production [57]; thus we need to study whether Shh regulates *pax6* gene expression in the developing retina in the near future [58, 59].

Expression of region-specific transcription factors clarified the properties of cloned *pax6*-transfected cells. The cells expressed diencephalon-associated markers, including otx2, six3, emxl and emx2 (fig. 2A), consistent with authentic RGCs.

We found that cloned *pax6*-transfected cells exhibited a rapid increase in intracellular Ca^{2+} in response to KCl stimulation, and that the intracellular Ca^{2+} increase was mainly due to calcium influx via Ca^{2+} channels and marginally due to the release of Ca^{2+} from intracellular stores in cloned *pax6*-transfected cells. We previously reported a similar intracellular Ca^{2+} increase in neurons induced from mouse ESCs [22]. We found that cloned *pax6*-transfected cells expressed NMDA receptors by RT-PCR (fig. 5A). Detailed functional study of glutamate and NMDA receptors are ongoing in our laboratory using several cloned *pax6*-transfected cell lines.

Electron microscopic examination disclosed the appearance of neurotubules and neurofilament in cloned *pax6*-transfected cells in vivo, suggesting their neuronal differentiation. Using cell clones, we are planning to study the differentiation requirements of retinal progenitors/RGC precursors to fully mature RGCs.

Taken together, we report here that cloned *pax6*-transfected cells include cells of retinal neuron progenitors, including RGC-like cells.

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Original Article

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Generation of Spinal Motoneurons from Mouse Induced Pluripotent Stem Cells

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Abstract

Severe spinal cord injury and diseases involving motoneuron loss cause miserable outcomes in clinical settings, and no therapy can reverse the patients' disabilities in the final stages of the disease. Cell replacement therapy, however, is a possible approach for treating irreversible damage and loss of spinal motoneurons. Embryonic stem cells have been considered for use as graft cells and analyzed for efficient cell transplantation. Recently, induced pluripotent stem (iPS) cells have been considered a viable alternative source because they provide easily accessible grafts without posing a risk of immunological rejection. Further more, the use of these cells does not involve ethical problems. In this study, we established methods to generate spinal motoneurons from mouse iPS cells and assessed their neural function *in vitro*. Quantitative PCR analysis revealed that sonic hedgehog (Shh) plus retinoic acid treatments doubled the level of HB9 mRNA expression as compared to no treatment. The treatment efficiently induced Tuj- and HB9-double-positive spinal motoneurons, and deletion of Shh dramatically reduced HB9-positive neurons. To increase spinal motoneuron induction, we also analyzed the effects of bone morphologic protein (BMP) as an induction pathway different from Shh signaling. We found that BMP-4 inhibited HB9-positive neuron induction. Therefore, additional treatment involving the blockade of BMP by using a recombinant receptor protein resulted in three times higher number of HB9-positive neurons. The generated neurons showed a neuron-specific Ca^{2+} influx upon KCL stimulation, similar to electrically active neurons *in vitro*. Thus, we successfully generated HB9-positive spinal motoneurons from iPS cells, which could be a potential source for cell replacement.

Key words

Induced pluripotent stem (iPS) cell, Spinal motoneuron, Retinoic acid (RA), Sonic hedgehog (Shh), Bone morphologic protein (BMP)

Introduction

Motoneurons represent a specialized class of neurons that are essential for the control of body movement. Motoneuron loss is the cause of a wide range of neurological disorders, including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy[1], and severe spinal cord injury[2]. Although several therapies for these diseases are available, there is a lack of effective therapies that can induce the functioning of motoneurons in a clinical setting, leading to severe disability for the patients. Although pulse steroids are recognized as an evidence-based therapy in the acute injury phase, an efficient

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therapy in the chronic phase is required for severe injuries to avoid a negative outcome. Cell replacement therapy is well-known as a potential therapy in clinical trials for Parkinson's disease, which is caused by a loss of dopamine-producing neurons[3]. Because of their reliability as a source for grafts, embryonic stem (ES) cells were expected to be an efficient donor source, even though there exists ethical problems pertaining to the use of eggs[4]. Stem cell transplantation is also an attractive strategy for motoneuron loss disease, and early successes in animal models of diseases have generated meaningful restorations of motor function[5]. Recently, induced pluripotent stem (iPS) cells have been considered an alternative donor source because of their easier accessibility and freedom from ethical issues. Dimos *et al.* reported successful generation of patient-specific iPS cells from ALS patients[6]. Here, we demonstrate the successful generation of electrically active motoneurons from iPS cells and the feasibility of the motoneurons as graft cells in cell replacement therapy.

