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SHORT COMMUNICATION

Androgenetic/biparental mosaicism in a girl with Beckwith–Wiedemann syndrome-like and upd(14)pat-like phenotypes

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This report describes androgenetic/biparental mosaicism in a 4-year-old Japanese girl with Beckwith–Wiedemann syndrome (BWS)-like and paternal uniparental disomy 14 (upd(14)pat)-like phenotypes. We performed methylation analysis for 18 differentially methylated regions on various chromosomes, genome-wide microsatellite analysis for a total of 90 loci and expression analysis of *SNRPN* in leukocytes. Consequently, she was found to have an androgenetic 46,XX cell lineage and a normal 46,XX cell lineage, with the frequency of the androgenetic cells being roughly calculated as 91% in leukocytes, 70% in tongue tissues and 79% in tonsil tissues. It is likely that, after a normal fertilization between an ovum and a sperm, the paternally derived pronucleus alone, but not the maternally derived pronucleus, underwent a mitotic division, resulting both in the generation of the androgenetic cell lineage by endoreplication of one blastomere containing a paternally derived pronucleus and in the formation of the normal cell lineage by union of paternally and maternally derived pronuclei. It appears that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of BWS-like and upd(14)pat-like phenotypes, but not for the occurrence of other imprinting disorders or recessive Mendelian disorders.
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INTRODUCTION

A pure androgenetic human with paternal uniparental disomy for all chromosomes is incompatible with life because of genomic imprinting.[1,2] However, a human with an androgenetic cell lineage could be viable in the presence of a normal cell lineage. Indeed, an androgenetic cell lineage has been identified in six liveborn individuals with variable phenotypes.[3–7] All the androgenetic cell lineages have a 46,XX karyotype, and this is consistent with the lethality of an androgenetic 46,YY cell lineage.

Here, we report on a girl with androgenetic/biparental mosaicism, and discuss the underlying factors for the phenotypic development.

CASE REPORT

This patient was conceived naturally to non-consanguineous and healthy parents. At 24 weeks gestation, the mother was referred to us because of threatened premature delivery. Ultrasound studies showed Beckwith–Wiedemann syndrome (BWS)-like features,[8] such as macroglossia, organomegaly and umbilical hernia, together with polyhydramnios and placentomegaly. The mother repeatedly received amnioreduction and tocolysis.

She was delivered by an emergency cesarean section because of preterm rupture of membranes at 34 weeks of gestation. Her birth weight was 3730 g (+4.8 s.d. for gestational age), and her length 45.6 cm (+0.7 s.d.). The placenta weighed 1040 g (+7.3 s.d.).[9] She was admitted to a neonatal intensive care unit due to asphyxia. Physical examination confirmed a BWS-like phenotype. Notably, chest roentgenograms delineated mild bell-shaped thorax characteristic of paternal uniparental disomy 14 (upd(14)pat),[10] although coat hanger appearance of the ribs indicative of upd(14)pat was absent (Supplementary Figure 1). She was placed on mechanical ventilation for 2 months, and received tracheostomy, glossectomy and tonsillectomy in her infancy, due to upper airway obstruction. She also had several clinical features occasionally reported in BWS[8] (Supplementary Table 1). Her karyotype was 46,XX in all the 50 lymphocytes analyzed. On the last examination at 4 years of age, she showed postnatal growth failure and severe developmental retardation.

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MOLECULAR STUDIES

This study was approved by the Institutional Review Board Committee at the National Center for Child health and Development, and performed after obtaining informed consent.

Methylation analysis

We first performed bisulfite sequencing for the *H19*-DMR (differentially methylated region) and KvDMR1 as a screening of BWS[11,12] and that for the IG-DMR and the *MEG3*-DMR as a screening of upd(14)pat,[10] using leukocyte genomic DNA. Paternally derived clones were predominantly identified for the four DMRs examined (Figure 1a). We next performed combined bisulfite restriction analysis for multiple DMRs, as reported previously.[13] All the autosomal DMRs exhibited markedly skewed methylation patterns consistent with predominance of paternally inherited clones, whereas the *XIST*-DMR on the X chromosome showed a normal methylation pattern (Figure 1a).

Genome-wide microsatellite analysis

Microsatellite analysis was performed for 90 loci with high heterozygosities in the Japanese population.[14] Major peaks consistent with paternal uniparental isodisomy and minor peaks of maternal origin were identified for at least one locus on each chromosome, with the minor peaks of maternal origin being more obvious in tongue and tonsil tissues than in leukocytes (Figure 1b and Supplementary Table 2). There were no loci with three or four peaks indicative of chimerism. The frequency of the androgenetic cells was calculated as 91% in leukocytes, 70% in tongue cells and 79% in tonsil cells, although the estimation apparently was a rough one (for details, see Supplementary Methods).

Expression analysis

We examined *SNRPN* expression, because *SNRPN* showed strong expression in leukocytes (for details, see Supplementary Data). *SNRPN* expression was almost doubled in the leukocytes of this patient (Figure 1c).

DISCUSSION

These results suggest that this patient had an androgenetic 46,XX cell lineage and a normal 46,XX cell lineage. In this regard, both the androgenetic and the biparental cell lineages appear to have derived from a single sperm and a single ovum, because a single haploid genome of paternal origin and that of maternal origin were identified in this patient by genome-wide microsatellite analysis. Thus, it is likely that after a normal fertilization between an ovum and a sperm, the paternally derived pronucleus alone, but not the maternally derived pronucleus, underwent a mitotic division, resulting both in the generation of the androgenetic cell lineage by endoreplication of

