Lab Image Gauge software (Fujifilm, Japan). The primers and PCR conditions used for the RT-PCR assays are shown in Table 3.

Results

Methylation states of genes with or without CpG islands and of the HERV-K loci

We chose four genes, *DRD1* (Minowa et al. 1993), *DRD2* (Samad et al. 1997), *COMT* (Tenhunen et al. 1994), and *NCAM* (Hirsch et al. 1991) that harbor CpG islands in the regulatory or flanking regions of the gene (CpG island genes) and four genes, *DRD3* (D'Souza et al. 2001), *HTR2A* (Zhu et al. 1995), *HTR3A* (Bedford et al. 1998), and *HCRT* (Waleh et al. 2001), that do not have CpG islands in the corresponding regions (non-CpG island genes). All eight genes are predominantly and abundantly expressed in brain. In addition, methylation of the HERV-K family, one of the HERV families classified as human retrotransposons, was examined (Löwer et al. 1996).

We first examined the methylation states of the 5'-flanking regions of the genes by Southern blot hybridization using methylation-sensitive restriction enzymes. The CpG island genes all showed completely cleaved patterns with exception *COMT* in one of the PBL samples (Fig. 1). The partial methylation pattern of *COMT* in PBL sample C is most likely the result of incomplete digestion due to insufficient purity of the sample, as well as the enzymatic characteristics of *Sac* II. A similar partial methylation pattern in *DRD1* was observed in some PBL samples (data not shown) using *Sac* II, even though the bisulfite method did not detect any methylated CpG within the *Sac* II recognition sequence. We confirmed by bisufite-modified DNA sequencing that all, or almost all, cytosine residues in the CpG sequences analyzed were unmethylated, as were cytosine residues in non-CpG sequences (Fig. 1).

In contrast, Southern blot hybridization of the non-CpG island genes showed a variety of methylation patterns, depending on the tissue and the gene analyzed (Figs. 2 and 3). One of the prominent trends is the methylation status in brain: methylation was very low or absent in all four genes in each of the three individuals. Placental DNA showed the next lowest levels of methylation in the non-CpG island genes, with the exception of *HCRT*, while liver DNA revealed a trend towards hypermethylation. PBL also showed hypermethylated patterns of *HCRT* and *HTR3A*. *DRD3* was unexpectedly extremely

hypomethylated in all tissues and individuals examined. Interestingly, differences in the degree of methylation between individuals were observed in *HTR2A* and *HCRT* in the placental samples: the methylation levels of *HTR2A* and *HCRT* were lower in Sample a than in Samples b and c (Fig. 2). Although the Southern blot method can reveal the methylation status only of the CpG sequence within a recognition sequence of a methyl-sensitive restriction enzyme, the results obtained with the bisulfite method were largely consistent with the results of Southern blot hybridization. The inter-individual differences in methylation in *HTR2A* and *HCRT* were also detected by the bisulfite method.

In addition to the single copy genes, we analyzed the methylation states of a repetitive sequence region of HERV-K by Southern blot hybridization. When the CpG within the *Sma* I recognition sequence in the 5' LTR of HERV-K is unmethylated, a *Pst* I-*Sma* I fragment of approximately 1.8-kb is produced from multiple HERV-K loci (Fig. 4, top panel). The blot hybridized with a probe prepared from the gag region yielded numerous signals with various sizes, among which the signal at 1.8 kb was most intense in the *Pst* I plus *Xma* I-cleaved lanes, indicated by a black arrowhead in the middle panel in Fig. 4. In contrast, the signal indicated by an open arrowhead at approximately 4.3 kb in size disappeared in those lanes. Therefore, the 1.8 kb fragment results in part from the 4.3 kb fragment. When cutting with *Pst* I and *Sma* I, a methyl-sensitive isoschizomer of *Xma* I, this inverse relation was clearly recognized in the placental samples. We measured the intensities of the 1.8 kb and 4.3 kb signals and normalized them relative to an internal control (not shown). Inter-individual differences in intensities were observed in both signals in the placental samples, and an inverse relation between the 1.8 kb and 4.3 kb signals was evident (Fig. 4, bottom panel). Although the inter-individual differences in intensity were not detected in the 1.8 kb signal in the liver samples, possibly due to overall weak signal intensity, differences could be clearly seen in the 4.3 kb signal intensity, and the trend was similar to that in the placental samples. Sample a was most strongly methylated and Sample c most hypomethylated in both the placenta and liver.