Materials and Methods

iPS cell culture

We obtained mouse iPS cells from the RIKEN cell bank[7]. iPS cells were maintained on a 0.1% gelatin-coated dish in DMEM supplemented with 10% fetal calf serum (FCS), 0.1 mM non-essential amino acids (NEAA) (Invitrogen, Carlsbad, CA), 0.1 mM 2-mercaptoethanol (Sigma, Tokyo, Japan), 100 units/ml penicillin (Invitrogen), 100 μg/ml streptomycin (Invitrogen), and 1000 units/ml LIF (Chemicon, Temecula, CA) (standard medium). To the standard medium without LIF (differentiation medium) for neural induction, we added 10% knockout serum replacement (KSR) instead of FCS. This was because FCS is a well-known strong inhibitor of neural induction from ES cells[8]. To maintain the undifferentiated status, nanog-GFP expressions were monitored.

Induction of spinal motoneurons

To initiate differentiation, we used a modified embryoid body formation method described previously[9]. Briefly, iPS cells were detached and dissociated into single cells by using 0.05% trypsin-EDTA. Next, single cells at a dose of 2×10^5 cells /ml were seeded on a Petri dish in 10 ml of the standard medium without LIF (*Stage I*, **Fig. 1A**). On day 4, cell aggregates (CAs) were transferred to a differentiation medium supplemented with 10 nM sonic hedgehog (Shh, R&D Systems, Minneapolis, MN) and 1 μM all-trans retinoic acid (RA, Sigma) for another 6 days (*Stage II*)[10]. To promote further maturation, CAs, which were partially dissociated by trypsin-EDTA, were plated on gelatin-coated dishes with factors for an additional 10 days (*Stage III*). During the evaluation of the effects of bone morphologic protein (BMP) on spinal motoneuron induction, either 5 nM BMP-4 (R&D Systems) or 150 ng/ml BMP receptor 1A recombinant protein (BMPR; R&D Systems) was added to the differentiation medium supplemented with RA and Shh in *Stages II* to *III*.

Immunocytochemistry

Colonies at the end of the culture (*Stage III*, Day 20) were fixed with 4% paraformaldehyde for 30 min, rinsed three times with PBS[11], and then incubated with 10% goat serum in PBS with 0.1% Triton X-100 for 60 min. Primary antibodies (1: 200, rabbit anti-HB9; Hybridoma Bank, IA, and 1: 400, mouse anti-Tuj; Covance, Princeton, NJ) were incubated overnight at 4°C followed by 3 h incubation with Alexa488 or 594-conjugated secondary antibodies (1:800, goat anti-rabbit IgG and goat anti-mouse IgG; Invitrogen) for visualization. A confocal microscope (Zeiss, Jena-gottingen, Germany) was used to confirm nuclei and cell body stainings.

PCR analyses

Colonies at the end of culture (*Stage III*, Day 20) were treated with trypsin-EDTA, and then total RNA was extracted from the collected cells using Trizol (Invitrogen). RNA samples, in the amount of 2 μg, were reverse transcribed by using random primers (SuperScript III First-Strand kit; Invitrogen). The resultant cDNA was amplified by PCR (GeneAmp PCR System 9700; Perkin Elmer, Norwalk, CT). For amplification of cDNA encoding beta-actin, Tuj, and Islet1, the forward (F) and reverse (R) primer sequences listed in **Table 1** were designed for the unique sequences of the target mRNA by using the Primer3 program (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

For quantitative PCR, TaqMan gene expression assays for HB 9 (cat # Mm 00658300_g 1, Mm 01222622_m1) were purchased from Applied Biosystems (Foster City, CA), and mRNA levels were

Table 1. Primer Sequences

	Forward	Reverse	Annealing Temp	PCR Product (bp)
β - actin	gatgacgatatcgctgcgctg	gtacgaccagaggcatacagg	59	440
Tuj	tagtggagaacacagacgaga	ctgctgttcttactctggatg	59	442
Islet1	atgggagacatgggcgat	tgcctagcggagatgggt	63	500

quantified using the 7300 Real-Time PCR system (Applied Biosystems). The concentration of HB9 mRNA in RA + Shh-treated cells was calculated by subtracting the Ct values of untreated cells from those of the treated cells, and the relative concentration was determined ($2^{-\Delta\Delta Ct}$ method).

