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Extracellular cAMP-dependent protein kinase (ECPKA) in melanoma

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Abstract

Melanoma is one of the fastest rising malignancies in the United States. When detected early, primary melanomas are curable through surgery. However, despite significant improvements in diagnosis and surgical, local and systemic therapy, mortality rate in metastatic melanoma remains high. Furthermore, genetic alterations associated with the development and stepwise progression of melanoma, are still unclear. Previous reports show that the catalytic kinase subunit of the cAMP-dependent protein kinase is secreted by tumor cells and can be detected in the serum of cancer patients. We examine in this report the clinical significance of this secreted C subunit kinase termed extracellular protein kinase (ECPKA) in melanoma patients. Our results showed the presence of ECPKA activity in the serum of melanoma patients and correlate with the appearance and size of the tumor. Most importantly, surgical removal of melanoma causes a precipitous decrease in ECPKA activity in the sera of patients, suggesting that ECPKA may be a novel predictive marker in melanoma.

Keywords: Catalytic subunit; Serum; Biomarker; Diagnosis

1. Introduction

The incidence of malignant melanoma has increased dramatically in recent years worldwide [1]. Transformation of melanocytes into malignant melanoma involves multiple genetic alterations [2,3]. The complexity and heterogeneity of the array of genetic and environmental factors that may contribute to the etiology of malignant melanoma is further confounded by lack of information on specific genetic mutations and gene expressions associated with the initiation and progression of the disease [3]. Some genetic alterations

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including those of p53, $p16^{ink4}$, β-catenin, Lef/Tcf transcription factors, Mel-CAM/MUC18, chondroitin sulfate proteoglycan, and gangliosides GD3, which may be associated with the transition from normal melanocytes to nevi, have been described [3]. Nevertheless, the underlying stepwise progressive genetic and biochemical defects of the malignant transformation of normal melanocytes to a highly invasive and often fatal tumor are still poorly defined.

Phosphorylation mediated by the cAMP signal transduction pathway is an important regulatory mechanism that affects cell growth and transformation, and gene expression [4,5]. The action of cAMP is mediated mostly by the cAMP-dependent protein kinase (PKA), which is composed of two regulatory (R) and two catalytic (C) subunits in a R_2C_2 holoenzyme complex. PKA is predominately an intracellular enzyme.

Recently, the presence of PKA activity in the form of free C subunit was found in cancer patients' sera as well as in extracellular media of cancer cell culture [6–8]. This anomalous extracellular protein kinase (ECPKA), phosphorylates Kemptide, a PKA-specific synthetic peptide substrate containing the consensus phosphorylation site of PKA [9]. The ECPKA activity is specifically inhibited by PKI, a PKA specific peptide inhibitor [10], but not by the PKC-specific peptide inhibitor, and is not activated by cAMP [6,8]. In addition, the C subunit kinase can be detected by immunoblotting in the serum and conditioned culture media, thus suggesting that the ECPKA characterized in these studies is a secreted free C subunit kinase, independent of the PKA holoenzyme. The biochemical mechanisms and the physiological significance of ECPKA or the secreted C subunit kinase are not clear at present.

We further examined in this report the clinical relevance of ECPKA in melanoma and found that greater than 90% of melanoma patients' serum samples were positive for the C subunit kinase activity and seems to correlate with tumor size. Notably, surgical resection of melanoma resulted in significant decrease in ECPKA activity in patients' sera, suggesting that cancer cells secrete the C subunit kinase, and ECPKA may be a useful tumor biomarker for diagnosis, screening, and monitoring of progression or regression of tumors.

2. Materials and methods

2.1. Serum preparation and ECPKA assay with patient samples

Serum was obtained from 35 melanoma patients who had histologically confirmed melanoma. The study was approved by the Institutional Review Board at the Robert Wood Johnson Medical School and all patients were required to give written informed consent. Some of these patients had undergone surgery to remove the melanoma. Serum from normal healthy volunteers was also included as negative control. Ninety microlitre aliquots of the serum were used for the ECPKA activity assay as described later.

2.2. PKA assay

ECPKA activity was determined by measuring the transfer of ^{32}P from [γ-^{32}P] ATP to Kemptide, a specific peptide substrate, as described previously [8]. In brief, the reaction mixture contained 27.5 mM 4-morpholinepropanesulfonic acid; 2-(*N*-morpholino)ethanesulfonic acid, 1.0 mM EDTA, 2.75 mM NaF, 5.5 mM magnesium acetate, 1.25 mg/ml BSA, 0.1 mg per ml of kemptide, and 5.0 μCi of [^{32}P] ATP in a total volume of 160 μl. One set of reactions contained 10 μM PKI, a specific PKA inhibitor. The reaction was initiated by the addition of serum samples. After 10 min of incubation at 30 °C, 40 μl aliquots were immediately spotted onto phosphocellulose discs, washed four times in 3% phosphoric acid, dried, and quantitated by scintillation counting. A no-enzyme blank was subtracted from the total incorporation.

2.3. Statistical analysis

Results of serum ECPKA activity from patients were logarithmically transformed and then the repeated measurement models were used to assess the differences in the ECPKA levels between the normal and the melanoma serum samples. The compound–symmetric correlation structure was used to account for the within-subject correlation. Linear regression model was used to assess the association between the log ECPKA activity and tumor size.

3. Results

To further evaluate the clinical importance of the secreted C subunit kinase in cancer, we examined here the presence of ECPKA in serum samples obtained from patients with melanoma. We evaluated the serum samples from a total of 24 melanoma patients for ECPKA activity. Serum samples from five normal individuals showed low and negligible levels of ECPKA activity (Table 1). In contrast, ECPKA activity was markedly elevated in the serum of melanoma patients and the kinase activity is inhibited by PKI, a PKA specific peptide inhibitor [10] (Table 1). Statistical analysis of the log ECPKA activity from normal individuals was significantly lower than those from melanoma patients ($P = 0.0073$). On average, the ECPKA activity from normal individuals was 42.98% of that from melanoma patients. The log PKI inhibited kinase activity from normal individuals was also significantly lower than that of melanoma patients ($P = 0.0107$); and the average PKI inhibited activity from normal individuals was 27.86% of the melanoma patients.

We found that >90% ($n = 22/24$) of the serum samples from melanoma patients exhibit ECPKA activities that are significantly higher than the normal controls. We further observed that 50% ($n = 13/24$) of these patients' serum samples have ECPKA activities that were at least two-fold more than those from normal volunteers (Table 1).

The serum samples were obtained from individuals with various stages of melanoma. Our results showed that the log ECPKA activity have a positive correlation with tumor size (Fig. 1A), significantly so for patients whose melanoma that were not necrotic ($P = 0.0385$) (closed diamonds); but not significant for the patients whose tumors were necrotic ($P = 0.354$) (open circles), due to small sample size ($n = 4$). The association of ECPKA with tumor size was further evaluated in three individual patients, whose serum samples were serially collected at different times during the course of the disease, and ECPKA activities increased as the disease progressed with time (Fig. 1B). These findings suggest that increasing amount of the C subunit of PKA was secreted as tumor grew, thus resulting in increased levels of ECPKA in the serum.