Expression analysis by semi-quantitative RT-PCR

To examine the relationship between DNA methylation and gene expression, we

estimated steady state levels of mRNA transcribed from each of the eight genes by semi-quantitative RT-PCR. The amount of RT products used as a template for PCR was adjusted relative to the internal control glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*). Primer pairs were located in exons of the corresponding genes.

Among the four genes with CpG islands, all but *COMT* were exclusively or predominantly expressed in the brain (Fig. 5). *COMT* was actively expressed in all of the tissues examined, consistent with the results of Tenhunen et al. (1994). The PCR products of *DRD1* and *DRD2* were detected in placental samples three to six cycles later than in the brains. The *NCAM* PCR product was barely detected in the other tissues after the maximum number of cycles. Thus, the transcriptional activity of the three CpG island genes, *DRD1*, *DRD2*, and *NCAM*, was much lower in the liver, placenta, and in PBL than in the brain, and these same genes showed little or no methylation in their promoter regions.

The non-CpG island genes were also expressed most abundantly in the brain among the four tissues examined (Fig. 5). *HTR2A* was expressed in the other three tissues, albeit at very low levels. *HCRT* and *DRD3* showed low levels of expression in the placenta and PBL, respectively. Unlike these genes, *HTR3A* was expressed in the liver and PBL at levels similar to that found in brain, even though the gene was heavily methylated in both tissues. The discrepancy between the expression and methylation of *HTR3A* prompted a database search in which we found an EST (GenBank accession number, BG341613) from primary B cells from tonsils that was located between the first and second exons of *HTR3A* (Ex1b in Fig. 6). Using new primers designed from Ex1b and a downstream exon produced a PCR product of the expected size in the liver and PBL, but no product was detected in the brain. In contrast, another primer from Ex1a yielded a PCR product detected in the brain, and in the liver at very low levels (Fig. 6). These data suggest that *HTR3A* may have at least two promoters: an upstream promoter used in the brain and a downstream one used in PBL and liver.

Discussion

In spite of the general recognition that DNA methylation plays an important role in biological functions, studies implicating methylation in the pathogenesis of inherited

diseases, especially psychiatric disorders, are still very limited. Here, we investigated methylation states in or near the promoter regions of eight single copy genes and one type of repetitive sequence, HERV-K, by the bisufite and/or Southern blot method(s).

The results obtained by the two methods were largely consistent. Each method has some inherent limitations, for example, artifactual partial digestion in the Southern blot method (Fig. 1, *COMT* in the PBL Sample c) and biased amplification and cloning in the bisulfite method. The Southern blot method was useful to identify the presence of biases and had the additional advantage of providing a visual representation of methylation states spanning several kilobases. Nevertheless, both methods are time-consuming and, laborious, limiting the extent of the analysis. Genome-wide analyses of DNA methylation need more robust methods, such as demonstrated in a recent study (Weber et al. 2005).

Half of the eight single copy genes had CpG islands in the 5' flanking regions (CpG island genes), and these CpG sequences were unmethylated in the four tissues examined. Among the four CpG island genes, all but *COMT* were predominantly expressed in the brain. *COMT* was abundantly expressed in all four tissues examined. Thus, the cytosines in the CpG islands were protected from methylation, regardless of the transcriptional state of the gene, which is consistent with the general recognition of hypomethylation in CpG islands except for either allele of the X chromosome genes in females and imprinted genes (Yoder et al. 1997).