Calcium imaging

Intracellular-free calcium levels were evaluated using a confocal microscope as described[12]. Briefly, the RA, Shh and a BMP antagonist treated cells (migrated outside the colonies at the end of *Stage III*) were loaded with 1 μM of fluo-3-acetoxymethyl (AM) ester (Dojindo Lab, Kumamoto, Japan) for 30 min at 37°C. Endogenous esterase converts the non-fluorescent fluo-3-AM into fluorescent fluo-3. After washing, the cells were kept in a calcium mobilization buffer containing 130 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 5.5 mM d-glucose, and 10 mM HEPES (pH 7.3) at room temperature until measurement was performed. Fluo-3 dye was excited with an argon laser at 488 nm, and fluorescence emission at $\lambda > 510$ nm from a single cell with neuronal morphologies was captured every 5 s. The duration of monitoring ranged between 0.8 and 2 s. Differential interference contrast images were also recorded. To see the voltage-gated calcium influx, 100 mM KCl was added after recording the base emission intensity.

Statistical Analysis

A student's *t* test was conducted to analyze the obtained data, which is expressed as means ± S.E.M.

In all statistical analyses, the level of significance was set at $P<0.05$.

Results

Induction of motoneurons from mouse iPS cells using floating cell aggregation method

We used mouse iPS cells generated from fibroblasts that had a nanog-GFP reporter gene (a gift from Shinya Yamanaka). To initiate differentiation using CA or embryoid body-like method, single iPS cells were cultured on petri dishes containing a medium without LIF (**Fig. 1A**, Day 0, *Stage I*). After four days, CAs were cultured with the differentiation medium supplemented either with or without factors such as RA and Shh for six days (*Stage II*). Disappearing GFP expressions in the CAs represent the differentiation status of the cells (**Figs. 1B, C,** and **D**; undifferentiated CAs). During this period, nanog-GFP expressions disappeared gradually. The GFP expressions decreased in approximately 70% of the CAs supplemented with the factors (**Fig. 1E**). Thereafter, partially dissociated CAs were placed onto adherent dishes containing the factors for 10 days in order to promote further maturation (*Stage III*). At the end of the culture, GFP expressions of the attached colonies were almost completely eliminated (**Fig. 1E**; RA + Shh treatment: 7.1% ± 0.6%). There were no significant differences in the expressions between groups with or without factors (no treatment: 22.2% ± 2.2 %).

Induction of spinal motoneurons using RA and Shh

To initiate spinal motoneuron differentiation from undifferentiated iPS cells in *Stages II to III*, we tested two treatment groups: RA (1 μM) and RA plus Shh (10 nM). We compared the groups to each other and to a group with no treatment because of the spontaneous differentiation of cells, including neurons, by using a previously described CA method[7]. For evaluation of neural induction and spinal motoneuron differentiation, dual labeling for Tuj, a neuron-specific marker, and HB9, a spinal motoneuron-specific marker, was performed for the treated colonies. Partially dissociated colonies treated with or without the factors were attached to the culture dishes, and cells had later migrated outside the colonies (**Fig. 2B**). The no treatment and RA treatment groups failed to induce HB9-positive cells in and around the colonies, while many Tuj-positive cells were found. We only found HB9 expression in the Tuj-positive colonies treated with

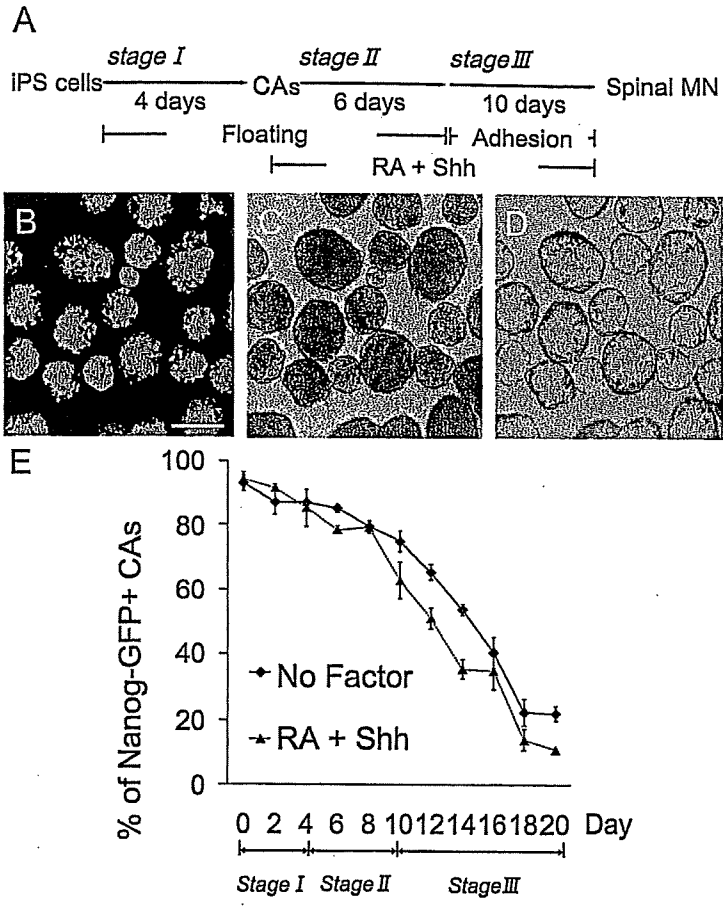

Figure 1. Expression of GFP driven by the nanog promoter in iPS cell aggregates.