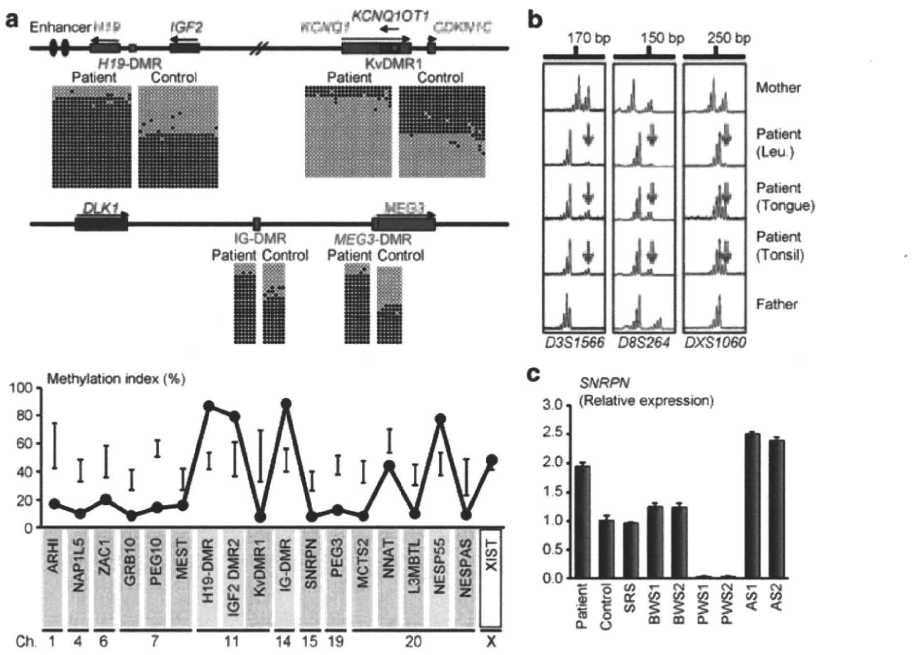

Figure 1 Representative molecular results. (**a**) Methylation analysis. Upper part: Bisulfite sequencing data for the *H19*-DMR and the KvDMR1 on 11p15.5, and those for the IG-DMR and the *MEG3*-DMR on 14q32.2. Each line indicates a single clone, and each circle denotes a CpG dinucleotide; filled and open circles represent methylated and unmethylated cytosines, respectively. Paternally expressed genes are shown in blue, maternally expressed gene in red, and the DMRs in green. The *H19*-DMR, the IG-DMR, and the *MEG3*-DMR are usually methylated after paternal transmission and unmethylated after maternal transmission, whereas the KvDMR1 is usually unmethylated after paternal transmission and methylated after maternal transmission.[10,11] Lower part: Methylation indices (the ratios of methylated clones) obtained from the COBRA analyses for the 18 DMRs. The DMRs highlighted in blue and pink are methylated after paternal and maternal transmissions, respectively. The black vertical bars indicate the reference data (maximum – minimum) in leukocyte genomic DNA of 20 normal control subjects (the *XIST*-DMR data are obtained from 16 control females). (**b**) Representative microsatellite analysis. Major peaks of paternal origin and minor peaks of maternal origin (red arrows) have been identified in this patient. The minor peaks of maternal origin are more obvious in tongue and tonsil tissues than in leukocytes (Leu.). (**c**) Relative expression level (mean ± s.d.) of *SNRPN*. The data are normalized against *TBP*. SRS: an SRS patient with an epimutation (hypomethylation) of the *H19*-DMR; BWS1: a BWS patient with an epimutation (hypermethylation) of the *H19*-DMR; BWS2: a BWS patient with upd(11)pat; PWS1: a Prader-Willi syndrome (PWS) patient with upd(15)mat; PWS2: a PWS patient with an epimutation (hypermethylation) of the *SNRPN*-DMR; AS1: an Angelman syndrome (AS) patient with upd(15)pat; and AS2: an AS patient with an epimutation (hypomethylation) of the *SNRPN*-DMR. The data were obtained using an ABI Prism 7000 Sequence Detection System (Applied Biosystems).

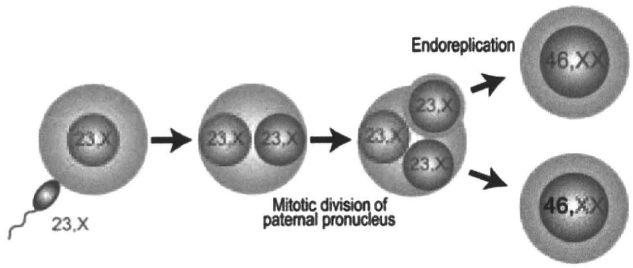

Figure 2 Schematic representation of the generation of the androgenetic/biparental mosaicism. Polar bodies are not shown.

one blastomere containing a paternally derived pronucleus and in the formation of the normal cell lineage by union of paternally and maternally derived pronuclei (Figure 2). This model has been proposed for androgenetic/biparental mosaicism generated after fertilization between a single ovum and a single sperm.[5,15,16] The normal methylation pattern of the *XIST*-DMR is explained by assuming that the two X chromosomes in the androgenetic cell lineage undergo random X-inactivation, as in the normal cell lineage. Furthermore, the results of microsatellite analysis imply that the androgenetic cells were more prevalent in leukocytes than in tongue and tonsil tissues.

A somatic androgenetic cell lineage has been identified in seven liveborn patients including this patient (Supplementary Table 1).[3–7] In this context, leukocytes are preferentially utilized for genetic analyses in human patients, and detailed examinations such as analyses of plural DMRs are necessary to detect an androgenetic cell lineage. Thus, the hitherto identified patients would be limited to those who had androgenetic cells as a predominant cell lineage in leukocytes probably because of a stochastic event and received detailed molecular studies. If so, an androgenetic cell lineage may not be so rare, and could be revealed by detailed analyses as well as examinations of additional tissues in patients with relatively complex phenotypes, as observed in the present patient.

Phenotypic features in androgenetic/biparental mosaicism would be determined by several factors. They include (1) the ratio of two cell lineages in various tissues/organs, (2) the number of imprinted domains relevant to specific features (for example, dysregulation of the imprinted domains on 11p15.5 and 14q32.2 is involved in placentomegaly[9,17]), (3) the degree of clinical effects of dysregulated imprinted domains (an (epi)dominant effect has been assumed for the 11p15.5 imprinted domains[18]), (4) expression levels of imprinted genes in androgenetic cells (although *SNRPN* expression of this patient was consistent with androgenetic cells being predominant in leukocytes, complicated expression patterns have been identified for several imprinted genes in both androgenetic and parthenogenetic fetal mice, probably because of perturbed *cis*- and *trans*-acting regulatory mechanisms[19]) and (5) unmasking of possible paternally inherited recessive mutation(s) in androgenetic cells. Thus, in this patient, it appears that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of BWS-like and upd(14)pat-like body and placental phenotypes, but remained below the threshold level for the occurrence of other imprinting disorders or recessive Mendelian disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Parthenogenetic chimaerism/mosaicism with a Silver-Russell syndrome-like phenotype

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ABSTRACT

Introduction We report a 34-year-old Japanese female with a Silver-Russell syndrome (SRS)-like phenotype and a mosaic Turner syndrome karyotype (45,X/46,XX).

Methods/Results Molecular studies including methylation analysis of 17 differentially methylated regions (DMRs) on the autosomes and the *XIST*-DMR on the X chromosome and genome-wide microsatellite analysis for 96 autosomal loci and 30 X chromosomal loci revealed that the 46,XX cell lineage was accompanied by maternal uniparental isodisomy for all chromosomes (upid(AC)mat), whereas the 45,X cell lineage was associated with biparentally derived autosomes and a maternally derived X chromosome. The frequency of the 46,XX upid(AC)mat cells was calculated as 84% in leukocytes, 56% in salivary cells, and 18% in buccal epithelial cells.