The non-CpG island genes revealed a variety of methylation patterns that were different between tissues, genes, and even between individuals (Fig. 2, placental Samples a vs. b and c in *HTR2A* and *HCRT*). The only common feature was hypomethylation in the brain samples in all four non-CpG island genes. In general, methylation in regulatory regions of a gene suppresses the transcriptional activity of that gene. However, *HTR3A* was heavily methylated in the liver and PBL samples, and was actively transcribed in these tissues. We subsequently identified an EST whose sequence was located between the 1st and 2nd exons of *HTR3A*. RT-PCR with a primer located in the authentic exon 1 (Ex1a in Fig. 6) and a common primer amplified a product from brain cDNA, but much less abundantly from liver samples, and nothing was detected after the maximum number of cycles in the PBL and placenta samples. In contrast, RT-PCR with a novel exon-specific primer (Ex1b in Fig. 6) and the common primer did not produce a PCR product from brain, while amplification was clearly observed in the liver and PBL samples. Thus, *HTR3A* may be transcribed from a brain-specific promoter in the brain

and from a different promoter in PBL and liver. *HTR2A* may also possess tissue-specific promoters, although *HTR2A* expression in the liver and PBL was much lower than in brain. Bunzel et al. (1998) reported polymorphic imprinting in expression of *HTR2A* in the human adult brain. The methylation pattern in the PBL, albeit not in the brain, seemed consistent with a differentially methylated region as found in imprinted genes (Robertson 2005). The methylation states in *DRD3* were also inconsistent with the expression status. Despite lacking the CpG island, *DRD3* was not methylated in the liver and placenta, where the *DRD3* transcript was not detected. Thus, our data suggest that for non-CpG island genes 5'-flanking regions are not methylated in any tissues in which the genes are actively expressed, but the converse is not necessarily true.

We are interested in the presence of methylation differences between individuals. In this study, we found inter-individual differences in methylation in two genes, *HTR2A* and *HCRT*, in the placental samples. Sample a showed much lower methylation in both these genes than in Samples b and c. We were unable to determine the methylation-expression relationship of *HTR2A* and *HCRT* because RNA was not available from Sample a.

To examine multiple loci and the relationship between methylation in single copy genes and repetitive sequences, we examined the HERV-K methylation status and identified two signals that showed inter-individual differences in signal intensity in placental and liver samples. The two signals possibly consist of multiple loci, with the larger one having a *Sma* I recognition sequence (CCCGGG) and the smaller one resulting from *Sma* I digestion of this locus. The inter-individual differences were most distinctive in the placental samples, with methylation highest in Sample a, moderate in Sample b, and lowest in Sample c, which is the reverse of the trend seen in *HTR2A* and *HCRT*. In addition, this order was the same as found in the liver samples. The reasons for inter-individual methylation differences remain to be ascertained, but here we could exclude a developmental effect on DNA methylation because we found opposite trends in different genes and our previous study revealed that methylation levels undergo global changes in the developing placenta (Fuke et al. 2004).

To date, the most widely used methods for DNA methylation analysis are Southern blot hybridization in combination with methyl-sensitive restriction enzymes and bisulfite-modified DNA sequencing. Both methods have intrinsic weaknesses, an artifactual partial methylation in the former and biased amplification and cloning in the latter. In our present study on DNA methylation, these methods were complementary and

provided very reliable basic data on methylation status within or near the promoter region of genes whose activities are essential to development and brain function. We additionally determined the relationship between methylation and expression of these genes. Furthermore, we identified inter-individual differences in DNA methylation, which may be helpful in elucidating methylation mechanisms. It is especially worth noting that the difference in HERV-K methylation levels between individuals clearly observed in the placenta was also detected in the liver. This finding may support our approach and hypothesis to explore the possible implications of epigenetics in the molecular etiology of psychiatric disorders (Fuke et al., 2004; Nakamura et al., 2003, and unpublished data). Recently, a genome-wide methylation analysis was performed using a novel combination of existing methods (Weber et al. 2005), and this approach will contribute to rapid progress in understanding the etiological roles of DNA methylation in the pathogenesis of common diseases, especially psychiatric disorders.

Acknowledgments

We thank Drs. K. Higashimoto, H. Soejima, K. Miura, and T. Miyamoto for their help. Prof. Y. Okazaki was supported by a Grant-in-Aid for Scientific Research (A) (No. 13307027).