(A) Protocol for spinal motoneuron differentiation from mouse iPS cells. *Stage I*: CA formation, *Stage II*: treatment with various factors under the floating condition, and *Stage III*: culture adherent on the plate after treatment with factors.

To understand the undifferentiated states of mouse iPS cells during differentiation, we monitored nanog-GFP expressions in CAs. Representative images of nanog-GFP expression in the CAs at the end of *Stage II* the 6-day culture (B), bright field (C), and merged (D). There is no significance between the treatment (RA plus Shh) group and the no treatment group (E). The 20-day culture, including the adherent step, was enough to obtain many differentiated CAs (>90%). The bar indicates 50 μm. The data consisted of four samples for each group.

RA plus Shh. The treatment-generated colonies had numerous Tuj- and HB9-double-positive cells in and around the colonies (Figs. *2A*, *B*, and **C**) at the end of the culture (**Fig. 2D**; 14.6% ± 4.6%, *Stage III*, Day 20). It was thus possible to generate spinal motoneurons from mouse iPS cells by using RA plus Shh treatment.

Analysis of mRNA expressions of the generated spinal motoneurons

We analyzed the mRNA expressions of the generated cells by RT-PCR. Islet-1 mRNA is expressed in cranial and spinal motoneurons and insulin-producing cells[13], while HB9 is a specific marker of spinal motoneurons[14]. Therefore, the Tuj- and HB9-double-positive cells are likely to be

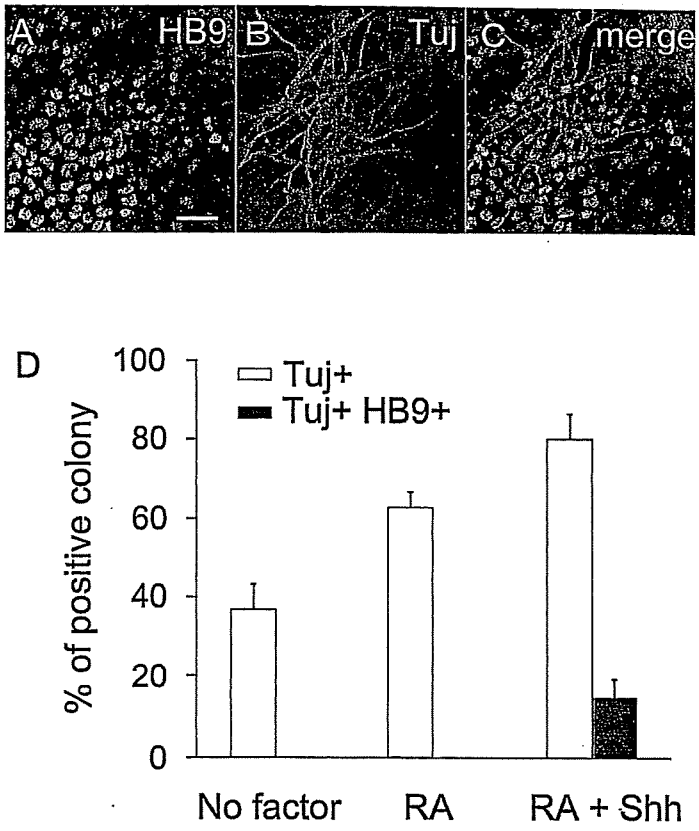

Figure 2. Dual labeling of the treated colonies by using HB9 and Tuj.

Representative images of the spinal motoneuron marker HB9 (**A**), the neuronal marker Tuj (**B**), and merged image (**C**) of the RA- plus Shh-treated colonies. (**D**) The number of double-positive colonies indicated the proportion of spinal motoneurons that were differentiated from iPS cells. The bar indicates 50 μm. The data consisted of four wells of four independent experiments.

categorized as spinal motoneurons in ES cell culture and differentiation[15]. Cells subjected to RA plus Shh treatment showed a spinal motoneuron phenotype that was positive for Tuj, Islet1, and HB9 (**Fig. 3A**). More importantly, HB9 expression was only found in the treated cells. In contrast, Islet1 expression was also seen in the RA alone-treated cells, indicating the differentiation of nonspecific motoneurons and/or insulin-producing cells. To evaluate the efficacy of RA plus Shh treatment in specific induction of spinal motoneurons, the expression level of HB9 was compared between RA plus Shh treatment group and other treatment group by using quantitative PCR (**Fig. 3B**). HB9 expression in the RA plus Shh treatment group was twice the level in the no treatment group, which is for a basal level of the expression because of spontaneous differentiation, as determined using the CA method. Therefore, the RA plus Shh treatment played a critical role in the induction of spinal motoneurons from mouse iPS cells.

