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# 論　文　内　容　の　要　旨

| 氏　名　（　陳露　CHEN LU　） | |
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| 論文題名 | Study on the role of neuropeptide receptor VPAC2 overactivation in schizophrenia and its potential therapeutic target<br>（統合失調症における神経ペプチド受容体VPAC2の過剰活性化の役割とその治療標的としての可能性に関する研究） |

論文内容の要旨

Schizophrenia is a chronic disabling brain disorder affecting approximately 1% of the world's population, and nearly 10% of those with schizophrenia have an affected first-degree relative. Schizophrenia is mainly characterized by positive symptoms such as hallucinations and delusions, negative symptoms such as decreased motivation, and cognitive dysfunction. Approximately one-third of patients with this diagnosis will attempt suicide, and eventually, about only one in ten of these will be successfully treated. New treatments are needed to lessen the suffering of patients and their families and to decrease the economic cost. The advent of the genomic era has led to the discovery of linkages of several genes and pathways to schizophrenia that may serve as new biomarkers or therapeutic targets for this disease. Accumulating evidence indicates that a number of rare copy number variants (CNVs), including both deletions and duplications, have been strongly associated with schizophrenia. Among the most highly penetrant genetic risk factors for neuropsychiatric disorders, clinical studies have shown that microduplications at 7q36.3, containing *VIPR2*, confer significant risk for schizophrenia. *VIPR2* encodes VPAC2, a seven transmembrane heterotrimeric G protein-coupled receptor (GPCR) that binds two homologous neuropeptides with high affinity, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP). PACAP and its receptors (PAC1, VPAC1, and VPAC2) are expressed in various organs, including the sensory, digestive, and reproductive organs, in addition to the central and peripheral nervous systems, and thus PACAP exhibits a variety of biological activities in a distinct manner. VIP binds to two of the three receptors, VPAC1 and VPAC2, with an affinity similar to that of PACAP. Lymphocytes from patients with 7q36.3 microduplications exhibited higher *VIPR2* gene expression and VIP responsiveness (cAMP induction), demonstrating the functional significance of the microduplications. These suggest that overactivation of the VPAC2 receptor signaling is involved in the etiology of schizophrenia and provides large-scale genetic evidence for a specific receptor-mediated (and potentially drug targetable) signaling pathway linked to schizophrenia that is distinct from dopaminergic, glutamatergic, and serotonergic systems. However, the mechanism by which overactive VPAC2 signaling may lead to schizophrenia is unknown. In the present study, to investigate how increased *VIPR2* dosage might predispose to psychiatric disorders, we have tried to develop a bacterial artificial chromosome (BAC) transgenic (Tg) mouse model of *VIPR2* CNV that recapitulates the genetic architecture of the susceptibility allele. Furthermore, since the VPAC2 receptor is considered an attractive drug target for development to treat schizophrenia, we have tried in another study to develop a selective VPAC2 receptor antagonist peptide.

BAC is a useful resource for long-range analysis of the genomic organization and gene function. On average, BAC inserts contain 130-200 kb of mouse genomic DNA. Ideally, during BAC transgenesis, BAC DNA introduced by pronuclear injection is integrated as intact BAC molecules into the mouse genome. Tg mice containing intact BAC molecules are likely to show physiologically-relevant gene expression patterns and copy number-dependent expression levels. We generated mice with additional integrated copies of the mouse *VIPR2* gene. Potential F0 founder and future progeny were screened by PCR. Southern blot analysis revealed three copies of the transgene in hemizygous F1 generation mice. Lines with documented germline

transgene transmission were maintained by breeding hemizygous transgenic animals to C57BL/6J wild-type animals. Then, expression of VPAC2 receptor mRNA significantly increased in the prefrontal cortex, cerebral cortex, and hippocampus of *VIPR2* BAC Tg mice (F2 generation) compared with wild-type littermates. Interestingly, the expression of PAC1 and VPAC1, but not dopamine-$D_2$, receptor mRNA in the prefrontal cortex of *VIPR2* BAC Tg mice was significantly less than that of wild-type mice. *VIPR2* BAC Tg mice showed enhanced locomotor activity, disruption in prepulse inibition of the acoustic startle, decrease in social preference, and cognitive impairment. These findings suggest that the VPAC2 receptor link to mental health disorders may be due in part to overactive VPAC2 signaling at a time when neural circuits involved in cognition and social behavior are being established. We have previously found that activation of the VPAC2 receptor impaired axon outgrowth and decreased dendritic arborization in mouse cortical neurons. Thus, alternatively or additionally, VPAC2 overactivity may disrupt ongoing synaptic plasticity during the processes of learning and memory.