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Figure legends

Fig. 1 Methylation analyses of genes with CpG islands. The three panels (a-c) presented for each gene show the following. **a** Schematic of the region analyzed where *filled boxes* indicate probes for Southern blot analysis, *open boxes* indicate the first exon of the gene, and *arrowheads* indicate positions of the primer sets for bisulfite-modified DNA sequencing. Lines under the restriction maps are possible fragments generated by restriction digestion. **b** The results of Southern blot analysis. *Letters* above the lanes indicate samples from different individuals. Samples *a*, *b*, and *c* were obtained from fetal tissues and placentas with gestational ages of 16, 19, and 21 weeks, respectively. **c** Schematic of methylation states of all cytosines in the CpG sequence as analyzed by the bisulfite method. Each nucleotide position is represented by a circle summarizing the results of 10 clones analyzed. *Blacksectors* indicates the percentage of methylated cytosine. Each number on the line indicates the position of the CpG region analyzed. The number in the *black box* indicates the position of the cytosine (in CpG) in the recognition sequences for methyl-sensitive restriction enzymes. P *Pst* I, SII *Sac* II, E *Eco*R I, S *Sma* I, D *Dra* I, *COMT,* catechol-O-methyltransferase; *DRD1* dopamine receptor D1, *DRD2* dopamine receptor D2, *NCAM* neural cell adhesion molecule, PBL peripheral blood leukocytes.

Fig. 2 Methylation analyses of genes without CpG islands. Description of panels **a-c** for each gene and *symbols* as in Fig. 1. P *Pst* I, H *Hpa* II, SI *Sac* I, Mb *Mbo* I, Hh *Hha* I, *HTR2A* 5-hydroxytryptamine receptor 2A, *HCRT* hypocretin (also called orexin).

Fig. 3 Methylation analyses of genes without CpG islands. *Open boxes* indicate the first exons of the genes; the first exon of *HTR3A* corresponds to Ex1a described in Fig. 6. Description of panels **a-c** for each gene and *symbols* as in Fig. 1. B methyl-sensitive restriction enzyme *Bst*B I. *HTR3A* 5-hydroxytryptamine receptor 3A, *DRD3* dopamine receptor D3.

Fig. 4 Methylation analysis of human endogenous retrovirus (HERV)-K. Part of a typical HERV-K (5' LTR and gag region) is shown at the top. The flanking sequence is depicted

by a thin line. A *black bar* indicates the location of the probe used for Southern blot hybridization. The *middle panels* show the Southern hybridization results. *Letters* above the lanes indicate samples from different individuals. Two bands that showed inter-individual differences in signal intensity are indicated by *open* and *filled triangles*. The intensities of these two signals were quantified by the Science Lab Image Gauge software (Fujifilm, Japan) and normalized relative to an internal control (not shown). The *bottom panel* shows the normalized intensities for each band.

Fig. 5 Semi-quantitative analysis of the expression of the eight single copy genes. The amount of cDNA (reverse transcription products) used for PCR was adjusted relative to that of an internal control gene, glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*). Equal volumes of PCR reaction mixes at the cycles indicated by the numbers above the lanes were fractionated in 4% polyacrylamide gels and stained with ethidium bromide. RNA from placental Sample a was not available. *GAPDH,* glyceraldehydes-3-phosphate dehydrogenase. Gene *symbols* as shown in Fig. 1.

Fig. 6 *HTR3A* transcription from a brain-specific promoter and a novel putative promoter. *Upper panel HTR3A*-gene structure. The *boxes* on the line indicate the relative size and location of the exons. The transcript-specific primers 1 and 2, and the common primer 3 are shown schematically. Ex1a is the first exon of *HTR3A* and Ex1b is the alternative exon 1 that is possibly transcribed from another promoter. *Lower panel* tissue specificity of the transcripts generated with Primers 1 and 3 or from Primers 2 and 3. *Numbers* above the lanes indicate the number of amplification cycles and + or – below the lanes indicates whether reverse transcriptase was included in the reverse transcription reaction followed by the PCR reaction. *Letters* indicate samples from different individuals.