To determine whether changes in ECPKA activity are associated with the development of melanoma, we next examined the kinase activity in patients' serum samples taken before and after surgical resection of the melanoma. We found that serum C subunit kinase activity was significantly reduced post-surgery in patients (Fig. 2). In one incidence, a patient's (#0024) serum initially exhibited 594% increase in kinase activity compared to normal

Table 1
ECPKA activity in serum samples of melanoma cancer patients

Patients	Average EcPKA activity (cpm)	Average PKI inhibited activity (cpm)	% EcPKA activity
N1	656 ± 74	65 ± 22	
N2	1059 ± 96	54 ± 26	
N3	921 ± 60	84 ± 27	
N4	680 ± 75	60 ± 23	
N5	906 ± 47	50 ± 12	
EcPKA-0025	1160 ± 169	132 ± 24	137
EcPKA-0031	1676 ± 175	120 ± 24	199
EcPKA-0028-1	1119 ± 48	113 ± 43	132
EcPKA-0021	1415 ± 147	122 ± 18	168
EcPKA-0032	1895 ± 214	279 ± 96	224
EcPKA-0033	1583 ± 43	188 ± 25	188
EcPKA-0029	865 ± 175	53 ± 17	102
EcPKA0035	1128 ± 141	110 ± 57	134
EcPKA-0042	6267 ± 651	663 ± 194	742
EcPKA-0043	2628 ± 381	325 ± 157	311
EcPKA-0034	9224 ± 800	2386 ± 505	1092
EcPKA-0045	3787 ± 718	1039 ± 242	448
EcPKA-0030-1	2505 ± 383	394 ± 176	297
EcPKA-0038	1288 ± 77	120 ± 45	153
EcPKA-008	1379 ± 144	137 ± 55	163
EcPKA-0010	1802 ± 45	169 ± 39	213
EcPKA-0011	1193 ± 187	98 ± 18	141
EcPKA-0012	734 ± 232	53 ± 22	87
EcPKA-0013	1735 ± 138	131 ± 49	206
EcPKA-0015	2097 ± 229	232 ± 97	248
EcPKA-0016-1	5231 ± 409	1979 ± 347	620
EcPKA-0017	1204 ± 115	88 ± 14	143
EcPKA-0018	3334 ± 578	271 ± 117	395
EcPKA-0019	2818 ± 302	549 ± 284	334

ECPKA activity derived from phosphorylation activity that is specific for Kemptide (Average EcPKA activity after subtracting total kinase activity from PKI inhibited activity), and inhibited by PKI (Average PKI inhibited activity). The percent serum ECPKA activity is inhibited by PKI and compared to ECPKA activity from normal healthy volunteers. N, serum samples from normal healthy volunteers; EcPKA, serum samples from melanoma patients.

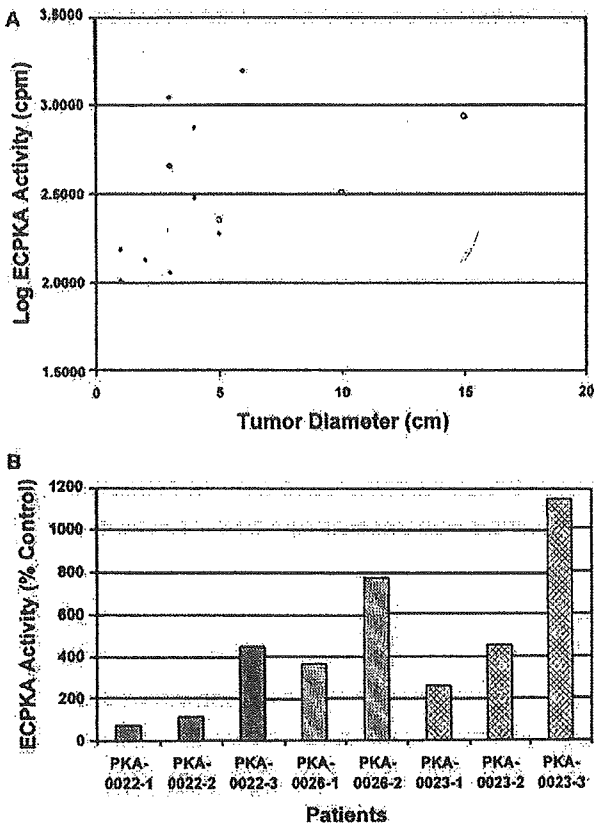

Fig. 1. Increased serum ECPKA activity with increasing tumor size. A, scatter plot of melanoma size relative to serum ECPKA activity. Open circle (O), melanoma with necrosis, closed diamonds (◆), melanoma without tumor necrosis. B, correlation of increasing tumor size and serum ECPKA activity relative to tumor progression in serially sampled serum from melanoma patients at different times during the course of the disease.

healthy control, and the serum ECPKA activity significantly lowered to 146% a week following surgical resection of the melanoma. However, 2 months after surgery, the individual relapsed and showed two-fold increase in serum ECPKA activity (Fig. 2). These results suggest that ECPKA activity may be useful for monitoring the progression of melanoma.

4. Discussion

We have shown in the present study that the C subunit of PKA can be secreted extracellularly in melanoma (Table 1). The amount of secreted C subunit kinase or ECPKA activity seems to correlate in part with increasing tumor size (Fig. 1), thus suggesting that secretion of the C subunit kinase increases as melanoma progresses with time into more advanced stages. Most importantly, serum ECPKA activity decreased significantly to levels almost close to those of normal control after surgical resection of the melanoma (Fig. 2). Therefore, our results further suggest that the secreted C subunit kinase activity may be a tumor biomarker for monitoring the progression of melanoma and predicting treatment response and relapse of the tumor.

Our study here with melanoma further underscores the clinical relevance and significance of the changes in serum ECPKA activity as an important tumor biomarker in cancer. A number of proteins, hormones, and antigens have been studied as potential tumor markers [11–13]. However, due to the lack of sensitivity and specificity, no single marker has been established as a practical cancer-screening tool either in a general healthy population or in high-risk populations.

Our finding that the levels of serum ECPKA activity correlating with tumor size in general suggests that the amount of secreted C subunit kinase is proportional to the size or mass of the tumor. Therefore, when the tumor is surgically resected, it is anticipated that ECPKA activity would be reduced precipitously. Indeed in three independent cases examined, we found significant decrease in ECPKA

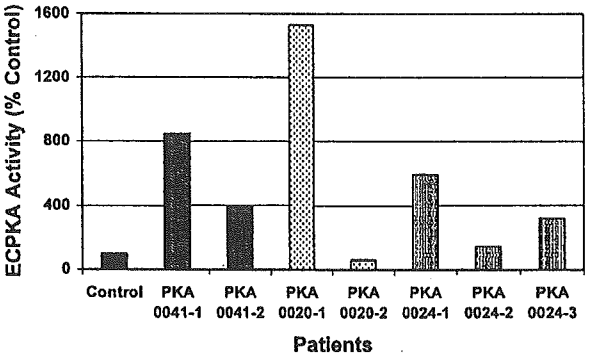

Fig. 2. Serum ECPKA activity before and after surgical resection in melanoma. Serum ECPKA activity was monitored before and approximately 1 week after surgical removal of melanoma from patients. In patient, PKA-0024, an additional serum sample was obtained during relapse approximately 2 months after the surgery.

activity in post-operative serum samples. When the tumor relapsed eventually in one of the patients, serum ECPKA levels increased accordingly. These observations support that serum ECPKA activity is a direct result from the secretion of the C subunit kinase by tumor cells. It remains to be determined whether serum ECPKA activity can be modulated or altered in response to chemotherapeutic treatment of melanoma, which can be used for predicting treatment response [14].

Previous studies show significant serum ECPKA activity in prostate [8] and a number of other cancers [6]. The characteristics of the serum ECPKA activity are consistent with a free C subunit kinase that is independent of the holoenzyme complex, because: (i) it phosphorylates the PKA-specific synthetic peptide substrate Kemptide; (ii) its activity is inhibited by PKI, a PKA-specific peptide inhibitor, but not by the PKC-specific peptide inhibitor; and (iii) it is not activated by cAMP, thus suggesting that it is not in a R_2C_2 holoenzyme configuration. In addition, by monitoring the presence of the intracellular enzyme marker, lactate dehydrogenase (LDH), in the sera or conditioned culture media, we also rule out the possibility that the ECPKA activity may have been a result of cell lysis, as evident from the low LDH activity in the sera and conditioned media that were comparable to controls [6,8].

Taken together, our results here with melanoma and those of previous findings [6–8], show that the C subunit of PKA may be secreted by cancer into the extracellular milieu. The prevalence of serum ECPKA activity in various cancers suggests that it may have roles in the tumorigenic progression of cancer, and hence may be a clinically important molecular biomarker as well as a target of therapeutics in cancer. Even though the functional significance of serum ECPKA or the secretion of the C subunit kinase is unclear, further studies clearly are warranted to explore the biochemical mechanisms of its secretion and the physiological consequence of the secretion of the C subunit kinase in cancer.