As mentioned above, the VPAC2 receptor might be an attractive drug target for the treatment of schizophrenia because both preclinical and clinical studies have demonstrated a strong link between high expression/overactivation of the VPAC2 receptor and schizophrenia. Despite these backgrounds, the proof-of-concept of VPAC2 inhibitors has not been examined clinically. A reason might be that the VPAC2 receptor belongs to class-B GPCRs, and the discovery of small-molecule drugs against class-B GPCRs is generally difficult. Another issue is the structural properties of the VPAC2 receptor. PACAP also binds tightly to its specific receptor PAC1 and high-affinity receptors for VIP, namely VPAC1 and VPAC2. VPAC1, VPAC2, and PAC1 have moderate amino acid sequence similarities (about 50%) with each other and highly three-dimensional structural homology. VIP selectively activates VPAC1 and VPAC2, and PACAP activates all three receptors. Therefore, these molecular features have made it difficult to discover VPAC2-selective small molecule drugs. Under these circumstances, an artificial 16-mer cyclic peptide VIpep-3, Ac-c(CPPYLPRRLC)TLLLRS-OH, which antagonizes human and rodent VPAC2 signaling pathways in vitro has been reported. In this study, we performed amino acid substitutions and structural optimization of VIpep-3 and obtained its derivative, named KS-133. It was highly stable in rat plasma for at least up to 24 h and showed a VPAC2-selective and potent antagonistic activity. KS-133 had $IC_{50}$ value of 24.8 nM against VPAC2, which was stronger antagonistic activity than parental VIpep-3 (40.6 nM), even though the molecular weight was reduced from VIpep-3 (1941.1 g/mol) to KS-133 (1558.8 g/mol). KS-133 did not antagonize VIP-VPAC1 and PACAP-PAC1 signaling pathways up to 5 $\mu$M. Subcutaneous and intranasal administration of KS-133 suppressed phosphorylation of CREB, a biomarker downstream of the VPAC2 receptor, in the prefrontal cortex of neonatal and adult mice, respectively. We also found that repeated administration of Ro 25-1553, a selective VPAC2 receptor agonist, during postnatal days 1-14 in mice caused cognitive impairment in adulthood and simultaneous treatment with KS-133 prevented this effect. The same postnatally restricted Ro 25-1553 treatment reduced the total branch number and length of apical and basal dendrites of the prefrontal cortex neurons in mice. These morphological abnormalities were counteracted by concomitant administration of KS-133 with Ro 25-1553.

In conclusion, we successfully generated a BAC Tg mouse model of *VIPR2* CNV and developed a novel peptide KS-133 with a potent and selective VPAC2 receptor antagonist activity that counteracts cognitive decline in a mouse model of psychiatric disorders. Our studies would provide a useful mouse model for uncovering the causative pathogenic role of *VIPR2* CNV on cognitive circuits and other behavioral manifestations in schizophrenia. KS-133 might contribute to both the development of a novel drug candidate for the treatment of psychiatric disorders such as schizophrenia and the acceleration of fundamental studies on the VPAC2 receptor.