特集：精神疾患におけるエピジェネティクス

87-93

ヒト内在性レトロウイルスと統合失調症

小田　高也*

Key words：HERV，retrotransposon，schizophrenia，human genome，repeat sequence

1．はじめに

統合失調症は，幻覚，妄想，思考障害といった陽性症状や，感情の平板化，寡動，意欲・自発性の低下といった陰性症状など多彩な症状を特徴とし，主に20代に発症し以後長期にわたり経過する疾患である[1]。本人および家族への影響や経済的損失からみても，大きなインパクトを持つ病気の1つとされている。発症に男女差は認められず頻度は地域によっては多少の違いは認めるものの，一般人口に対して約1％とされており稀な疾患ではない[14]。厚生労働省による「平成14年患者調査の概況」によれば全国に73.4万人の患者がいることがわかっている。

疾患の原因は未だ不明ながら，遺伝的因子の関与が双生児および養子研究よりわかってきている。同胞（危険率は約10％）の場合と同程度の確率で同じ遺伝子型の組み合わせを持つと考えられる二卵性双生児の場合，統合失調症発症の一致率が10-25％であるのに対して，同じ遺伝型を持つ一卵性双生児の場合は一方が発病した場合もう一方が発病する危険率は40-60％と高くなる。しかし，この場合統合失調症の発病が遺伝的因子にのみ規定されていると考えるならば100％一致することが期待されるため，たとえ遺伝型が同じでも60-40％は発病しないことが示唆されたことになる[18]。また，養子研究からは統合失調症でない母親から生まれて養子に出された子供の罹病危険率は1％であるのに対して，統合失調症の母親から生まれた新生児では統合失調症の既往歴のない家族に養子に出された場合の罹病危険率が10％となることがわかった。つまり，統合失調症の発病には遺伝的因子の寄与が高く，良好な養育環境でも統合失調症の遺伝因子を持った子供は発病しやすいということが示された[18]。

都市に多いことや，都市化と共に増加したらしいこと。また，高緯度地域に多いなどといった疫学的に知られる生育環境に加えて，統合失調症発症のリスクと考えられている環境因子としては仮死などの周産期障害やウイルス感染がある[24,38]。母体へのウイルス感染と統合失調症発症との因果関係が推察される背景には，1918年に起こった「スペインかぜ」と呼ばれるインフルエンザの大流行後に統合失調症の発症が増えたとの報告があることや[22]，疫学的に統合失調症が冬生まれの者に多いという事実がある。最

HERV and schizophrenia

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近もBrown et al.は，64例を対象にしたコホート調査を行い，妊娠第2三半期および第3三半期のインフルエンザウイルスへの暴露は統合失調症のリスクに影響を及ぼさなかったものの，第1三半期中の暴露は統合失調症のリスクを7倍に上昇させたと報告している[4]。

さて，統合失調症の責任遺伝子を同定しようという試みは1970年代に始まり，治療薬の薬理作用から疾患との因果関係が期待されたドーパミン受容体をはじめ多くの候補遺伝子に関して変異解析が行われてきている[6]。しかし，今のところドーパミン受容体D2に関して報告されたCys311Ser多型のように，疾患との関連性を示唆する報告はあるものの，発症との因果関係を明確にできるには至っていない[11]。また，連鎖解析法という他の遺伝性疾患の責任座位の決定に用いられてきた検索法も導入され，最近のヒトゲノム計画の進展を背景に，広範囲のゲノム規模の分析が可能となってきている。現在までに，いくつかの染色体で疾患との連鎖を示す遺伝子座検出の報告があるものの，再現性の確認が困難な場合も多い[3,25,40]。しかしながら，これらの遺伝子座に相当する領域は組み換えDNA技術で取り扱うほど狭い範囲にまでは絞り込まれていないのが現状である。最近では候補遺伝子の特徴の解析を細かく行いながら，連鎖不平衡法解析を使って関連すると思われる領域を絞り込む研究が精力的に行われている[12]。

2．ヒト内在性レトロウイルス

かつて10万とも予想されていたヒトの遺伝子は，近年のヒトゲノム計画の進展に伴いはるかに少ない数しか存在しないことが明らかとなってきた。ヒトは，ショウジョウバエが持つ僅か2倍あまりの遺伝子で，10万種以上ある酵素やホルモンなどのタンパク質を作り分けており，更に言語を操るといった能力に代表される脳の高次機能を実現しているのは注目に値する[34]。「少ない遺伝子が複雑な生命活動を支える仕組みを解明すること」は，生命科学の今後の重要な課題の1つともいえよう。

さて，タンパク質をコードする遺伝子がゲノムに占める割合は1.5%ほどである一方，45％もの領域は同じ塩基配列の繰り返し（リピート）で構成されている。この膨大な非遺伝子領域を構成するリピート（配列）は，かつて存在意義が不明なため意味をなさない存在，“ジャンク”と考えられていたが，ヒトゲノム計画が進むにつれて遺伝子の発現調節との関与やクロマチン構築およびヒトの進化における役割という観点から関心が高まってきている[13,20]。