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CANCER GENETICS AND CYTOGENETICS

Short communication

Prediction of chemotherapeutic response in ovarian cancer with DNA microarray expression profiling

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Abstract Ovarian carcinoma is a leading cause of gynecologic cancer death in women. Despite treatment, a large number of women with ovarian cancer eventually relapse and die of the disease. Hence, recurrent ovarian cancer continues to be a therapeutic dilemma, possibly a result of the emergence of drug resistance during relapse. Recent advances in expression genomics enable global transcript analysis that leads to molecular classification of cancers and prediction of outcome and treatment response. We did a cDNA microarray examination of the expression profiles of eight primary ovarian cancers stratified into two groups based on their chemotherapeutic response. We applied a voice–speech–pattern recognition algorithm for microarray data analysis and were able to model and predict the response of these patients to chemotherapy from their expression profiles. Hence, gene expression profiling by means of DNA microarray may be applied diagnostically for predicting treatment response in ovarian cancer.

1. Introduction

Early-stage ovarian cancer tends to be asymptomatic and most ovarian cancers are diagnosed at advanced stages [1]. Despite treatment, a vast majority of ovarian cancer patients eventually relapse and die of their disease [2]. Drug resistance is a major cause of treatment failure, resulting in death for more than 90% of patients with metastatic disease. The poor prognosis has prompted major efforts to identify prognostic factors, improve surgical staging, and develop adjuvant therapies that could improve patient outcome [3].

Tumorigenic progression of ovarian cancer is accompanied by multiple genetic changes at the molecular level [4,5]. Traditional clinicopathologic features based on morphology cannot reliably classify the clinical characteristics and behavior of the cancer, nor can they predict response to various treatment modalities. Treatment is given to ovarian cancer patients even though a large number of them ultimately fail to respond, because oncologists have no way of predicting who might or might not respond to therapy.

Completion of the human genome sequence promises practical advances in genomics medicine, whereby increasingly physicians will be able to prescribe the right drug at the right dose for the right person [6]. Recent advances in expression genomics through global transcript analysis have led to molecular classification of cancers [7–13] and prediction of outcome and treatment response [14,15]. Molecular classification of ovarian cancer by means of transcription profiling has shown normal ovarian tissue clearly distinguishable from malignant tissues, and three types of tumors were further identified [16,17], thus suggesting that DNA microarray can be applied for disease diagnosis.

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We examined the expression profiles of eight primary epithelial ovarian cancers with DNA microarray and correctly predicted the response of these patients to chemotherapy. Our results here provide the basis for further study to identify the genetic changes that may be the mechanisms of clinical multidrug resistance in ovarian cancer.

2. Materials and methods

2.1. Patients and tissue specimens

Primary cancer tissues were obtained for expression profiling analysis from eight patients with invasive epithelial ovarian carcinoma at the Department of Obstetrics and Gynecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong. All patients were staged according to the guidelines of FIGO, the International Federation of Obstetrics and Gynecology [18], and had undergone treatment between 1996 and 2000.

Tumor tissue was dissected and embedded in Optimal Cutting Temperature (OCT) compound (Ted Pella Inc, Redding, CA) for frozen sectioning and staining with hematoxylin and eosin. In all the tumor tissues, the proportion of malignant cells was greater than 70%. All tumors were histologically classified and graded, based on the degree of histologic differentiation according to the criteria defined by the World Health Organization. The eight epithelial ovarian carcinomas examined included two serous, four endometrioid, one clear cell adenocarcinoma, and one undifferentiated epithelial tumor. The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. The study involved no potential risk to the patients, in that only archival samples without identifier labels were used.

2.2. RNA extraction and microarray analysis

RNAs were isolated using the RNeasy Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Expression profiling with cDNA microarray was done as previously described [19], using arrays custom printed on 7.62 × 25.4 cm (3 × 10 inch) nylon membrane. These arrays contained ~10,692 DNA elements, comprising expressed sequence tags that either correspond to human transcripts with known function in the GenBank database (~7000), or are anonymous (>3000).

2.3. Data analysis

Supervised clustering methods, involving training and testing of ovarian cancer gene expression profiles and clinical treatment response were applied to predict and correlate gene expression patterns with treatment response, based on an algorithm developed for voice–speech recognition as previously described [19]. In brief, we applied a statistical approach to pattern recognition using a combination of linear discriminant analysis for training set, feature extraction by means of Bayesian parameter estimation, decision by means of nearest-neighbor classification, and classifier performance evaluation. For training and feature selection, we selected three patients from each of two categories; no evidence of disease (sensitive to chemotherapy) and died of disease (resistant). Testing was then conducted with the remaining samples from each category.

To further test the validity of our data analysis, our result was compared with randomized data sets by assigning each of the eight samples arbitrarily to different clinical groups (sensitive or resistant) as previously described [19].

3. Results and discussion

We evaluated the expression profiles of eight patients with epithelial ovarian cancer to predict their response to chemotherapy. The clinical and histopathologic characteristics of the patients were summarized in Table 1. Each patient underwent exploratory laparotomy with bilateral salpingo-oophorectomy, hysterectomy, and infracolic omentectomy and maximal tumor debulking as part of the treatment for ovarian cancer, and no patient received any drug treatment

Table 1
Clinicopathologic characteristics of patients with ovarian carcinoma

					Survival time (months)	
Patient code	Age (years)	Histologic type	Clinical stage	Clinical status	Disease-free	Total
O147A	56	Serous	3b	DOD	11	24
O204A	69	Undifferentiated	3c	DOD	22	39
O182A	68	Endometrial	4	AWD	28	55
O171A	29	Endometrial	3c	DOD	11	39
O215A	42	Clear cell	3c	NED	32	32
O241A	42	Endometrial	3c	NED	18	18
O218A	50	Endometrial	3b	NED	31	31
O140A	64	Serous	3b	NED	67	67

Abbreviations: AWD, alive with disease (resistant to chemotherapy); DOD, died of disease (resistant to chemotherapy); NED, no evidence of disease (sensitive to chemotherapy).

before surgery. All of the patients were treated postoperatively with platinum-based regimen chemotherapy: five patients were treated with cisplatin–cyclophosphamide, two with carboplatin–paclitaxel, and one with cisplatin–paclitaxel.

The response to chemotherapy was defined as (*a*) sensitive, if no relapse or progression was noted within 6 months after the last cycle of chemotherapy, or (*b*) resistant, if the patient relapsed or progressed within 6 months after the last cycle of chemotherapy. Follow-up ranged from 18 to 67 months. At evaluation of the microarray data, three deaths had occurred, and one patient was alive with disease; hence, this group was considered chemotherapy resistant. The remaining four patients showed no evidence of disease (sensitive to chemotherapy). Supervised clustering analysis of the expression profiles using a pattern-recognition algorithm as previously described [19] correctly predicted the response of the two groups of patients as either chemotherapy sensitive or chemotherapy resistant (Fig. 1).

To further understand the prediction model, the top 100 genes in the signature cluster were extracted for bioinformatics searches. These genes have a wide spectrum of cellular functions (Table 2). Notably, a cluster of nucleic acid binding proteins, as well as proteinases, showed elevated expression in tumors of patients classified as resistant to chemotherapy.

It is well known that tumor heterogeneity probably results from multiple genetic changes accumulated during neoplastic transformation [20,21]. Hence, tumor characteristics can vary greatly from one patient to another. Note also that there is significant heterogeneity of tumor cells within a tumor. Consequently, it is not possible to characterize individual tumor by means of a single, or even several molecular markers. The properties of cancer are likely the sum of the functions of all the expressed genes in the cancer cells, thus yielding a tumor that may be intrinsically resistant to treatment or poised to undergo further changes to acquire drug resistance [22,23]. There are currently no clinical markers or technologies that enable oncologists to accurately predict, a priori, whether patients would respond to treatment.