Discussion The results imply that a parthenogenetic activation took place around the time of fertilisation of a sperm missing a sex chromosome, resulting in the generation of the upid(AC)mat 46,XX cell lineage by endoreplication of one blastomere containing a female pronucleus and the 45,X cell lineage by union of male and female pronuclei. It is likely that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of SRS phenotype, but not for the occurrence of other imprinting disorders or recessive Mendelian disorders.

Although a mammal with maternal uniparental disomy for all chromosomes (upd(AC)mat) is incompatible with life because of genomic imprinting,[1] a mammal with a upd(AC)mat cell lineage could be viable in the presence of a co-existing normal cell lineage. In the human, Strain *et al*[2] have reported 46,XX peripheral blood cells with maternal uniparental isodisomy for all chromosomes (upid(AC)mat) in a 1.2-year-old phenotypically male patient with aggressive behaviour, hemifacial hypoplasia and normal birth weight. Because of the 46,XX disorders of sex development, detailed molecular studies were performed, revealing the presence of a normal 46,XY cell lineage in a vast majority of skin fibroblasts and a upid(AC)mat 46,XX cell lineage in nearly all blood cells. In addition, although the data are insufficient to draw a definitive conclusion, Horike *et al*[3] have also identified 46,XX peripheral blood cells with possible upd(AC)mat in a phenotypically male patient through methylation analyses for plural differentially methylated regions (DMRs) in 11 patients with Silver–Russell syndrome (SRS)-like phenotype. This patient was found to have a normal 46,XY cell lineage and a triploid 69,XXY cell lineage in skin fibroblasts.

However, such patients with a upd(AC)mat cell lineage remain extremely rare, and there is no report describing a human with such a cell lineage in the absence of a normal cell lineage. Here, we report a female patient with a upid(AC)mat 46,XX cell lineage and a non-upd 45,X cell lineage who was identified through genetic screenings of 103 patients with SRS-like phenotype.

MATERIALS AND METHODS

Case report

This Japanese female patient was conceived naturally and born at 40 weeks of gestation by a normal vaginal delivery. At birth, her length was 44.0 cm (−3.1 SD), her weight 2.1 kg (−2.9 SD) and her occipitofrontal head circumference (OFC) 30.5 cm (−2.3 SD). The parents and the younger brother were clinically normal (the father died from a traffic accident).

At 2 years of age, she was referred to us because of growth failure. Her height was 77.7 cm (−2.5 SD), her weight 8.45 kg (−2.6 SD) and her OFC 43.5 cm (−2.5 SD). Physical examination revealed several SRS-like somatic features such as triangular face, right hemihypoplasia and bilateral fifth finger clinodactyly. She also had developmental retardation, with a developmental quotient of 56. Endocrine studies for short stature were normal as were radiological studies. Cytogenetic analysis using lymphocytes indicated a low-grade mosaic Turner syndrome (TS) karyotype, 45,X[3]/46,XX[47]. Thus, a screening of TS phenotype[4] was performed, detecting horseshoe kidney but no body surface features or cardiovascular lesion. Chromosome analysis was repeated at 6 and 32 years of age using lymphocytes, revealing a 45,X[8]/46,XX[92] karyotype and a 45,X[12]/46,XX[88] karyotype, respectively. On the last examination at 34 years of age, her height was 125.0 cm (−6.2 SD), her weight 37.5 kg (−2.0 SD) and her OFC 51.2 cm (−2.8 SD). She was engaged in a simple work and was able to get on her daily life for herself.

Sample preparation

This study was approved by the Institutional Review Board Committees at National Center for Child health and Development. After obtaining written informed consent, genomic DNA was extracted from leukocytes of the patient, the mother and the brother and from salivary cells, which comprise ~40% of buccal epithelial cells and ~60% of leukocytes,[5] of the patient. Lymphocyte metaphase spreads and leukocyte RNA were also

obtained from the patient. Leukocytes of healthy adults and patients with imprinting disorders were utilised for controls.

Primers and probes

The primers utilised in this study are summarised in supplementary methods and supplementary tables 1–3.

DMR analyses

We first performed bio-combined bisulfite restriction analysis (COBRA)[6] and bisulfite sequencing of the *H19*-DMR (A) on chromosome 11p15.5 by the previously described methods[7] and methylation-sensitive PCR analysis of the *MEST*-DMR (A) on chromosome 7q32.2 by the previously described methods[8] with minor modifications (the methylated and unmethylated allele-specific primers were designed to yield PCR products of different sizes, and the PCR products were visualised on the 2100 Bioanalyzer (Agilent, Santa Clara, California, USA)). This was because hypomethylation (epimutation) of the normally methylated *H19*-DMR of paternal origin and maternal uniparental disomy 7 are known to account for 35–65% and 5–10% of SRS patients, respectively.[9 10] In addition, fluorescence in situ hybridisation (FISH) analysis was performed with a ~84-kb RP5-998N23 probe containing the *H19*-DMR (BACPAC Resources Center, Oakland, California, USA). We also examined multiple other DMRs by bio-COBRA. The ratio of methylated clones (the methylation index) was calculated using peak heights of digested and undigested fragments on the 2100 Bioanalyzer using 2100 expert software.

Genome-wide microsatellite analysis

Microsatellite analysis was performed for 96 autosomal loci and 30 X chromosomal loci. The segment encompassing each locus was PCR-amplified, and the PCR product size was determined on the ABI PRISM 310 autosequencer using GeneScan software (Applied Biosystems, Foster City, California, USA).

PCR analysis for Y chromosomal loci

Standard PCR was performed for six Y chromosomal loci. The PCR products were electrophoresed using the 2100 Bioanalyzer.

Expression analysis

Quantitative real-time reverse transcriptase PCR analysis was performed for three paternally expressed genes (*IGF2*, *SNRPN* and *ZAC1*) and four maternally expressed genes (*H19*, *MEG3*, *PHLDA2* and *CDKN1C*) that are known to be variably (usually weakly) expressed in leukocytes (UniGene, http://www.ncbi.nlm.nih.gov/sites/entrez?db=unigene), using an ABI Prism 7000 Sequence Detection System (Applied Biosystems). *TBP* and *GAPDH* were utilised as internal controls.