Antagonism of BMP signals by BMPR enhanced spinal motoneuron induction

To further increase HB9-positive motoneurons from iPS cells, we analyzed another signaling pathway. It has already been reported that BMP-4 inhibits neuronal and motoneuron differentiation from mouse ES cells[8]. A lack of antagonized signals from the BMPs also inhibit motoneuron generation in the embryo[16]. Therefore, we focused on the effect of BMP antagonists by adding a BMP

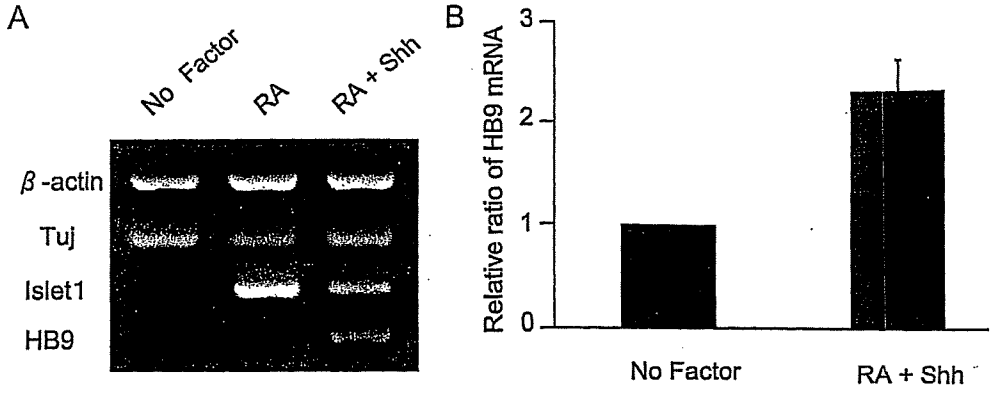

Figure 3. Expressions of motoneuron-related transcripts in cell aggregates.
(A) Representative PCR images for internal control (ß-actin), Tuj, Islet1, and HB9. (B) Quantitative PCR of the HB9 transcript was performed, because HB9 is a specific spinal motoneuron marker. The data consisted of two samples of two independent experiments.

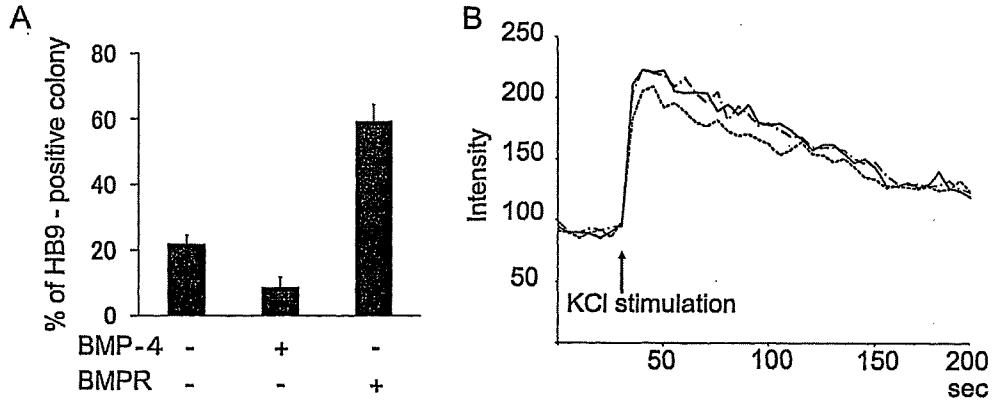

Figure 4. Antagonism of the BMP pathway for further induction of spinal motoneurons.
(A) Counting HB9-positive colonies in the RA- and Shh-treated colonies either with or without a recombinant BMP-4 or BMP receptor 1A (BMPR). The data consisted of four samples of two independent experiments. (B) Representative three separate experiments of Ca influx imaging of the RA + Shh + BMP antagonist-treated cells upon KCl-induced depolarization by a confocal microscope. The increasing green fluorescent intensity of the Ca2+ indicator dye fluo-3-AM demonstrated electro-physiologically active neurons.