ヒト内在性レトロウイルス（HERV: Human Endogenous Retrovirus）は，L1に代表されるLINE（long interspersed nuclear element）や*Alu*に代表されるSINE（short interspersed nuclear element）と共にリピートを構成しておりレトロトランスポゾンと呼ばれている。植物ゲノムで観察される動く遺伝子（トランスポゾン）が主にカットアンドペーストの様式をとってゲノム中を移動するのに対して，HERVなどはRNAが逆転写酵素によってcDNAに変換された後に宿主ゲノムに組み込まれるというコピーアンドペーストの転移様式を取ることからこう呼ばれている。HERVはゲノム全体の8％を構成し，gag, pol, envという構造タンパクをコードする遺伝子がプロモーター・エンハンサー活性を持つlong terminal repeat（LTR）に囲まれた基本構造をしている[7]。

ヒトゲノム計画の進展により提供された膨大な配列情報を解析することによりゲノム中のHERVの大多数は核酸の欠損や挿入などの変異を伴い，ウイルスとしては不完全な構造をしている事が明らかとなってきた。かつてウイルス粒子を構成するのに使われていたタンパク質の読み取り枠には致命的な停止コドンが散見され，あたかも“死んで”いるかのように見える一方で，少数ではあるが変異の程度が低く現在もウイルス粒子を作る可能性が指摘されるHERVもある[33,41]。

感染性レトロウイルス由来であるというその性質上，近年までHERVは主として複製の際に

プライマーとして使用されるホストのtRNAに対応するアミノ酸表記文字にちなんで分類されてきた。しかし，近年ヒトゲノム計画で得られた配列情報を利用した系統的分類が試みられている。2000年にTristemは，gagおよびenv領域と比べて比較的変異率の低いpol領域に注目してHERVの系統樹解析を行った。彼は，新規に提唱した6種を含めてHERV全体を22種類（ファミリー）に分類し，各ファミリーを構成するゲノム中のHERVの個数（十数個～数百個）も概算している[39]。

さて，このようにゲノム中には数多くのHERVが存在することが確認できるようになった一方で，HERVの転写に関しては限られた知見しか得られていない。正常組織では組織特異的な発現が，限られた数のHERVで報告されているのみである。正常組織での発現が確認できたHERVを詳細に解析してみても，何故そのHERVが類似する（転写調節に関わるとされるLTR内に確認される転写因子の結合配列などを含めて）同一ファミリーに属する他のメンバーのHERVと比べて選択的に発現しているかの理由は明確にはできていない[27]。しかし，正常組織では発現が確認されないHERVでも，奇形癌の細胞株ではそのHERVの発現が転写レベルのみならず翻訳レベルでも確認された報告があることから，HERVの発現は正常組織では厳格に制御（抑制）されていると考えられる[21]。レトロトランスポジションによって，ホストにとって致死的ともなるゲノムの構造変化をおこしかねないHERVの活動を抑制するのは，個体が生き残るための防御機構として理解しやすい。さらに，ホストゲノムで発現抑制のかかったHERVは，複製の際にはそのまま娘細胞に分配される事になるため，DNAの一次配列によらずに子孫に伝搬可能な情報を伝えるDNAのメチル化といったエピジェネティックな機構が関与していると推察される[15]。*in vitro*の系では，環境要因（培地へのステロイドホルモンの添加）が特定の遺伝子におけるDNAのメチル化に影響を与える事が示されている[37]。このことは，ヒトにおいても何らかの環境要因が，エピジェネティックな制御機構に“揺らぎ”を生じさせ，それがジェネティックスでは説明が困難な遺伝形式を示す疾患の発症に関与する可能性を示唆していると思われる[15]。