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# 論文審査の結果の要旨及び担当者

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## 論文審査の結果の要旨

統合失調症は、世界の人口の約1%が罹患している慢性的な脳機能障害であり、患者の一親等親族の発症リスクは約10%になります。統合失調症は主に、幻覚や妄想などの陽性症状、意欲の低下などの陰性症状、および認知機能障害を特徴としています。しかし統合失調症の発症や治療・寛解機構の詳細は依然不明です。そこでこれまでの臨床研究で明らかになった神経ペプチド受容体VPAC2（遺伝子名VIPR2)のコピー数増加と統合失調症との関連に着目し、病態メカニズム解明のための新しいモデルマウスとして、VIPR2コピー数多型マウスを作製しました。また、精神疾患モデルマウスの認知機能低下を抑制する強力かつ選択的なVPAC2受容体阻害活性を持つ新規二環状ペプチドKS-133を開発しました。

ヒトの病態に近い形での内因性VPAC2受容体が発現増加しているマウスを作製するに当たり、VIPR2遺伝子の全長を含むゲノムDNA断片を大腸菌人工染色体(BAC, Bacterial Artificial Chromosome)に導入したBACクローンを作製し、それをマウス受精卵前核にインジェクションする事で、VIPR2 BAC Tgマウスを得ました。まず、得られたVIPR2 BAC Tgマウスは、マウスVIPR2遺伝子が3コピー導入されていることを明らかにしました。このVIPR2 BAC Tgマウスでの VPAC2受容体mRNAの発現は、野生型同腹仔と比較して、前頭前野、大脳皮質、および海馬において有意に増加していました。一方で、VIPR2 BAC Tgマウスの前頭前野では、VPAC2受容体と内因性リガンド(VIPおよびPACAP)を共有するPAC1受容体、VPAC1受容体の発現が野生型マウスよりも有意に低下していました。しかし、既存の統合失調症治療薬の標的となっているドパミンD2受容体の発現には変化がありませんでした。またVIPR2 BAC Tgマウスは、自発運動量の増加、聴覚性驚愕反応におけるプレパルス抑制の低下、社会的嗜好性の低下、および認知機能障害を示しました。これらの発見は、VPAC2受容体の発現増加に因る過剰なシグナル伝達が、認知や社会的行動の形成に関与する神経回路に影響を与えることを示唆しており、統合失調症におけるVPAC2受容体の病態的役割を示す新しい知見と評価されます。

次に、VPAC2受容体が統合失調症を治療するための有望な創薬ターゲットになると考え、VPAC2受容体選択的アンタゴニストペプチドの開発を試みました。VPAC2受容体は、セクレチン受容体ファミリーが属するクラスB GPCRに分

類されており、またVIPやPACAPは、他の受容体サブタイプであるVPAC1、PAC1受容体にも結合するため、立体構造の類似性などの問題から、VPAC2受容体選択的な低分子化合物の開発は難航していました。その様な中、近年既知のVPAC2受容体アンタゴニスト活性を有するペプチドVIpep-3の配列について、VPAC2受容体の細胞外ドメインの構造、VIPのC末端構造、そしてVIPとVPAC1受容体の複合体構造モデルなどの情報を用いて最適化し、新規のVPAC2受容体アンタゴニストペプチドを見いだしました。候補ペプチドの各種VIP/PACAP受容体に対する阻害活性とVPAC2受容体選択性を評価し、またその中枢作用について検討した結果、VIpep-3 (MW＝1941.1, IC50＝40.6 nM)と比較して、新規に創製した人工ペプチドの一つであるKS-133は、分子量が小さく(MW＝1558.8)、かつ、より高いVPAC2受容体阻害活性を示しました(IC50＝24.8 nM)。一方VPAC1、PAC1受容体に対しては、そのIC50値は5 μM以上でした。KS-133は血漿中において高い安定性を示し、新生仔マウスでは、皮下投与においてVPAC2受容体アゴニストによる大脳皮質でのリン酸化CREBの増加を抑制し、さらに成熟マウスでは、経鼻投与において同リン酸化CREBの増加を抑制しました。このような条件下、KS-133がVPAC2受容体活性化によるマウス大脳皮質での錐体細胞樹状突起の未成熟と認知機能障害の発症を抑制することを明らかにしました。

以上、本研究は、統合失調症のモデルマウスとしてのVIPR2 BAC Tgマウスを作製し、その行動学的解析を通じて統合失調症におけるVPAC2受容体の病態生理的役割の一端を明らかにする事に成功しました。またKS-133がVPAC2受容体に対して高い選択性と阻害活性を示し、in vivoにおいても薬効を発揮することから、新しい統合失調症治療薬候補物質になる可能性を示しました。以上の成果は、博士（薬科学）の学位論文に値するものと評価します。