receptor 1A protein ⌜BMPR⌟ in the RA plus Shh treatment in order to study an alternative induction pathway. Recombinant soluble BMPR attaches to the BMPs, including BMP-4, and acts as an antagonist *in vitro*[17]. BMP-4 treatment of the colonies treated with RA and Shh (**Fig. 4A**) dramatically blocked HB9 expression (from 21.6% ± 2.7% in the RA plus Shh-treated colonies to 8.8% ± 3.1% in the BMP-4 treated colonies; $p<0.05$), even though a strong promoting factor, Shh[18], was added to the culture. More importantly, about 60% of the colonies showed dual Tuj expression and HB9 expressions when the cells were cultured with RA plu Shh and BMPR (**Fig. 4A**; BMPR, 59.3% ± 5.1% RA plus Shh, 21.6% ± 2.7%; $p<0.05$). Thus, BMP antagonist, BMPR was efficient in causin further induction of spinal motoneurons fron mouse iPS cells.

Functional properties of the spinal motoneurons i vitro

To understand the functional property of th generated neurons, electro-physiological analysi using Ca influx imaging was performed for th

BMP antagonist-treated neurons at the end of the culture (*Stage III*). Levels of fluo-3 (green fluorescent) expression before and after depolarization by KCl stimulation are sensitive to Ca influx in functional neurons[19]. The up-regulated intensity of fluo-3 expression after depolarization (**Fig. 4B**) confirmed the presence of electrically active neurons *in vitro*.

Discussion

RA and Shh are well-known factors that induce the generation of motoneurons *in vitro* and *in vivo*[20]. The RA plus Shh treatment of ES cells is thought to be a standard method for inducing spinal motoneuron differentiation[21]. In this study, we applied this treatment to iPS cells and generated HB 9-positive spinal motoneurons, as confirmed by quantitative PCR and immunostaining. Furthermore, we established a novel method that involved the use of RA, Shh, and a BMP antagonist to induce numerous neurons that had a specific spinal motoneuron phenotype. The generated neurons were also electrically active, as confirmed by Ca imaging.

HB9 encodes for a transcription factor specifically expressed by mature motor neurons[10], and Karumbayaram *et al.* also showed that HB9 expression along with Tuj expression is strongly related to the spinal motoneuron phenotypes in human iPS cells[22]. In our preliminary data, most of the HB9 -positive neurons that differentiated from iPS cells had other spinal motoneuron phenotypes, such as Islet1 and ChAT, as confirmed by immunostaining. The generated motoneurons survived and maintained the HB9 phenotype after transplantation into an injured spinal cord model (unpublished data, under preparation for other journal). Therefore, we could efficiently generate spinal motoneurons from mouse iPS cells using our novel method, and the motoneurons were usable for cell replacement therapy.

Neurulation is a part of organogenesis in vertebrate embryos. The process begins when the notochord induces the formation of the central nervous system by signaling the ectoderm germ layer to form the neural plate. The neural plate folds in upon itself to form the neural tube, which later differentiates into the spinal cord and the brain. RA is a posteriorization factor[23], and Shh is a ventralization factor in the neurulation[24]. Shh is also known as an inducer of dopamine neurons in the ventral midbrain[25]. To further increase the generation of spinal motoneurons from iPS cells, we added a BMP antagonist to the culture that was treated with RA plus Shh. Because BMP is a well-known dorsalization factor of neural tubes, we hypothesized that the antagonist acts against the dorsalization signals produced by BMPs in our culture system, and finally induces a ventral neural tube phenotype, which includes spinal motoneurons. From our results, we inferred that antagonized BMP signals are synergistic with Shh signals with regard to spinal motoneuron induction without causing neuronal inhibition. The results also supported the notion that initiation of ventral spinal motoneuron differentiation from the neural tube by antagonized signals of BMPs[26] is also essential for the differentiation of neurons from iPS cells.

To understand the function of the generated neurons, we performed Ca influx imaging using live cells. These cells have typical neuronal morphologies, such as long dendrites and bipolar cells. We wanted to prevent cell death before the imaging; therefore, we did not choose spinal motoneurons because they cannot be stained with the motoneuron marker HB9 and/or the neuronal marker Tuj, without any fixation and permeabilization. We attempted to stain the monitored neurons with HB 9 and Tuj dual staining after obtaining images, but the shrunken neurons were not viable and hence further analyses could not be performed. Therefore, we chose the typical single neurons around the individual colonies, which had a neural rosette-like structure before the images were taken, because the colonies enriched with HB9- and Tuj-positive neuron had the same structure in other analyses. Thus, we showed that the generated neurons are electrically active and are likely to be spinal motoneurons.

To prevent overestimation and/or underestimation, we counted the HB9/Tuj-positive colonies but not the positive cells, because the colonies included many positive cells in the 3D structure. Sasai *et al* had also applied this counting system for their ES cell culture and differentiation[27]. We and other research groups could not exclude the existence of unnecessary and unexpected cells in the 3D colonies. Therefore, we need to establish a method to purify spinal motoneurons from other cells in and around the colonies for their use in cell replacement therapies in the near future.