To address this complex tumor phenotype that confers drug resistance, we showed here the application of expression genomics by means of DNA microarray for monitoring or predicting ovarian cancer patients' response to chemotherapy based on the gene expression profiles from the primary cancer tissues. We correctly predicted the response of the ovarian cancer patients as either sensitive or resistant to chemotherapy (Fig. 1), using a supervised pattern-matching algorithm that has previously been applied for predicting radiotherapy response in cervical cancer [19]. Even though sample size in both studies was small, our results nevertheless suggest that it may be possible in future to use gene expression profiles derived from primary cancer tissues to predict treatment response. Such an approach will spare a large number of patients who might not respond to therapy from unnecessary exposure to the toxic side effects of chemotherapy, and alternative treatment might be sought to personalize drug regimen and so yield better efficacy and response for these patients.

We identified some predictor genes that may be the signature expression pattern of drug-resistant cancers (Table 2), from the patients who did not favorably respond to treatment. Note that a significant number of these genes are functionally involved in the regulation of gene expression, including transcription factors that bind DNA. The spectrum of gene changes in the tumor tissue from patients resistant to chemotherapy is reminiscent of the expression patterns found in ovarian cancer cell lines exposed to cisplatin (data not shown), thus suggesting that these nucleic acid binding proteins may have an important role in conferring either intrinsic or acquired drug resistance in ovarian cancer. In addition, increased glutathione *S*-transferase expression, which is known to confer resistance to cisplatin, was also observed in the tumors of drug-resistant patients (Table 2) [24].

It has been shown in in vitro cell culture studies that overexpression of phosphoinositide-3-kinase (PI3K) may be associated with increased cisplatin resistance in ovarian cancer [25]. We, however, found a significant decrease in PI3K levels in tumors of patients resistant to cisplatin-based regimen of chemotherapy. Whether decreased expression of PI3K is associated with drug-resistant tumor remains to be determined in future studies with a larger cohort of ovarian

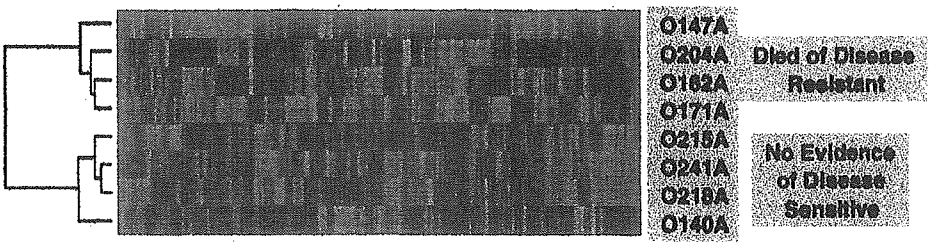

Fig. 1. Dendrogram of signature expression pattern that predicts treatment response. A pattern-matching algorithm was applied for array data analysis. The top 300 genes that correctly predicted treatment response were used for clustering and were displayed using the Cluster version 2.20 and TreeView version 1.60 software suite. Codes on the right identify patients (see Table 1) who either responded to or failed treatment.

Table 2
Predictor genes differentially expressed between ovarian cancer patients who responded (sensitive) or failed to respond (resistant) to platinum-based chemotherapy

Accession no.	Gene name	Ratio, resistant/ sensitive
AA281784	Phosphoinositide-3-kinase, catalytic, delta polypeptide	0.11
AI151105	Syntaxin binding protein 2	2.4
AA670200	Procollagen C-endopeptidase enhancer	22.5
AA864479	Proteasome subunit beta type 5	9.5
T56021	Carboxypeptidase D	10.2
AA410517	Serine proteinase inhibitor	2.3
AI262370	WWW domain binding protein 2	7.5
AA779480	Bone morphogenetic protein 8	8.2
AI636025	Zinc finger protein	3.3
AI367095	RET finger protein	5.7
AA485226	Vitamin D receptor	3.7
AA465353	Histone deacetylase 1	14.0
AA262080	Zinc finger protein 91	35.0
AA425419	Short stature homeobox	7.6
T90374	KIAA0798, hypothetical zinc finger protein	0.4
AA142971	Glutathione *S*-transferase M2	3.3

cancer patients. Nevertheless, which of the differentially expressed genes in our current study represent the etiologic causes of drug resistance or treatment failure must be further determined to confirm their association with chemotherapy resistance.

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＜特集関連情報＞

わが国の母子感染によるHIV/AIDSの現状

はじめに

わが国の母子感染によるHIV感染者・AIDS患者の最新の累積数は，厚生労働省エイズ動向委員会報告・感染経路別の項目（http://api-net.jfap.or.jp/mhw/survey/mhw_survey.htm）で閲覧できる。一方，私どもは全国の小児科診療施設に対する継続的な疫学アンケート調査*を行うことによって，HIV感染妊婦から出生した小児全体に関する情報を収集してきた。その結果，2003（平成15）年末現在，わが国のHIV陽性女性から出生した児の累積数は221例であり，内訳は感染35例（推定捕捉率は76%），非感染158例，未確定不明28例であることがわかった。

HIV感染妊婦より出生した児の実態調査の概要

（1）年次別出生数と児の感染状況（表1）：1984年に初めての出生があり，1992年までの出生数は年間1ないし5であった。1993～1997年は10から19，1998年以降は20と増加傾向にあるが，後述する予防対策**によって，年次ごとの感染児数は1995年の7をピークに減少傾向にあり，2001年以降は0である。

表1. 年次別出生数と児の感染状況

年	出生数	感染	非感染	未確定・不明
1984	1	1	0	0
1987	1	1	0	0
1988	1	0	1	0
1989	4	0	3	1
1990	1	0	1	0
1991	4	3	0	1
1992	5	2	2	1
1993	11	5	6	0
1994	12	2	10	0
1995	19	7	11	1
1996	15	3	11	1
1997	19	5	13	1
1998	21	2	16	3
1999	23	1	21	1
2000	21	3	13	5
2001	24	0	23	1
2002	25	0	18	7
2003	14	0	9	5
合計	221	35	158	28

（2）地域別出生数：ブロック別で関東甲信越・東海・近畿の順に集中し，次いで九州・外国に分布している。北海道4例，東北8例，北陸3例と中四国1例であり，報告0のブロックは存在しないが，中四国・九州ブロックには報告0県が多い。

（3）年次別母子感染予防対策と効果（表2-1，次ページ表2-2）：分娩方法と母児への抗ウイルス薬投与の別によって感染率を比較した。予定帝王切開は158例中4例＜母児とも投薬あり106例中1例（0.9%），母児とも投薬なし23例中1例（4.3%），母のみ投薬12例中1例（8.3%），児のみ投薬8例中0（0%），不明9例中1例（11%）＞であった。緊急帝王切開は15例中4例（27%）＜母児とも投薬なし4＞，経膣42例中22例（52%）＜母児ともなし21，不明1＞，様式不明6例中5例（83%）＜母児ともなし4，不明1＞であった。

（4）非感染児が周生期に受けた抗ウイルス治療（ART）の短長期的影響について：母子感染予防に用いられた核酸系逆転写酵素阻害剤によるミトコンドリア機能障害が欧州から報告されていることから，非感染と診断された児の成長過程を調査した。母体妊娠中ARTについて23例で記載があり，AZT群6例に有害事象はなかったが，HAART群17例には突然死が2例あった；生後2カ月と3カ月であり，どちらも急変して病院搬入された時は既に心肺停止であり，かつ剖検が得られず詳細な死因が不明であった。

（5）感染児35例のまとめ（次ページ表3）：感染児が医療機関を初診した時の年齢別症状発現率は，0歳16例中9例（56%），1歳4例中2例（50%），2歳7例中5例（71%），4歳1例中1例（100%），5歳3例中2例（67%），6歳1例中0例（0%），7歳2例中1例（50%），11歳1例中1例（100%）であった。症状には年齢特異性がみられ，3歳未満では呼吸器障害が8例と多く，他に歩行障害2例，体重増加不良2例，