3．HERV研究の背景

図1にYolken et al.が提唱したHERVが関わる疾患の発症仮説を示す[44]。レトロトランスポジションによるHERVのホストゲノムへの挿入は機能遺伝子のタンパク質コード領域やプロモーター領域を問わず起き得るため，そのタンパク質の欠如または機能不全が生じる。また，挿入されたHERVのLTRが新たなプロモーター・エンハンサーとして働くため挿入部位の下流に位置する遺伝子の発現制御に干渉する可能性も持つ。また，Woodsが推察する如くゲノムへのHERVの挿入によりある個体が発生初期に受けた因子の影響が，その後時間が経過した後に表れるような機構を作り出しているのかもしれない。この仮説は，統合失調症の発症時期と疫学的に発症との関連が示唆されている胎生期でのウイルス感染という2つのイベントの関連を説明するのに適していると思われる[43]。

HERVの挿入がホストの遺伝子の発現に影響を与えたと推察される複数例の報告がある。その1例として中枢神経系における細胞膜の形成に重要な役割を果たしている酵素の1つであるホスホリパーゼA2と相同性の高い転写物の発現が，HERVによって制御されている事があげられる[19]。現在までにヒトの疾患発症に関してHERVとの明確な因果関係が示された例はないものの，I型糖尿病患者の膵臓培養上清中よりHERV-Kが単離されて，スーパー抗原活性を持つことが示されたことなど疾患との関連を示唆する報告は多い[5]。

また，感染性のウイルスに関してではあるがHIVやHTLVに感染した場合，認識機能障害等の数々の統合失調症様な症状を示す症例が経験されている[28,31,42]。ウイルス感受性については，

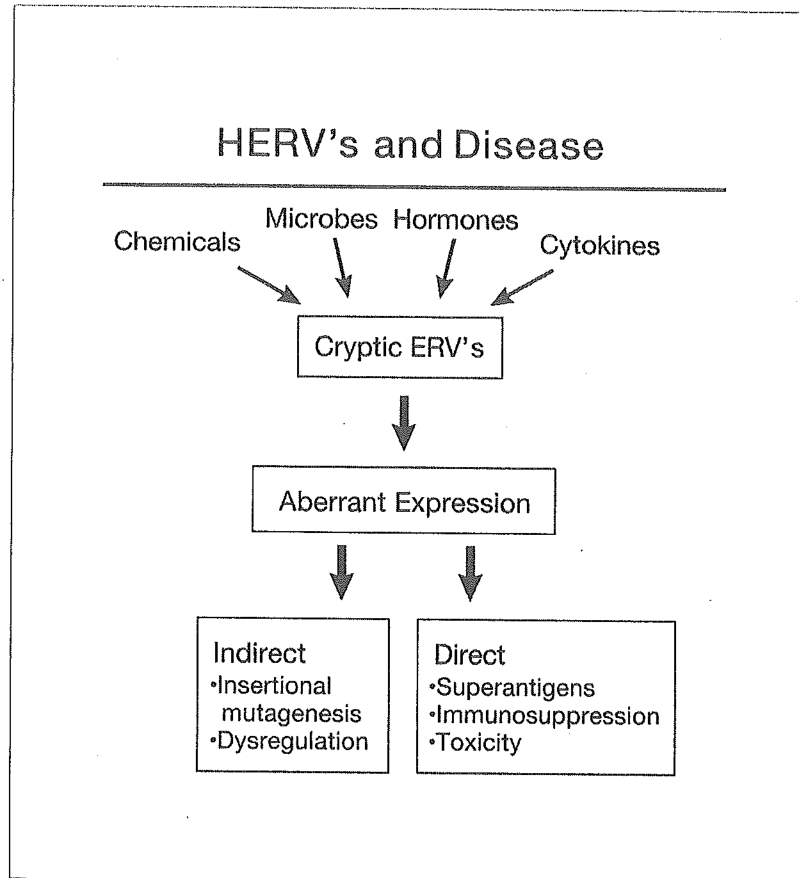

図1 Yolken et al. が提唱する HERV による疾患の発症モデル
種々の環境因子の働きにより HERV の発現が誘発され，スーパー抗原活性の提示（直接的関与）やゲノムへのコピーの挿入（間接的関与）といった機序を介して発症につながると想定している（文献[44]より引用)。

最近ケモカイン受容体-5 の遺伝子が関与しているとの報告がなされ，この受容体はレトロウイルスの受容に関連しており，この遺伝子の変異型では HIV へほとんど感染しなくなるといわれる[8)32)]。