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マウス誘導多能性幹細胞からの脊髄運動神経の分化誘導

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抄 録

脊髄変性疾患や外傷による脊髄損傷において，失われた機能を回復する有効な治療法は現在皆無であり，神経系細胞移植など新規治療法の開発が期待されている。なかでも倫理的に問題なく拒絶反応がないiPS細胞を用いた治療が注目されている。今回我々はマウスのiPS細胞から脊髄運動神経への効果的な分化誘導法について検討した。

【方法】Nanog-iPS 細胞から脊髄運動神経（sMN）を分化誘導するために Retinoic acid (RA), Sonic Hedgehog (Shh) を用いた。PCR法で神経系およびsMNマーカーであるIslet-1, Tuj, HB9のmRNA発現を確認し，蛍光二重染色によりsMNに特異的であるHB9陽性Tuj陽性細胞比率を従来のRAとShhのみを用いた分化誘導法と比較した。脊髄運動神経への分化を抑制するBMPを分化誘導時に添加することにより，BMPのantagonistであるBMPRの有用性を評価した。【結果】Nanog-GFP持続発現は分化誘導後10日目まで認めた。RT-PCR法では上記神経系およびsMNマーカーの発現を確認した。免疫染色によるHB9とTuj二重陽性のsMN数は，RA, Shhに BMPRを加することにより，約20%から約60%へと増加した。【結論】RA, Shhに BMPRを付加することで高効率にsMNを分化誘導することができた。脊髄運動神経への分化誘導にはRA, Shhが必須であること，BMPを阻害することが重要であることが示唆された。

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老年期うつ病と認知症の共存と連続性

山口　登

要旨：老年期うつ病の発症には，心理・社会的要因（病苦・経済苦，離別による孤独感などのストレス）と生物学的要因（脳・身体要因）が関与する．老年期においては，生物学的要因としての脳血管病変やその危険因子はうつ病発症に大きく関与し，うつ病と認知症が併存する症例や連続性のスペクトラムとして考えられる症例がみられる．したがって，診断学的には一時点における横断的観察ばかりでなく，縦断的な経過観察が重要となる．血管性病変の危険因子と考えられる基礎疾患（高脂血症，高血圧，糖尿病）がAlzheimer病（AD）併存やその進行とも関連し，その治療薬（スタチン類や降圧薬など）がADの予防や症状悪化の抑制に効果が期待できるという報告もある．したがって，生活習慣の是正，運動や身体機能の維持等により生活習慣病の治療や予防が老年期うつ病や認知症の予防や症状進行抑制に重要である．

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Key words：うつ病，認知症，共存，連続性，老人病学
depression, dementia, coexistence, continuity, geriatrics

1．うつ病の原因

うつ病の原因は不明でその特定は困難であるが，現状では内因（個人の素質に基づく要因）と誘因（ストレスなど）の複合で引き起こされると考えられている．現在使われている内因の概念とは，「疾患に罹患しやすい個人の素質に基づくもの」であり，先天的（遺伝的＋胎生的）素質に後天的（生育的）影響が積み重なったものと考えられる．内因の詳細は非常に不明確だが，今後の分子生物学的精神医学研究の進展（疾患脆弱性遺伝子の発見など）とともに明確となれば，内因の概念が変わる可能性がある．内因が強力な場合には明確な誘因がなくても発症し，内因が弱い場合には強力な精神的・身体的負荷（ストレス）が誘因として加わりはじめて発症するものと考えられる．したがって，現実には同じストレスが加わっても障害が生じる人もいれば生じない人もいる．すなわち，内因としてのvulnerability（脆弱性）とresilience（罹患抵抗力，回復力）が問題となる．基本的には，発症しやすい個人の内因と発症を促す誘因との複合で発症すると考えられる．

近年，Magnetic Resonance Imaging（MRI）の導入により，うつ病患者の中には脳虚血性病変を合併する患者の存在が明らかとなり，1997年Krishnanら[9]ならびにAlexopoulosら[1]はVascular Depression（VD）の概念を提唱した．これは，脳器質性変化の関与すなわち脳血管障害あるいは脳血管障害危険因子が臨床上あるいは検査上認められるものを言う．VDは，脳血管障害の発作後にうつ病を発症したPost-stroke depressionと，うつ病と診断された患者にMRI画像上脳梗塞（無症