RESULTS

DMR analyses

In leukocytes, the bio-COBRA indicated severely hypomethylated *H19*-DMR, and bisulfite sequencing combined with *rs2251375* SNP typing for 30 clones revealed maternal origin of 29 hypomethylated clones and non-maternal (paternal) origin of a single methylated clone in this patient (figure 1A). Thus, the marked hypomethylation of the *H19*-DMR was caused by predominance of maternally derived clones rather than hypomethylation of the *H19*-DMR of paternal origin. FISH analysis for 100 lymphocyte metaphase spreads excluded an apparent deletion of the paternally derived *H19*-DMR or duplication of the maternally derived *H19*-DMR (Supplementary figure 1). Methylation-sensitive PCR amplification for the *MEST*-DMR delineated a major peak for the methylated allele and a minor peak for the unmethylated allele (figure 1B). This also indicated the predominance of maternally derived clones and the co-existence of a minor portion of paternally derived clones. Furthermore, autosomal DMRs invariably exhibited markedly abnormal methylation patterns consistent with predominance of maternally inherited DMRs, whereas the methylation index of the *XIST*-DMR on the X chromosome remained within the female reference range (figure 1C). The abnormal methylation patterns were less obvious in salivary cells (thus, in buccal epithelial cells) than in leukocytes, except for the methylation index for the *XIST*-DMR that mildly exceeded the female reference range (figure 1A–C).

Microsatellite analysis

Major peaks consistent with maternal uniparental isodisomy and minor peaks of non-maternal (paternal) origin were identified for at least one locus on each autosome, with the minor peaks of non-maternal origin being more obvious in salivary cells than in leukocytes (figure 1D and supplementary table 4). Furthermore, the frequency of the upid(AC)mat cells was calculated as 84% in leukocytes, 56% in salivary cells and 18% in epithelial buccal cells, using the area under curves for the maternally and the non-maternally inherited peaks (supplementary note). Such minor peaks of non-maternal origin were not detected for all the 30 X chromosomal loci examined.

PCR analysis for Y chromosomal loci

PCR amplification failed to detect any trace of Y chromosome-specific bands in leukocytes and salivary cells (Supplementary figure 2).

Expression analysis

Expression analysis using control leukocytes indicated that, of the seven examined genes, *SNRPN* expression alone was strong enough to allow for a precise assessment (Supplementary figure 3). *SNRPN* expression was extremely low in this patient (figure 1E).

DISCUSSION

These results imply that this patient had a upid(AC)mat 46,XX cell lineage and a non-upd 45,X cell lineage. Indeed, methylation patterns of the *XIST*-DMR is explained by assuming that the two X chromosomes in the upid(AC)mat cells undergo random X-inactivation and that 45,X cells with the methylated *XIST*-DMR on a single active X chromosome[11] are relatively prevalent in buccal epithelial cells. Furthermore, lack of non-maternally derived minor peaks for microsatellite loci on the X chromosome is explained by assuming that the two X chromosomes in the upid(AC)mat cells and the single X chromosome in the 45,X cells are derived from a common X chromosome of maternal origin, with no paternally derived sex chromosome. It is likely, therefore, that a parthenogenetic activation took place around the time of fertilisation of a sperm missing a sex chromosome, resulting in the generation of the 46,XX cell lineage with upid (AC)mat by endoreplication (the replication of DNA without the subsequent completion of mitosis) of one blastomere containing a female pronucleus and the 45,X cell lineage with biparentally derived autosomes and a maternally derived X chromosome by union of male and female pronuclei (figure 2), although it is also possible that a paternally derived sex chromosome was present in the sperm but was lost from the normal