表2-1. 年次別母子感染予防対策

出生年	出生数	予定帝王切開分娩						緊急帝王切開分娩				
		母児とも投薬なし	予防投薬 母のみ	予防投薬 児のみ	予防投薬 母と児	投薬不明	合計	母児とも投薬なし	予防投薬 児のみ	予防投薬 母と児	投薬不明	合計
1984	1 (1)	0	0	0	0	0	0	0	0	0	0	0
1987	1 (1)	0	0	0	0	0	0	0	0	0	0	0
1988	1	1	0	0	0	0	1	0	0	0	0	0
1989	4	1	0	0	0	0	1	0	0	0	0	0
1990	1	1	0	0	0	0	1	0	0	0	0	0
1991	4 (3)	1 (1)	0	0	0	0	1 (1)	0	0	0	0	0
1992	5 (2)	2	0	0	0	0	2	0	0	0	0	0
1993	11 (5)	3	0	0	0	0	3	1	0	0	0	1
1994	12 (2)	3	2	0	0	0	5	2	0	0	0	2
1995	19 (7)	3	2 (1)	0	0	4	9 (1)	1	1	0	0	2
1996	15 (3)	3	3	1	1	1	9	2 (1)	0	0	0	2 (1)
1997	19 (5)	3	3	2	6 (1)	1 (1)	15 (2)	1 (1)	0	0	0	1 (1)
1998	21 (2)	2	2	1	13	0	18	1 (1)	0	0	0	1 (1)
1999	23 (1)	0	0	1	19	1	21	1	0	0	0	1
2000	21 (3)	0	0	1	14	1	16	1 (1)	0	0	0	1 (1)
2001	24	0	0	1	20	1	22	1	0	0	0	1
2002	25	0	0	1	20	0	21	0	0	2	0	2
2003	14	0	0	0	13	0	13	0	0	1	0	1
総数	221 (35)	23 (1)	12 (1)	8	106 (1)	9 (1)	158 (4)	11 (4)	1	3	0	15 (4)

（ ）内は児のHIV陽性数再掲

表2-2. 年次別母子感染予防対策

出生年	出生数	経腟分娩					分娩様式不明			
		母児とも投薬なし	予防投薬 児のみ	予防投薬 母と児	投薬不明	合計	母児とも投薬なし	予防投薬 母と児	投薬不明	合計
1984	1 (1)	0	0	0	0	0	1 (1)	0	0	1 (1)
1987	1 (1)	1 (1)	0	0	0	1 (1)	0	0	0	0
1988	1	0	0	0	0	0	0	0	0	0
1989	4	3	0	0	0	3	0	0	0	0
1990	1	0	0	0	0	0	0	0	0	0
1991	4 (3)	3 (2)	0	0	0	3 (2)	0	0	0	0
1992	5 (2)	3 (2)	0	0	0	3 (2)	0	0	0	0
1993	11 (5)	6 (4)	0	0	0	6 (4)	1 (1)	0	0	1 (1)
1994	12 (2)	4 (1)	0	0	1 (1)	5 (2)	0	0	0	0
1995	19 (7)	5 (4)	0	0	1	6 (4)	1 (1)	0	1 (1)	2 (2)
1996	15 (3)	3 (2)	0	0	1	4 (2)	0	0	0	0
1997	19 (5)	3 (2)	0	0	0	3 (2)	0	0	0	0
1998	21 (2)	1 (1)	0	1	0	2 (1)	0	0	0	0
1999	23 (1)	1 (1)	0	0	0	1 (1)	0	0	0	0
2000	21 (3)	2 (1)	0	0	0	2 (1)	1 (1)	1	0	2 (1)
2001	24	0	0	1	0	1	0	0	0	0
2002	25	0	1	1	0	2	0	0	0	0
2003	14	0	0	0	0	0	0	0	0	0
総数	221 (35)	35 (21)	1	3	3 (1)	42 (22)	4 (4)	1	1 (1)	6 (5)

( )内は児のHIV 陽性数再掲

肝脾腫・カンジダ症・肝機能障害・被虐待が各1例であった。4歳以上では耳下腺腫脹2例，カンジダ症・帯状疱疹・呼吸障害が各1例であった。

3歳未満の呼吸器障害8例の予後は不良で，7例までがAIDSまたは死亡となった。歩行障害の2例も1例はAIDSになり，1例はHAARTを受けたが死亡した。一方，5歳以上の7例中5例はHAARTが奏効し，免疫能が維持されている。

HAARTが実施された10例の組合せは（AZT or d4T)＋3TC＋NFVが5例，(AZT or d4T)＋3TC＋LPV/rが4例，d4T＋ABC＋LPV/rが1例であった。

HAARTの応用によって小児HIV/AIDSが慢性疾患として管理されるようになったことから，今後は服薬の長期毒性，耐性ウイルス出現，性教育，告知などさらなる問題の出現が予想される。

*平成15年度厚生労働省エイズ対策研究事業「HIV感染妊婦の早期診断と治療および母子感染予防に関する基礎的・臨床的研究」班（主任研究者：稲葉憲之）

表3. 感染児35例のまとめ

No	児出生年	分娩方法*	母乳	年齢(年) 初診	年齢(年) 終診	初診時の状態 症状	初診時の状態 臨床病期**	転帰 症状	転帰 CD4 (/μl)	転帰 VL (copies/ml)	転帰 ART
1	1984		不明	4.4	-	カンジダ症	B	死亡			
2	1987	v	有	2.0	16.2	検査目的	N?	AIDS			
3	1991	v	無	11.0	12.0	呼吸障害	B	無症状	15.4%	87	AZT・3TC・LPV/r
4		s-c/s	有	1.3	11.6	検査目的	N?	無症状	579	6700	d4T・ABC・LPV/r
5		v	有	0.2	0.6	呼吸器症状・体重増加不良	A	AIDS	505		
6	1992	v	無	2.2	6.6	歩行障害・カンジダ症	C	死亡	4	100000台	d4T・3TC・NFV
7		v	有	0.1	1.7	検査目的	N	死亡			
8	1993	v	無	0.2	0.3	呼吸困難	A	不明			
9		v	無	5.0	13.3	帯状疱疹	N?	無症状	211	690	d4T・3TC・LPV/r
10		不明	不明	1.2	2.5	呼吸器症状	C	死亡	12.5%		
11		v	有	0.3	1.8	体重増加不良	A	死亡			
12		v	無	7.0	14.3	検査目的	N	無症状			
13	1994	v	有	2.0	4.2	呼吸障害	C	AIDS			
14		v	有	2.2	9.1	検査目的	N	無症状	8.4%	33000	
15	1995	v	有	0.1	1.0	カンジダ症	A	ARS・帰国	1218 (14.4%)		AZT
16		不明	不明	6.8	8.2	-	N	N	1166	<400	d4T,3TC,NFV
17		v	有	7.5	8.5	耳下腺腫脹、全身リンパ節腫大	B	B	724	730000	AZT,3TC,LPV/r
18		不明	無	0.0	1.0	検査目的	N	無症状	18.0%		
19		v	有	2.0	4.6	被虐待児	N?	帰国			
20		v	無	0.0	5.3	検査目的	N	無症状	843	2500	d4T・3TC・NFV
21		s-c/s	無	0.1	0.6	検査目的	N	死亡			
22	1996	u-c/s	無	0.0	-	検査目的	N	不明			
23		v	有	0.7	5.4	検査目的	N	無症状			
24		v	有	0.3	0.8	呼吸障害	C?	死亡	140	750000	AZT
25	1997	v	有	1.0	2.7	呼吸障害	B?	死亡			
26		s-c/s	無	5.6	6.4	-	N	N	120	270000	開始未
27		u-c/s	有	0.5	4.0	肝機能障害	B	AIDS			
28		s-c/s	無	2.0	5.5	歩行障害	C	AIDS			
29		v	有	5.2	6.2	耳下腺腫脹,反復性肺炎、全身リンパ節腫大	B	B	209	730000	AZT,3TC,LPV/r
30	1998	u-c/s	有	0.9	4.5	呼吸障害	C	AIDS	1428	1300	AZT・3TC・NFV
31		v	有	2.0	5.2	検査目的	N	無症状	970	43000	AZT・ddI
32	1999	v	有	0.1	2.3	検査目的	N	リンパ・肝脾腫大	14.8%	110000	
33	2000	v	有	0.3	-	呼吸障害	B?	AIDS			
34		不明	有	1.8	2.0	-	N	N	19.8%	>110000	開始未
35		u-c/s	有	0.6	1.2	肝脾腫精査	C	死亡	840	1100000	AZT・3TC・NFV

* 分娩方法: v: 経腟, s-c/s: 予定帝王切開, u-c/s: 緊急帝王切開

**臨床病期: 小児HIV感染症(13歳未満)の臨床病期分類(1994, CDC)

の分担研究「HIV 感染妊婦より出生した児の実態調査とその解析」班（分担研究者：外川正生）による。

**HIV母子感染予防対策マニュアルは財団法人エイズ予防財団のホームページ：エイズ予防情報ネット（http://api-net.jfap.or.jp/）内の「資料室」からダウンロードできる。

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妊娠—診察—妊娠後半期

B型肝炎陽性妊婦の胎児・新生児管理はどうするか?

