Tosho®（東ソー）を用いてフリーT_4，TSH値を測定した。また，血清は-80℃で保存され，凍結融解による影響はないことを確認済みである。同研究は倫理委員会の承認を得て行った。

図に各キットごとの妊娠各期および産後のフリーT_4，TSHの2.5～97.5パーセンタイル値を示した※。また，以前に報告されている栗岡ら[3)]のエクルーシス®の同値も付け加えた。妊娠，各期のフリーT_4値は測定キットごとに妊娠中に低下する程度は異なっていたが，妊娠初期には非妊娠時より高値もしくはほぼ同等であったが，妊娠の進行に伴い低下した。TSH値については，妊娠中は非妊娠時より低値であり，特に妊娠初期ではその中央値は低値を示した。今回の結果の限界は，ルミパルス®の妊娠初期の値を除くと症例数が少ないこと，甲状腺自己抗体のチェックが行われていないことから，甲状腺自己抗体陽性者も含まれている可能性がある点である。しかし，妊娠中の甲状腺機能異常を診断する上で有用と思われたため，妊娠中の甲状腺評価のための基準参考値として示した。

※生データもしくはべき乗変換後に正規性が確認できたものについては平均値±2SDにより，正規性が確認できなかったものについてはノンパラメトリック法により算出した。



	free T4 (ルミパルス®, CLEIA)					free T4 (Architect®, CLIA)					free T4 (Tosoh®, FEIA)					free T4 * (エクルーシス®, CLEIA)				
	基準値 (156)	初期 (170)	中期 (79)	後期 (66)	産後1ヶ月 (50)	基準値	初期 (39)	中期 (40)	後期 (39)	産後1ヶ月 (35)	基準値 (618)	初期 (40)	中期 (40)	後期 (38)	産後1ヶ月 (34)	基準値	第1三半期 (119)	第2三半期 (132)	第3三半期 (135)	産後2〜3日 (136)
2.5パーセンタイル	0.68	0.64	0.57	0.52	0.61	0.70	0.96	0.86	0.84	0.91	0.82	0.82	0.69	0.69	0.86	1.00	1.16	0.89	0.77	0.81
50パーセンタイル		0.88	0.69	0.69	0.83		1.17	1.04	1.06	1.04		1.16	0.93	0.94	1.05		1.43	1.11	1.02	1.08
97.5パーセンタイル	1.26	1.12	0.90	0.90	1.05	1.48	1.39	1.23	1.32	1.18	1.63	1.49	1.23	1.19	1.23	1.80	1.95	1.39	1.27	1.34



	TSH (ルミパルス®, CLEIA)					TSH (Architect®, CLIA)					TSH (Tosoh®, FEIA)					TSH * (エクルーシス®, CLEIA)				
	基準値 (149)	初期 (174)	中期 (78)	後期 (63)	産後1ヶ月 (52)	基準値	初期 (41)	中期 (38)	後期 (36)	産後1ヶ月 (37)	基準値 (497)	初期 (40)	中期 (38)	後期 (38)	産後1ヶ月 (36)	基準値	第1三半期 (119)	第2三半期 (132)	第3三半期 (135)	産後2〜3日 (136)
2.5パーセンタイル	0.746	0.013	0.074	0.262	0.062	0.350	0.034	0.143	0.119	0.073	0.380	0.044	0.095	0.161	0.086	0.270	0.040	0.170	0.040	0.900
50パーセンタイル		0.53	0.91	1.00	0.77		0.57	0.88	1.07	0.92		0.71	1.07	1.28	1.08		1.05	1.51	1.23	2.96
97.5パーセンタイル	4.12	3.10	2.04	2.17	1.71	4.94	2.71	1.62	2.25	1.96	4.31	3.32	2.05	2.87	2.44	4.20	3.39	3.72	3.30	5.81

図. 各キット毎の妊娠・産後のfree T_4, TSH参考値

＊エクルーシス®のデータは Kurioka ら[3]の論文より引用し、甲状腺疾患既往のない単胎妊娠の各三半期と産後2〜3日の2.5〜平均値〜97.5パーセンタイル値を示す。非妊娠時のデータは各キットのメーカー基準値(2.5〜97.5パーセンタイル値)を示す。

初期:〜妊娠16週未満, 中期:妊娠16週〜28週未満, 後期:妊娠28週以降,
第1三半期:〜妊娠14週未満, 第2三半期:妊娠14週〜28週未満, 第3三半期:妊娠28週以降,
CLEIA: Chemiluminescent Enzyme Immunoassay, CLIA: Chemiluminescent Immunoassay,
FEIA: Fluorescence Enzyme Immunoassay

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文　献

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母子への環境影響Q&A
お母さんからの質問にどう答えるか

**お母さんにとって，赤ちゃんへの環境影響は大きな関心事です。
ここでは，母子の健康と環境の問題について
お母さんから寄せられた質問をピックアップし，専門家の方々が答えます。**

妊娠中や授乳中のお母さんにとって気になるのが，環境によって赤ちゃんの成長や健康にどのような影響が出るのかということではないでしょうか。

子どもに影響を与える環境要因を明らかにするため，環境省の「子どもの健康と環境に関する全国調査」(エコチル調査)が今年から本格的にスタートしましたが，すでに日常臨床の現場では，助産師をはじめとする専門職に対して，環境によるリスクについてお母さんたちから質問される機会が増えているようです。

そこで，本稿では，お母さんから実際に寄せられた質問にどう答えればよいかを，Q&A形式で解説します。いずれの回答も，エコチル調査の関係者をはじめ，「環境と健康」の問題にかかわる専門家の方々の執筆によるものです。環境リスクが健康に与える影響についてはまだ未確定の部分も多く，これから明らかになっていく事項もありますが，このQ&Aにおける回答は，現在の最新知見に基づいたものと言えます。回答とともに，さらに関連知識を深めるための参考文献もあげていただきました。

日々お母さんたちと相対する専門職が，環境に関する質問を受けたときの答え方や考え方のヒントになればと考えます。

妊娠とマグロ，キンメダイ

Q 妊娠したら，マグロなどの大型魚やキンメダイは食べてはいけないのですか。

A 魚介類にはメチル水銀がわずかながら含まれています。メチル水銀濃度は，マグロなどの大型の肉食魚やキンメダイでは，アジやイワシなどの小型魚より高いことが知られています。

妊婦さんがメチル水銀を多く摂取すると胎児の神経系の発達に影響を与えることが報告されており，食品安全委員会は，妊婦さんのメチル水銀耐容摂取量を1週間に体重1kgあたり2μg(1μgは100万分の1g)と定めました。耐容摂取量とは，その量を摂り続けていても悪影響が出ない量のことです。

その後厚生労働省から，「妊婦への魚介類の摂食と水銀に関する注意事項」が出されています。これは，耐容摂取量を超えないように魚介類を摂取するにはどう食べたらよいかの目安を示したものです。例えばクロマグロやキンメダイの摂取は(80g食べるとして)週に1回までとしています。魚を食べないようにするのではなく，(メチル水銀濃度の異なる)魚種ごとに食べる量に制限を設けたということです。

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