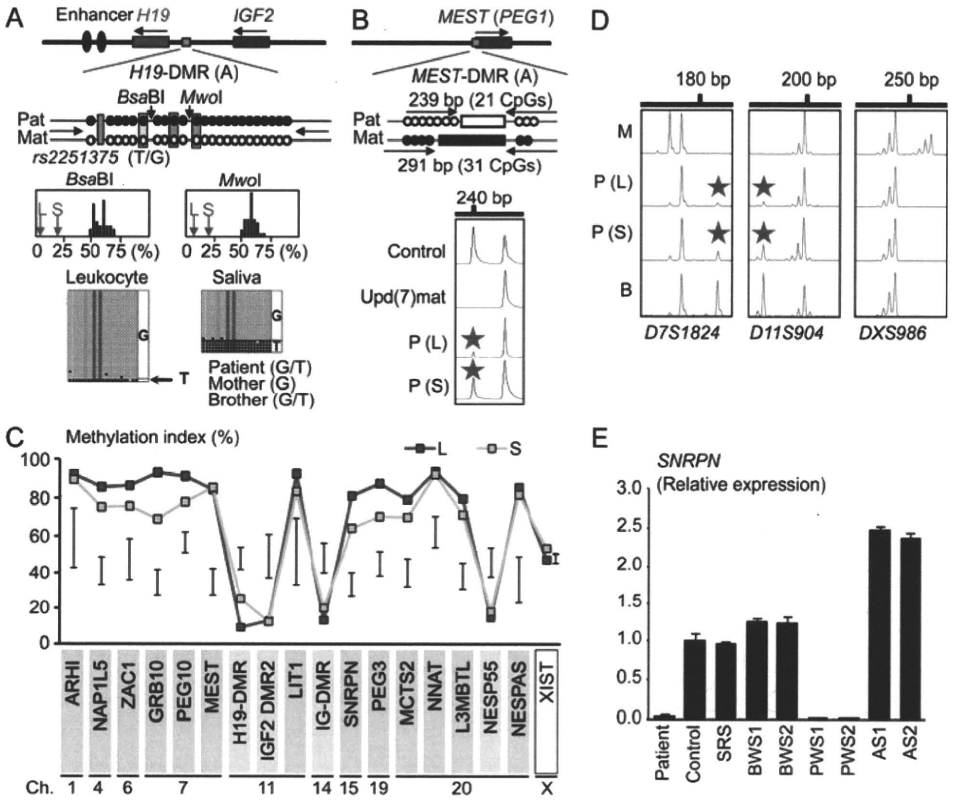

Figure 1 Representative molecular results. Pat, paternally derived allele; Mat, maternally derived allele; P, patient; M, mother; B, brother; L, leukocytes; and S, salivary cells. Filled and open circles in A and B represent methylated and unmethylated cytosine residues at the CpG dinucleotides, respectively. A. Methylation patterns of the *H19*-DMR (A) harbouring 23 CpG dinucleotides and the T/G SNP (*rs2251375*) (a grey box). The PCR products are digested with *Bsa*BI when the cytosine at the sixth CpG dinucleotide (highlighted in yellow) is methylated and with *Mwo*I when the two cytosines at the ninth and the 11th CpG dinucleotides (highlighted in orange) are methylated. For the bio-COBRA data, the black histograms represent the distribution of methylation indices (%) in 50 control participants, and L and S denote the methylation indices for leukocytes and salivary cells of this patient, respectively. For the bisulfite sequencing data, each line indicates a single clone. B. Methylated and unmethylated allele-specific PCR analysis for the *MEST*-DMR (A). In a control participant, the PCR products for methylated and unmethylated alleles are delineated, and the unequal amplification is consistent with a short product being more easily amplified than a long product. In a previously reported patient with upd(7)mat,[8] the methylated allele only is amplified. In this patient, major peaks for the methylated allele and minor peaks for the unmethylated allele (red asterisks) are detected. C. Methylation patterns for the 18 DMRs examined. The DMRs highlighted in blue and pink are methylated after paternal and maternal transmissions, respectively. The black vertical bars indicate the reference data (maximum–minimum) in 20 normal control participants, using leukocyte genomic DNA (for the *XIST*-DMR, 16 female data are shown). D. Representative microsatellite analysis. Minor peaks (red asterisks) have been identified for *D7S1824* and *D11S904* but not for *DXS986* of the patient. Since the peaks for *D7S1824* and *D11S904* are absent in the mother and clearly present in the brother, they are assessed to be of paternal origin. E. Relative expression level (mean ± SD) of *SNRPN* on chromosome 15. The data have been normalised against *TBP*. SRS, an SRS patient with an epimutation (hypomethylation) of the *H19*-DMR; BWS1, a BWS patient with an epimutation (hypermethylation) of the *H19*-DMR; BWS2, a BWS patient with upd(11)pat; PWS1, a PWS patient with upd(15)mat; PWS2, a PWS patient with an epimutation (hypermethylation) of the *SNRPN*-DMR; AS1, an Angelman syndrome (AS) patient with upd(15)pat; and AS2, an AS patient with an epimutation (hypomethylation) of the *SNRPN*-DMR.

cell lineage at the very early developmental stage. Hence, in a strict sense, this patient is neither a chimera resulting from the fusion of two different zygotes nor a mosaic caused by a mitotic error of a single zygote. In this regard, a triploid cell stage is assumed in the generation of a upid(AC)mat cell lineage, and such triploid cells may have been detected in skin fibroblasts of the patient reported by Horike *et al*.[3]

The upid(AC)mat cells accounted for the majority of leukocytes even in adulthood of this patient, despite global negative selective pressure.[12 13] This phenomenon, though intriguing, would not be unexpected in human studies because leukocytes are usually utilised for genetic analyses. Rather, if the upid(AC)mat cells were barely present in leukocytes, they would not have been detected. It is likely, therefore, that upid(AC)mat cells have occupied a relatively large portion of the definitive haematopoietic tissues primarily as a stochastic event. Furthermore, parthenogenetic chimera mouse studies have revealed that parthenogenetic cells are found at a relatively high frequency in some tissues/organs including blood and are barely identified in other tissues/organs such as skeletal muscle and liver.[13] Such a possible tissue-specific selection in favour of the preservation of parthenogenetic cells in the definitive haematopoietic tissues may also be relevant to the predominance of the upid(AC)mat cells in leukocytes. In addition, a reduced growth potential of 45,X cells[14] may also have contributed to the skewed ratio of the two cell lineages.

Clinical features of this patient would be determined by several factors. They include: (1) the ratio of two cell lineages in various tissues/organs, (2) the number of imprinted regions or DMRs relevant to the development of specific imprinting disorders (eg, plural regions/DMRs on chromosomes 7 and 11 for SRS[9 10] and a single region/DMR on chromosome 15 for Prader–Willi syndrome (PWS)),[15] (3) the degree of clinical effects of dysregulated imprinted regions/DMRs (an (epi)dominant effect has been

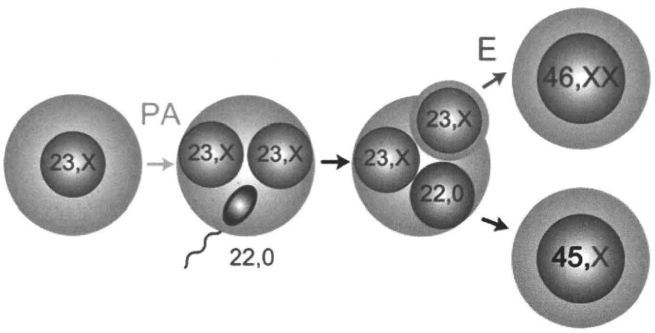

Figure 2 Schematic representation of the generation of the upid(AC) mat 46,XX cell lineage and the non-upd 45,X cell lineage. Polar bodies are not shown. PA, parthenogenetic activation; and E, endoreplication of one blastomere containing a female pronucleus.

assumed for the 11p15.5 imprinted regions including the *IGF2–H19* domain on the basis of SRS or Beckwith–Wiedemann syndrome (BWS) phenotype in patients with multilocus hypomethylation[16] and BWS-like phenotype in patients with a upid (AC)pat cell lineage,[17] a mirror image of a upid(AC)mat cell lineage), (4) expression levels of imprinted genes in upid(AC)mat cells (although *SNRPN* expression of this patient was consistent with upid(AC)mat cells being predominant in leukocytes, complicated expression patterns have been identified for several imprinted genes in androgenetic and parthenogenetic fetal mice, probably because of perturbed *cis*- and *trans*-acting regulatory mechanisms)[18] and (5) unmasking of possible maternally inherited recessive mutation(s) in upid(AC)mat cells.[19] Collectively, it appears that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of SRS phenotype and horseshoe kidney characteristic of TS[4] but remained below the threshold level for the occurrence of other imprinting disorders or recessive Mendelian disorders.

In summary, we identified a upid(AC)mat 46,XX cell lineage in a woman with an SRS-like phenotype and a 45,X cell lineage accompanied by autosomal haploid sets of biparental origin. This report will facilitate further identification of patients with a upid(AC)mat cell lineage and better clarification of the clinical phenotypes in such patients.

Acknowledgements We thank the patient and her family members for their participation in this study. We also thank Dr. Toshiro Nagai for providing us with blood samples of patients with Prader–Willi syndrome.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Institutional Review Board Committees at National Center for Child health and Development.

Contributors Drs Kazuki Yamazawa (first author) and Kazuhiko Nakabayashi (second author) contributed equally to this work.