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Key words：B型肝炎，HBV，HBV母子感染，胎児・新生児管理

背景

成人がB型肝炎(以下：HBV)に初感染すると急性肝炎もしくは劇症肝炎を発症するが，通常慢性化することはない。母子感染と小児期(3歳以下)の水平感染では，90%以上の確率で無症候性HBVキャリアになる。そしてキャリアの10%前後は慢性肝炎，肝硬変，肝癌へと進行する。つまり母子感染と小児期水平感染を予防すれば，HBV感染による肝硬変，肝癌を防ぐことが可能となる。実際には小児期に水平感染することは少なく，母子感染を予防できればHBV感染を予防できると考える。我が国では，HBV母子感染防止のため，1986年6月から厚生省(旧)が「B型肝炎母子感染防止事業」を開始させ，その後1995年4月に改定し，現在もHBV母子感染防止事業要綱にのっとってHBV母子感染の防止をはかっている(図)。

エビデンス

①HBV母子感染の自然経過は，キャリア妊婦がHBe抗原陽性の場合，児は生後4カ月以内に73%がキャリア化し，HBe抗原陰性の場合，一過性のHBs抗原陽転を除いてキャリア化する児は少ない。HBVキャリア妊婦からの平均キャリア化率は26.8%である。キャリア化児の約60%は肝機能異常(sALT>110 IU/ml)を繰り返したが劇症肝炎には至らなかった(稲葉，2003)[1]。

②HBs抗原陽性，HBe抗原陰性の妊婦からの出生児ではHBVキャリアになることはまれだが，6〜9%の児が生後2〜3カ月に急性肝炎，ときに劇症肝炎を発症する(白木，1995)[2]。この報告により1995年の改定でHBs抗原陽性妊婦の児全員に感染防止措置を施行することになった。

③宮城県で1989年から1996年の11年間，妊婦のHBs抗原検査促進と検査期間の精度管理，キャリア妊婦とワクチン被接種児の登録・追跡調査を行った。妊婦のHBs抗原スクリーニング受診率はおおむね95%以上で推移し，1995年からは99.9%である。妊婦HBs抗原陽性率は0.72〜1.41%であった。被接種児の抗体産生状況は1986年から1990年までは82.5%から98.2%とばらつきがあったが，リコンビナントワクチン(以下HBRVと略す)を使用した1991年からは90〜100%となり，1995年からは100%であった(白地，1998)[3]。

④HBIGの投与間隔を3カ月と4カ月の群に分け，児のキャリア化率を比較したところ3カ月群は2.4%，4カ月群は26.7%となり，HBIGは1回投与で少なくとも3カ月有効と判明した。新生児(n=25)に生後24時間以内，1カ月，3カ月の3回HBRVを接種し，血中HBs抗体を検査した。抗体獲得率は1回接種にて32%，2回接種にて最大96%，3回目のブースター接種では抗体力価は上昇したが96%にとどまった。この結果よりHBIG1回，HBRV3回(24時間以内，1カ月，3カ月)法を考案し，厚生省方式と比較した。HBIGが一回少なくてすむにもかかわらず，同じ効果が得られるとの結果であった(稲葉，2002)[4]。多くの諸外国では24時間以内のHBRV投与法を取り入れており，日本でも上記のような提案がなされている。さらなる比較検討が必要と思われる。

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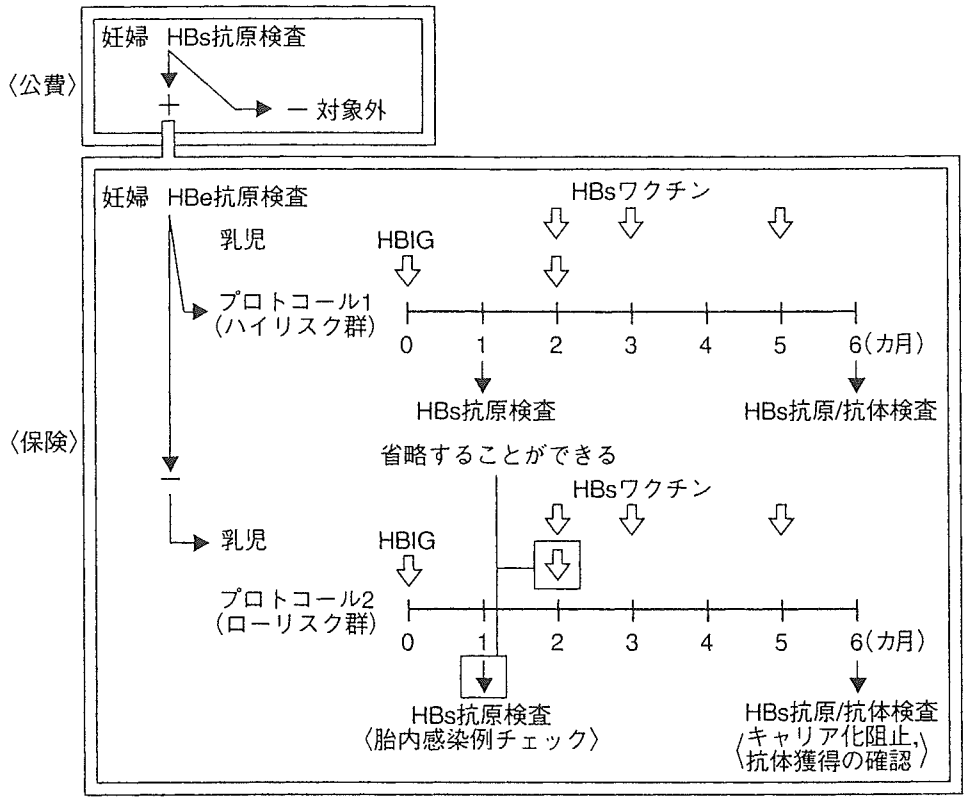

図 **HBV 母子感染防止事業の流れ**(B 型肝炎母子感染防止対策の手引き：厚生省心身障害研究，ウイルス性肝疾患の母子感染防止に関する研究，医療機関向けパンフレット，1995 より引用)

現時点における結論

HBV 母子感染防止事業の結果，母子感染による HBV キャリア率は事業開始前の 0.26%から(Shiraki, 1994)[5]，9 年後には 0.024%と約 10 分の 1 に低下したと推定されている(白木，1997)[6]。現行の事業では，妊婦は公費で HBs 抗原スクリーニングを受ける。HBs 抗原陰性であれば事業対象外となる。HBs 抗原陽性の場合，健康保険でさらに HBe 抗原・抗体・肝機能などを検査し経過観察する。HBV キャリアと妊娠合併症の関連にはさまざまな報告はあるが結論はでていない。HBs 抗原陽性妊婦から出生した児は，抗 HBs ヒト免疫グロブリン(以下 HBIG)を 2 回(出生直後(48 時間以内)と生後 2 カ月)，B 型肝炎ワクチンを 3 回(生後 2，3，5 カ月)施行する。臍帯血の HBs 抗原検査は施行しなくてよい。生後 1 カ月に HBs 抗原検査を施行し，陽性の場合母子感染が成立したと判断し以後の処置は行わない。児の感染病態は，妊婦が HBe 抗原陽性か陰性かによって異なる。HBe 抗原陽性の場合出生した児はハイリスク群，陰性の場合出生した児はローリスク群として識別しておく。ローリスク群は，生後 1 カ月の HBs 抗原検査と生後 2 カ月の HBIG の投与を省略し得る。生後 6 カ月の時点で両群に HBs 抗原・抗体検査を施行し，キャリア化阻止と抗体獲得を判定し，抗体獲得不良群にはワクチンをさらに追加する。これらの管理は健康保険の対象である。この事業が始まって 18 年が経ち，見直しを求める声もあるが，現時点ではそこまで至っていない。

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妊娠・診察―妊娠後半期

C型肝炎陽性妊婦の胎児・新生児管理はどうするか?