4．研究の進展

正常の脳組織を用いて行われた発現活性能を保持している HERV の検索が Nakamura et al.によって報告されている。彼らは Tristem の分類に基づき dbEST を利用して系統的に HERV の発現予測を行い，その予測を RT-PCR を用いて検証した結果 1q21-q22 および 22q12 の 2 ヵ所に存在する HERV は転写活性能を保持している事を示した[26)]。

また，Karlsson et al.は，RT-PCR を用いて発症初期の統合失調症および分裂感情障害の脳脊髄液（初回入院日より平均 14 日以内に採取）よりレトロウイルスの pol 領域を検出したと報告した[16)]。これは，対象 35 例中（18 ～ 48 歳，平均 25 歳）10 例でのことであったが，対照群 52 例（健常者 30 例，偽脳腫瘍 14 例，正常圧水頭症 8 例）から検出できた例は皆無であった。また，慢性期 20 例の患者脳脊髄液から 1 例ではあるがレトロウイルス DNA 断片を検出している。サンプル中の免疫グロブリン・アルブミン・細胞数等は比較群間で有意差は認めなかった。増幅された DNA 断片をクローン化して詳しく調べてみると HERV-W に属する配列であることがわかっ

た。また，統合失調症例の死後脳前頭葉では，対照群に比べて全例（5症例）でHERV-Wの発現亢進が確認できた。また，その後脳脊髄液を調べた35例中33例を含む54例（統合失調症，分裂感情障害および統合失調症様障害）中9例で，血漿中からレトロウイルスのgag領域を検出したと報告している[17]。

彼らは，脳脊髄液中からHERV-Wに属するDNA断片が得られたことに関しては，偶然にpol配列が類似するレトロウイルスが症例に感染していた可能性が否定できないとしながらも，HERV-Wの疾患発症への関与を慎重に取り扱うべきだと考察している。その理由の1つには，一卵性双生児の統合失調症不一致例からRDA法（representational difference analysis）で彼らと同じくHERV-Wに属するレトロウイルス関連DNA断片が検出されたことをあげている[9]。この例では，レトロウイルスの個体ゲノムへの挿入が*de novo*に起きた可能性が示唆されている。

7q21.2にローカスをもつシンシチンは，宿主であるヒトに新規の機能を与えたHERV（-W）としてはじめて認知された遺伝子である。シンシチンは，正常の胎盤での発現を認め，*in vitro*の系で細胞融合能を持つことが証明されたことにより，胎盤での合胞体形成に関わることが示唆されている[23]。また，多発性硬化症例の脳脊髄液からもMSRVとよばれるHERV-Wに属すると見られるDNA断片が得られている[29]。

統合失調症と多発性硬化症という共に発症原因が不明な疾患の患者から得られた脳脊髄液で，互いに相同性が高いレトロウイルス配列が検出されたことの意味合いは不明である。この2つの疾患は臨床的に明確に区別が可能な疾病であり，それぞれ固有の病理学的特徴を示す。しかし，中には発症年齢，出生時期，地域的特性など疫学的な共通点も認め[35][36]，症例によっては両疾患の臨床的症候を示す例の報告もある[10][30]。この2つの疾患に関して患者の中枢神経系で活性の亢進しているレトロウイルス関連の転写物およびその発現制御機構を引き続き研究する事は，ヒトの脳における疾患とレトロウイルスとの関係を明確にし，より優れた診断法と治療法の開発に役立つと思われる。

5．おわりに

2004年末，シンシチンに関連して興味ある研究が報告されている。元来シンシチンは，ノーザンレベルの発現が胎盤と精巣で確認されているのであるが，Antony et al.は，多発性硬化症症例の脳におけるシンシチンの発現を検討し，それが健常群に比べて3倍程度亢進していることを発見した。更に，シンシチンに対する特異抗体を用いた免疫組織染色では，シンシチンタンパクの発現が主として脱髄病変部で亢進していることが確認できた。*in vitro*の実験では，星状膠細胞でのシンシチンの強制発現により乏突起膠細胞の障害が誘発される事が明らかとなった[2]。正常の胎盤においてシンシチンが担うと考えられる細胞融合能と多発性硬化症病変部でシンシチンタンパクが果たしている役割の違いの理由を明らかにするためにも，今後の研究の成果が期待される。

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