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特集　産科医療における遺伝学

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秦　健一郎

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特集 産科医療における遺伝学

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秦　健一郎*

DNAメチル化をはじめとするエピジェネティックな遺伝子発現制御は，ヒトの発生に必須の機構である。特に，DNAメチル化によって制御されるゲノムインプリンティングは，胎盤と胎児の発生分化に深く関与している。エピジェネティックな制御機構はまた，長期間遺伝子発現の変化が固定されるような現象の分子基盤となりうることも示唆されている。ヒト疾患のエピジェネティクス研究はまだ端緒についたばかりであるが，われわれの研究をはじめ，産科疾患とエピジェネティックな異常の関連を示す解析結果が得られつつあり，今後の展開が待たれる。

はじめに

ヒトゲノムプロジェクトが2003年に終了し，約30億塩基対のゲノム配列標準データが公開され，マイクロアレイによる網羅的発現解析や一塩基多型解析などのハイスループットな解析手法が格段の進歩を遂げた。ヒト疾患の遺伝子解析はもはや，特殊な研究ではなくなった。一方で，「エピジェネティクス」も含め，遺伝子配列が判明しただけでは理解できない生命現象が数多く残されており，ヒト疾患との関連が注目されている。本稿では，産科領域にかかわるエピジェネティクスについての知見を俯瞰するとともに，われわれの行っている研究を簡潔に紹介する。

I. エピジェネティクスの背景

エピジェネティックな生命現象の典型例として，図1にDNAのメチル化を挙げる。DNAを構成する四つの塩基G，C，T，Aのうち，シトシンの5位の炭素にメチル基（CH_3）がくっつくという簡単な化学的修飾により，そのシトシンを含むゲノム領域の機能は原則として抑制される。例えば，ある遺伝子のプロモーター領域が何らかの理由でメチル化されると，その遺伝子の発現が抑制されてしまうことがある（図1の遺伝子A）。しかも，いったんメチル化されると，細胞分裂後も安定して娘細胞に伝達され，遺伝子の発現が抑制され続ける場合がある。このような現象は，点変異や欠失による遺伝子の機能喪失と見分けがつかないが，遺伝子配列に変化を伴わないという点が決定的に異なる。遺伝子配列に変化を伴わないため，その後DNAメチル化が何らかの理由で消失すると，それまで失われていたかにみえていた遺伝子機能が復活する。

上記で示した例は，遺伝子配列の変化を伴わずに遺伝子機能が変化し，しかも細胞分裂を経て安定して受け継がれるため，「遺伝子配列を介在しない遺伝情報」ととらえることができる。このような現象を，従来のgenetics（遺伝学）

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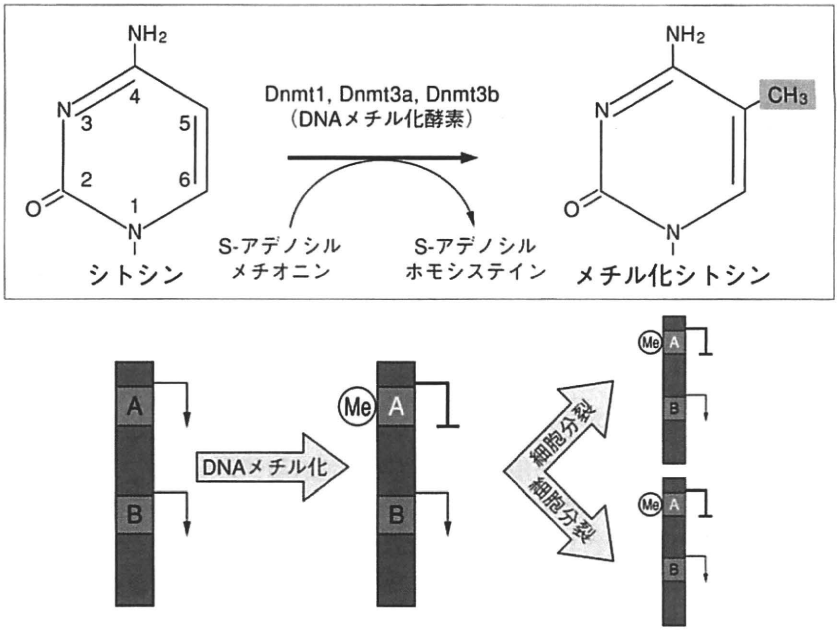

図 1　DNA のシトシンのメチル化によるエピジェネティックな遺伝子発現制御

という言葉に対し，epi-という接頭語を付け，epigenetics（エピジェネティクス）と呼ぶ。適切な日本語訳が存在しないため，本稿では以降エピジェネティクスと表記する。

II. エピジェネティクスと個体発生

DNA メチル化は，エピジェネティックな生命現象のなかでも比較的詳細の解明が進んでおり，しかも DNA メチル化によって制御されるゲノムインプリンティング現象は，産科領域と深い関連がある。

経験的に，ヒトが単為発生しないことはよく知られているが，1980 年代に行われた一連の巧妙な遺伝学的あるいは発生工学的な実験により，その理由の一端が示された。染色体の一部あるいは全部が片親由来である二倍体胚を作製したところ，正常な発生には，父と母それぞれに由来するゲノム両方が必要であることがわかった[1)2)]。実は，一部の遺伝子群（数百個と推定されている）は，父由来と母由来で DNA メチル化状態が正反対になっており，親の由来を区別することができる。そしてこれらの遺伝子は，通常の遺伝子発現とは異なり，厳密にどちらかの親由来の遺伝子だけが発現する（片親性発現する）。例えば単為発生が起こると，二倍体であってもすべてが母親由来の遺伝子なので，これらの遺伝子の発現量は正常時と比較してゼロまたは二倍と大きく乱れてしまい，単為発生胚は発生異常を呈する。このような DNA メチル化状態の違いは，親世代の精子と卵子の形成過程で確立され，受精後も生涯不変である（図 2 の破線）。DNA メチル化によって親の由来情報が「刷り込まれ」ていることから，このような片親性発現をゲノムインプリンティングと呼ぶ。

ゲノムインプリンティングは，胎盤を有する哺乳類に特有のエピジェネティックな現象である。非常に興味深いことに，インプリンティングが破綻すると，多くの場合胎児発育異常を伴う。例えば，インプリンティング異常疾患である Silver-Russell 症候群の一部は，インプリンティング遺伝子 *H19* 領域の低 DNA メチル化状態により発症するが，FGR（IUGR）が必発である。その逆に，Beckwith-Wiedemann 症候群の一部は，同じ *H19* 領域が高メチル化状態に

なっており，胎児は過形成・過成長を伴う。あるゲノム領域のメチル化状態が正反対で，関連する遺伝子発現も正反対になるため，一方の症候群では発育が悪く，他方は大きくなる，という因果関係が矛盾なく説明できる。ゲノムインプリンティングが，実際にヒト胎児発育に関与していることを示唆する典型的なモデルである。

III. 生殖細胞のエピジェネティクス

DNA メチル化酵素関連因子遺伝子である *Dnmt3L* 遺伝子を欠失させると，オスでは無精子症，メスでは不育症を呈する。いずれも，配偶子形成過程で確立されるインプリンティング遺伝子の DNA メチル化が失われていること[3)4)]，また，少なくともオス生殖細胞では，ある種の反復配列領域の DNA メチル化も失われることが示されている[5)6)]。ゲノムインプリンティングに必要なメチル化が，配偶子形成過程で「刷り込まれない」ために，*Dnmt3L* 遺伝子ホモ変異メスの卵子は一見正常に受精して着床するものの，主に胎盤の形成異常を原因として，すべて妊娠中期に致死となってしまう。