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Key words：C型肝炎，HCV，母子感染，経過観察

背景

C型肝炎(以下：HCV)感染は我が国の肝癌の原因の約75%を占め，小児，成人を問わずキャリア化する点がHBV感染とは異なる。現在のHCVキャリアや慢性C型肝炎のほとんどが母子感染以外の原因で感染していることから，HCV母子感染に関してはHBV母子感染のように国をあげての対策はとられてこなかった。小児HCV感染の原因はかつて，手術や血液疾患などで使用した輸血や血液製剤が主であった。しかし輸血血液のHCVスクリーニングの結果，それらが原因のHCV感染は激減し，今後母子感染によるHCV感染が主因になってくる可能性がある。一般妊婦HCV抗体陽性率は一般婦人抗体陽性率とほぼ同じで0.6～1%程度であり，その70%がHCV RNA陽性である。HCV母子感染率は報告によって差はあるものの数%から11%である。

エビデンス

①非HIV感染，非静脈麻薬常習者(以下IVDUと略す)のHCVキャリア妊婦の母子感染はおおむね数%から11%である(稲葉，2003)[1]。

②HIV/HCV重複感染はHCV母子感染のリスクファクターになる(Catalano D, 1991)[2]～(Europian Paediatric Hepatitis C Virus Network, 2001)[4]。

③母IVDUはHCV母子感染のリスクファクターになる(prospective)(Resti, 2002)[5]。

④キャリア妊婦の分娩時肝炎(sALT>110 IU/m*l*)はHCV感染のリスクファクターになる(稲葉，2003)[1]。

⑤HCV RNA量が2.5×10^6 copies/m*l*以上で児への感染が起こる(prospective)(Okamoto, 2000)[6]。

⑥分娩様式は経腟分娩や陣発後帝王切開術に比べて予定帝王切開術のほうが感染率が有意に低い(Gibb, 2000)[3]。しかしp=0.04であり，さらにHCV感染児の病態が必ずしも重症ではないので，現時点では帝王切開術の適応になるまでには至っていないと考える。

⑦HCVキャリア妊婦の破水から分娩までの時間が長いほうが母子感染率は高くなる(prospective)(Polywka, 1997)[7]。

⑧母乳栄養と人工栄養を比較して母子感染率に差がない(Gibb, 2000)[3]，(Okamoto, 2000)[6]，(Spencer, 1997)[8]，(Tajiri, 2001)[9]。これに反して，HCV RNA量が多い妊婦や母乳中HCV RNA陽性の場合，母乳が感染源になるとする報告もあるが，現段階で母乳栄養を禁止する根拠にはなっていない。

⑨HCV抗体陽性妊婦から出生した児は母親からの移行抗体で抗体陽性になるが，感染しなかった場合，13カ月までに95%で陰性になる(Gibb,

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2000)[3]。

⑩感染すると生後3カ月以内にHCV RNA陽性になるが，3歳まではおおよそ30%の児で陰転する(Spencer, 1997)[8](Sasaki, 1997)[10]。

現時点における結論

HCV母子感染はHBVと比較すると胎児・新生児管理に利用できるエビデンスは乏しい。母体のHCV感染を識別することは，母体と新生児の肝炎管理にとっては有意義であるが，母子感染の予防法はないので，HBVのように確立された胎児・新生児管理はない。感染時期は分娩時であろうと推測されているがはっきりとわかっていない。妊娠中の管理に関しては，妊娠初期～中期にHCV抗体をスクリーニングし，HCV抗体陽性例についてはHCV RNA定量，肝機能を検査し，その後も経過観察する。リスクファクターをもった妊婦の場合には新生児管理を慎重に行う。分娩様式は産科的適応がなければ経腟分娩である。授乳は母乳栄養でかまわない。新生児は定期的に肝機能，HCV抗体，HCV RNA量を検査し，経過を観察していく。経過観察の期間に関して定説はないが3歳までとする意見が多い。HCV感染の児は臨床的には無症状の場合が多く，発育もほぼ正常である。

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HIV の胎児への感染予防はどうするか？

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Key words：HIV，HIV 母子感染，抗ウイルス剤，選択的帝王切開

背景

HIV 母子感染の時期は妊娠中，分娩時，産後授乳時の三つの時期に分けられる。妊娠中の感染は主に妊娠後期に起こるとされ，陣痛により胎盤微小血管の損傷などで母児間輸血が起こり，児にウイルスが移行するなどの機序が考えられているが，その詳細は不明である。分娩時には産道で児がウイルスに汚染された母体血液に暴露されることによって感染が起こり，妊娠中に特に治療をせずに経腟分娩で出産すると約 1/4 の例で母子感染が発生する。出産後には母乳を介してウイルスが新生児に移行する。これらの感染ルートを断つことによって HIV 母子感染は予防される。

胎児感染予防のエビデンス

1．HIV 感染妊婦に対して妊娠中期より抗ウイルス剤を投与して母体血中ウイルス量を低下させる

エビデンス：あり

1994 年に発表された Pediatric Aids Clinical Trial Group (PACTG)の protocol 076 study (prospective study)[1]が有名である。CD4 が 200 cell/mm^{3}以上の HIV 感染妊婦を対象として妊娠中期から予防的に zidovudine (AZT)500 mg/day を連日投与，分娩時には AZT を静注～点滴で投与し，生まれた児に生後 6 週間 AZT シロップを 2 mg/kg で 6 時間毎に投与した場合，placedo 群で母子感染率が 25.5%だったのに対して，投与群では母子感染率は 8.3%に低下した。この報告の時代には，母体の血中ウイルス量は測定されておらず，AZT の母子感染防止の効果がウイルスを低下させることによるのかどうかの結論は出ていなかった。

その後，欧米の prospective cohort study で，1990 年から 2000 年の間の出産例を対象とした成績が報告された[2]。抗ウイルス療法を受けなかった HIV 感染妊婦 396 例における母子感染率は 20.0%だったのに対し，妊婦に AZT 単剤投与，あるいは 2 剤，さらには 3 剤以上の併用療法(HAART)が施行された 711 例，186 例，250 例の母子感染率はそれぞれ 10.4%，3.8%，1.2%と有意に低かった(**図 1**)。また，この study では母体の分娩時のウイルスレベルを調べており，ウイルスコピー数が 30,000 コピー /m*l* 以上では母子感染率は 23.4%，10,000～29,999/m*l* では 14.7%，3,500～9,999/m*l* では 9.3%，400～3,499/m*l* では 5.3%，400/m*l* 未満では 1.0%と分娩時のウイルス量が低いほど母子感染が抑制されていた。さらに，この発表では妊娠中投与期間が長いほうが感染率が低いことも示されている。すなわち，産道での HIV への暴露を防ぐことが胎児への母子感染を予防する上で重要なことが確認されている。