ヒストンのメチル化酵素である *G9a* 遺伝子（DNA は，ヒストンに巻きついて折りたたまれ，高次構造を構築している。ヒストンのアセチル化やメチル化は，高次構造の変化を介して遺伝子発現を制御するため，エピジェネティックな機構の一つと考えられている）を，生殖細胞で働かなくすると，やはりオスでは無精子症を呈し，メスでは著しい成熟生殖細胞の減少をきたす[7)]。

近年，蛋白質に翻訳されずに RNA のまま機能している non-coding RNA 分子（非翻訳 RNA 分子）の存在が注目を集めている。これらの分子は，DNA やヒストンの修飾と協調しながら，エピジェネティックな遺伝子発現制御に関与している。なかでも生殖細胞には，低分子の特殊な non-coding RNA 分子が存在し，レトロトランスポゾン遺伝子の DNA メチル化による抑制に関与していると考えられる。これらの機構に異常のあるマウスは，乏精子症を呈する[8)9)]。

ヒトでも，乏精子症の精子を解析すると，DNA メチル化異常が見つかってくることが報告されている[10)11)]。今のところ，このようなヒト症例で前述の *Dnmt3L* などのエピジェネティクス関連因子遺伝子を調べても，明らかな機能欠失変異は見つからない。今後，生殖細胞発生分化を制御するエピジェネティクス機構の理解が深まるとともに，未知のエピジェネティクス因子が介在する可能性も含め，ヒト症例との関連も示されていくものと期待される。

IV. 初期胚発生におけるエピジェネティクス

主にモデル生物を使った解析から，生殖発生過程における DNA メチル化状態の大まかな変動が明らかになっている。図 2 で示したように，配偶子形成過程と，受精後着床から原腸陥入期にかけて，ゲノム全域にわたり，大きな脱メチル化と再メチル化の波が 2 回観察される。インプリンティング遺伝子の DNA メチル化は，配偶子形成過程においてのみ消去され，自身の性に応じた DNA メチル化状態が構築される（図 2 の破線）。それ以外のゲノム領域の DNA メチル化状態は，受精後から胚盤胞期にかけて全体的に低 DNA メチル化状態となる。エピジェネティックな抑制が外れることで，その後の分化能を獲得していくと考えられている（図 2 の実線）。いわゆる ES 細胞（胚性幹細胞）は，低 DNA メチル化状態である胚盤胞期の内部細胞塊から樹立される。ただし，インプリンティング遺伝子の DNA メチル化状態は，詳細未知の機構によりこの脱メチル化過程を免れる（図 2 の破線）。その後，分化に応じて各細胞系譜・組織に特異的な DNA メチル化状態が獲得され，最終分化後はその状態が維持される（図 2 の実線と波線）。*PGC7/Stella* 遺伝子産物は，卵子に存在し，初期発生に必要な母性因子として知られている。同遺伝子を欠失したマウス卵

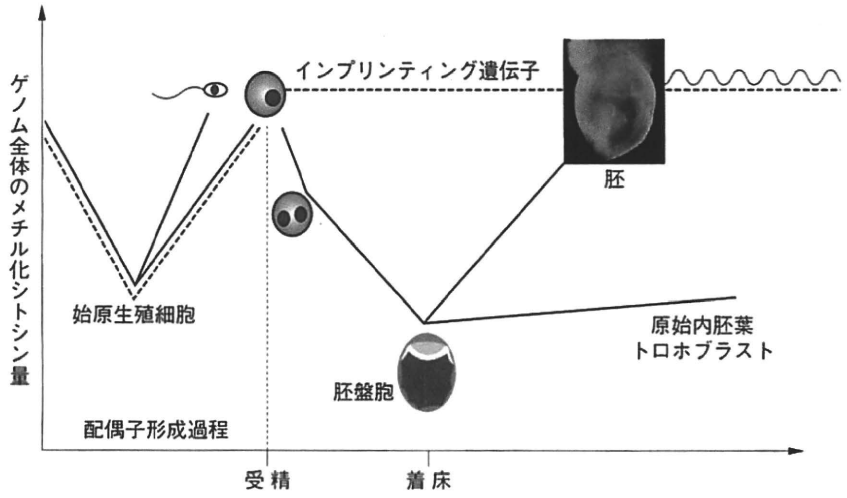

図２　発生過程における DNA メチル化状態の変化

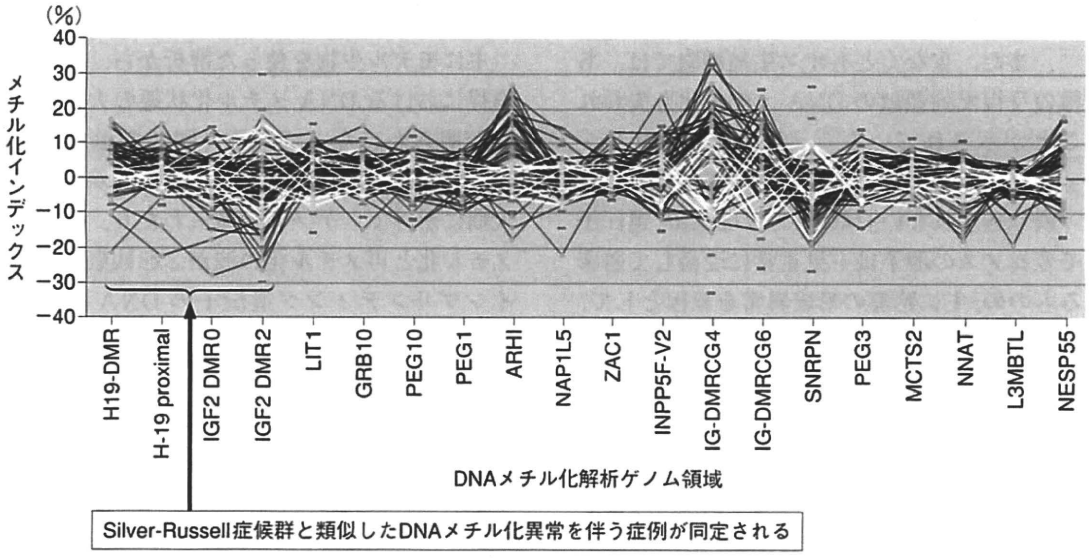

図３　FGR（IUGR）症例の胎盤 DNA メチル化解析例

子由来の胚では，一部の領域で異常な DNA メチル化消失が起きている。正常胚では，これらの遺伝子が，これらの領域を生理的脱メチル化からブロックしていると考えられる[12]。