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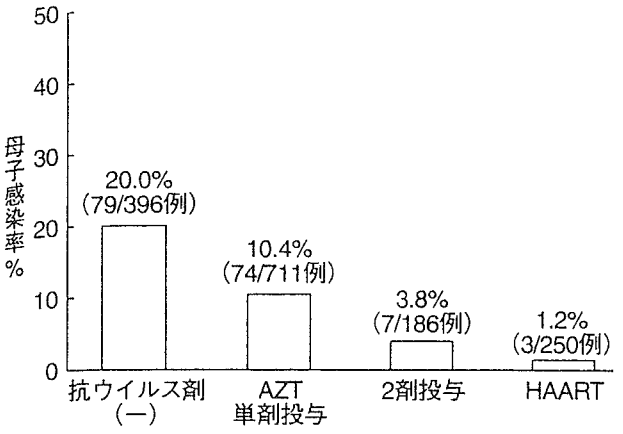

図1 抗ウイルス剤の投与と HIV 母子感染率(Cooperら，2002 より引用改変)[2]

表 我が国における HIV 感染妊婦の分娩様式と母子感染率

分娩様式	非感染	感染	感染率(%)	不明	合計
帝切	133	2	1.5(2/135)	26	161
経腟分娩	15	12	44.4(12/27)*	5	32
		5#	25.0(5/20)**		
合計	148	14	8.6(14/162)	31	193
		7#	4.5(7/155)		

#児の異常による受診を機に母親の感染が確認された 7 例を除く。
*p<0.0001　**p<0.0002　Fisher's exact test
(厚生労働省「HIV 感染妊婦の早期診断と治療および母子感染予防に関する基礎的・臨床的研究」班，2004 より)[4]

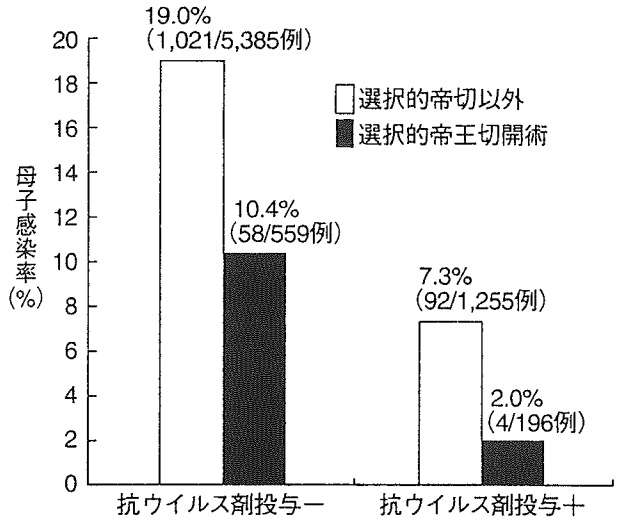

図2 分娩様式と HIV 母子感染率(The International Perinatal HIV Group, 1999 より引用改変)[3]

2. 分娩様式は陣痛発来以前に予定帝切を選択する

エビデンス：あり

分娩様式と母子感染を論じた文献は多い。その中で 1999 年に発表されたヨーロッパと北アメリカの 15 の prospective cohort study[3]の metaanalysis では HIV 感染妊婦 8,533 例という多数の症例について分析が行われている。分析は AZT の投与法や児の出生体重などについても行われているが，結論としては分娩様式を選択的帝切とそれ以外で比較し，妊婦への AZT の投与の有無との組み合わせた結果を示している。結果は図 2 にまとめたが，抗ウイルス剤が投与されない場合，選択的帝切以外の分娩群の母子感染率は 19.0%であるが，選択的帝切群では母子感染率は 10.6%に低下した。抗ウイルス剤が妊娠，分娩時および新生児に投与された場合には，選択的帝切を施行することによって母子感染率は 2.0%にまで抑制されている。また，この分析では，選択的以外の帝切における母子感染率は 16.2%となっており，陣痛発来により感染の起こるリスクが高まることも示されている。

我が国においては，平成 12 年度からの「HIV 母子感染予防の臨床的研究」班および平成 16 年度から引き継いだ「HIV 感染妊婦の早期診断と治療に関する基礎的・臨床的研究」班の retrospective な報告[4]がある。現在(2004 年 3 月)まで，我が国では 303 例の HIV 感染妊婦の存在が確認されている。そのうち 193 例について分娩様式が確認され，母子感染率は帝切群で 1.5%経腟群で 25.0%と感染率に有意の差が確認されている(表)。また，帝切群では 161 例中 49 例で抗ウイルス剤の投与が行われていなかったが，49 例中詳細の確認された 38 例の中で母子感染例は 1 例(2.5%)のみであった。個々の症例の母体血中ウイルス量は不明であるが，母子感染予防に帝切が有効であることは確認されている。

現時点における結論

以上，HIV 母子感染は，妊婦への抗ウイルス剤の投与(妊娠中および分娩時)によって，母体血中ウイルス量を低下させ，選択的帝切を施行することでほとんど予防可能である。しかし，胎内での感染の機序がいまだ明確でないことや，HAART 療法の児への影響が懸念されることなどの問題もある。さらに米国を中心に母体の血中ウイルス量を十分に低下させれば，経腟分娩でも母子感染は予防できるとする意見もあり，今後検討すべき問題が残されている。

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血清検査—HTLV-1抗体，HIV抗体

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HTLV-1抗体

1．目　的

human T-lymphotropic virus type I（HTLV-I）は成人T細胞白血病（adult T cell leukemia：ATL）の病因ウイルスであり，1981年に日本の日沼頼男が分離した。ATLは1977年に日本の高月らによって報告された疾患で，日本では九州，沖縄，四国地方に発生が多い。HTLV-IはHIV同様レトロウイルス科に属し，ヒトT細胞のDNAに自らのDNAを組み込んで生息している。感染は未感染リンパ球が感染リンパ球に直接接触することで成立する。主な感染経路は，①母子感染，②性行為感染，③輸血感染の三つである。感染すると無症候性キャリアとなり，約2,000人に1人の割合で，40〜50年の潜伏期を経てATLを発症する。ATLは5型に分類されるが，その中で急性型とリンパ腫型は通常1年以内に死亡し，現在の治療方法では予後の改善が図られていない。このようにATLは発症すると大変予後が悪いため，感染を防ぐことが大事である。輸血感染については1986年以降，献血血液の抗体検査が実施されている。性行為感染については感染力の弱さと潜伏期が長いことからATLが発症した報告はない。つまりATL患者は母子感染からの発症であり，この経路を遮断することが感染予防につながる。

具体的には，妊婦健診時にHTLV-I抗体スクリーニング検査を実施し，その後の精密検査で陽性であればキャリアと診断し，児への感染を防ぐことである。今回はそのスクリーニング検査と精密検査についてくわしく述べる。

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2．手技・原理

HTLV-Iを構成する蛋白質には，構造蛋白質であるgag蛋白（p19，p24，p15など）と外被糖蛋白質であるenv蛋白（gp46など）があり，これら2種類の蛋白質のことを以下HTLV-I抗原と略す。

1）ゼラチン粒子凝集法（paricle aggulutination：PA法）

不活化処理したHTLV-I抗原をゼラチン粒子に吸着させた感作粒子と希釈した検体との凝集反応によってHTLV-I抗体の有無を判定する。検査方法が簡単で，2時間程度で判定できるので多数検体のスクリーニングに適している。

2）酵素免疫測定法（ELISA法）

マイクロカップ内壁やビーズに吸着させたHTLV-I抗原に検体を加え，さらに酵素標識抗ヒトIgG抗体を加える。抗体陽性の場合，抗原・抗体・酵素標識抗ヒトIgG抗体の免疫複合体を形成し，さらに基質液を加えると発色するので発色度で判定する。スクリーニング検査に使用されている。

3）間接螢光抗体法（IF法）

HTLV-I感染細胞を固定したスライドグラスに検体を加え，さらにFITC標識抗ヒトIgG抗体を反応させる。抗体陽性の場合，抗原・抗体・螢光色素標識二次抗体の免疫複合物を形成し，螢光顕微鏡で螢光染色の有無で判定する。使用するHTLV-I感染細胞はすべてバックグランドが同じ