本節で述べた見解はすべてモデル動物の解析結果であり，必ずしもすべてヒトと同一ではない可能性があるが，ヒト初期胚を系統的に調べることは困難であり，今後もこれらの知見が，産科異常のエピジェネティクスを解析する際の重要な手掛かりとなるであろう。

V. 胎児発育のエピジェネティクス

ここまで示してきたように，エピジェネティックな遺伝子発現制御は，生殖や発生において重要な役割を担っていることが，様々なモデル動物の解析から示されている。特に，インプリンティング異常疾患（前述の Silver-Russell 症候群や Beckwith-Wiedemann 症候群など）の例から，ゲノムインプリンティングが破綻すれば，ヒトでも胎児発育異常を呈するこ

とを述べた。それでは逆に，FGR（IUGR）と診断された症例には，エピジェネティックな異常を伴う症例が存在しているのであろうか。

われわれは現在，「胎児や胎盤の発生分化・発育異常を呈する異常妊娠には，未知のゲノムインプリンティング異常，DNA メチル化異常などのエピジェネティクス異常を伴う症例が存在する」という仮説の下に研究を進めている。エピジェネティックな修飾状態を効率よくスクリーニングするために，ヒトインプリンティング遺伝子の調節領域，胎児胎盤の発生発育に深く関与していると考えられている領域を，過去の報告あるいはマウスゲノムとの比較により網羅的に抽出し，DNA メチル化状態を定量評価する解析系を独自に確立した（図 3，中林ら未発表データ）。

現在，この独自の解析系を用い，FGR（IUGR）と診断された症例の臍帯血および胎盤組織の DNA メチル化状態を網羅的に解析している。すでに解析を終えた百余症例から，2 症例に DNA メチル化異常を同定している（図 3）。同定されたメチル化異常は，Silver-Russell 症候群（FGR を伴うインプリンティング異常疾患）でも報告されている領域の低 DNA メチル化であることから，これらの FGR（IUGR）発症には，共通の分子病態が存在すると推測される。

また，これらの症例では，DNA メチル化異常は胎盤ゲノム DNA のみに認められ，同一児の臍帯血ゲノム DNA には認められなかった。発生学的な考察から，このような DNA メチル化異常が起こるとすれば，その起源は親の配偶子ではなく，受精後の，胚体外組織と胚体組織が非可逆的に分化した時点，おそらく胚盤胞期前後であろうと推測される。加えて，DNA メチル化異常が局所的であることから，DNA メチル化機構の異常による系統的な消失ではなく，偶発的なメチル化消失であることが示唆される。よって，今回われわれが同定した症例に関しては，出生後に DNA メチル化異常に起因する生理機能異常は伴わず，同胞や次世代に同様の異常が発生する可能性は極めて低いであろう，という予測が立つ。諸家からの報告も，解析対象領域と結論が限定的ではあるが，われわれの結果と矛盾しない程度の頻度でメチル化異常症例を検出している[13)~16)]。

そのほか，最近同定されたエピジェネティックな異常を伴うヒト発生異常疾患として，反復胞状奇胎が挙げられる。通常の完全胞状奇胎は雄核発生であり，父親の染色体のみを有する。ところが反復胞状奇胎は，組織学的には通常の完全胞状奇胎とまったく同一であるにもかかわらず，非常に奇妙なことに，両親由来の染色体を有する一見正常な二倍体である。同疾患の発症機序に多大な関心が寄せられていたが，その後の詳細な解析により，反復胞状奇胎組織は，正常な二倍体ではあるものの，インプリンティング遺伝子の DNA メチル化を失っていることが示された[17)]。連鎖解析により同定された原因遺伝子[18)]に，母でホモ変異があると，おそらく卵子形成時に DNA メチル化が確立されず，ゲノムインプリンティングに異常が生じ，雄核発生胚と同様の発生分化異常をきたすと考えられているが，その詳細は今後のさらなる解析が待たれる。

VI. 環境による胎児のエピジェネティックな変化

ここまで，産科領域に関係する生理的なエピジェネティクスあるいはその破綻による疾患について述べてきたが，最後に，母体環境による胚のエピジェネティックな変化をモデル生物で解析した例を紹介する。

マウスの体毛の色を決める遺伝子に viable yellow agouti と呼ばれる変異があると，兄弟間で文字どおり黄色から野生色まで様々な毛色を持つ個体が生まれてくる。この変異では，毛色を決める遺伝子の近くにトランスポゾンと呼ばれる配列が挿入されており，その挿入配列が DNA メチル化によって抑制されていると通常の野生色になり，逆に DNA メチル化の程度が低いと黄色くなる。母体に，妊娠前から授乳期

まで葉酸やビタミン B_{12}など（代謝産物がメチル基の供与体となるもの）を過剰摂取させ続けると，生まれてくる子どもは高い頻度で野生色を呈するようになる。予想どおり，これらの子どもでは挿入配列が高度にメチル化され，しかもその状態が成獣になっても維持されていた[19]。母体の食餌による胎児期の環境が，胎児のDNAメチル化状態に影響を与え，出生後も長期にわたり遺伝子発現を変化させたと考えられる。この実験結果の解釈には慎重を期さねばならないが，少なくとも「環境要因が除去されても影響が長期にわたって遺残する現象」に，エピジェネティックな分子メカニズムが介在している可能性は十分に考えられ，今後の検証が待たれる。

おわりに

以上，本稿では，産科領域に深くかかわっているエピジェネティックな遺伝子発現制御を簡潔に紹介してきた。産科疾患を丁寧に解析していくことで，産科異常の病態解明のみならず，実験が困難なヒト初期発生における分化制御機構の解明が期待される。得られる基盤的知見は，再生医療や腫瘍性疾患の研究にも貢献できるものと考える。また，本稿ではジェネティックな解析については割愛したが，エピジェネティックな生命現象の理解が深まるにつれ，なおさらのこととしてジェネティックな解析も併せて行い，ゲノム機能を多面的にとらえる研究が今後はますます重要になってくると予想される。

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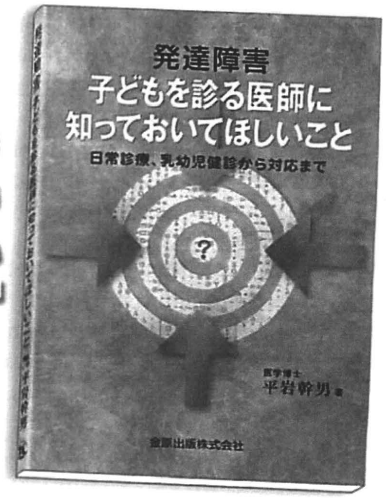

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