Coexistence and continuity of geriatric depression and dementia
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候性）が検出されるMRI-defined VDの二つに分類される．Coffeyら[3]によれば，老年期うつ病患者では大脳基底核と視床のMRI-T2強調画像での高信号が高率に認められ，神経症状を伴う患者では75%に，神経症状を伴わない患者でも40%にうつ症状が出現する．Chenら[2]によれば，抑うつ症状の発現は，Alzheimer病（AD）の初期に高率（17.6%）に見られ，AD発症に引き続き抑うつ症状が出現する可能性は，非認知症者に比べ，補正後のオッズ比で6.5であった．Lyketsosら[10]によれば，ADと大うつ病の共存率は22%，小うつ病との共存率は27%とであり，両者併せると約半数のAD患者の経過中に何らかのうつ状態が共存する．ADの前駆症状としての抑うつ状態の発現率も高い．非認知症（認知症の前段階）である軽度認知機能障害（mild cognitive impairment：MCI)においてうつ状態の発現率45%という報告もある[15]．Devanandら[4]は，60歳以上者の5年間の縦断的調査から，抑うつ気分はAD進展のリスクファクター(相対危険率2.94)であると報告している．このようにADの経過中，特に初期においてうつ症状を伴うこと，またADの前駆症状としてうつ状態が発現することがある．したがって，中高年期のうつ病発症には，脳血管性病変や初期AD等の変性疾患などの脳器質性変化が関与している可能性がある．

また，代謝性疾患(糖尿病，高脂血症など)，循環器性疾患（虚血性心疾患，高血圧など）が抑うつ状態を合併しやすく，更にその身体疾患の転帰にうつ状態が悪影響を与えることがある[5]．これら身体要因はresilienceの低下に大きく関与することが推測される．

うつ病発症に関する要因をまとめると，次の事項が挙げられる．

a. 病前性格

(1) メランコリー親和型性格（Tellenbach)：勤勉・誠実，秩序性，良心性，他者配慮性など

(2) 執着気質（下田光造)：仕事熱心，凝り性，徹底的，正直，几帳面など

(3) 循環気質(Kretschmer)：朗らか，親切，激しやすい，苦労性など

b. 社会・心理的要因

(1) ストレス（心理社会的縮小・喪失体験）

①脳・身体機能の衰退，罹患などによる健康感の喪失

②社会経済的問題；引退，社会的役割や経済的自立の喪失など

③人間関係の変化；配偶者の死，友人の喪失，核家族化(子供の自立，別居)，家庭内葛藤・孤立など

(2) 学習性無力感：逃れにくい慢性ストレスの曝露およびそこからの脱出の諦め

(3) 幼少期・若年期心的外傷体験：両親との死別・離別，虐待など

c. 身体要因

(1) 脳器質性変化

①脳血管障害あるいは脳血管障害危険因子

②老化性変化や変性疾患：Alzheimer病，Lewy小体型認知症など

(2) 身体疾患

循環器疾患，糖尿病，悪性新生物など

2. 診断上の問題点（うつ病と認知症)

うつ病の診断基準は，米国精神医学会による精神疾患の分類と診断の手引き第4版（Diagnostic and Statistical Manual of Mental Disorders, 4th edition；DSM-IV）の大うつ病エピソードや，WHOによる国際疾病分類第10版（International Classification of Disease, 10th revision；ICD-10）のうつ病エピソードが広く用いられている．これらは操作的診断基準であり，推定される原因(内因性，反応性，神経症性など)での分類をやめ，臨床症状に注目し，客観性および臨床や研究での診断一致度を高めることに重点を置いている．

老年期を代表する精神神経疾患として，うつ病は脳機能性疾患，認知症は脳器質性疾患として，二分的・対比的に論じられてきた．しかし，現実にはこの両者の鑑別は非常に困難なことがある．前述したように，うつ病から認知症に移行する症例あるいは両者が併存する症例を経験する．うつ病患者は記憶機能の低下および判断力や集中力の低下を訴えることが多く，認知症類似の状態（仮

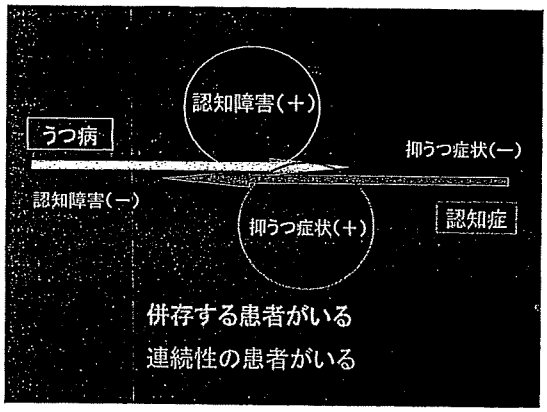

図1　うつ病と認知症のスペクトラム

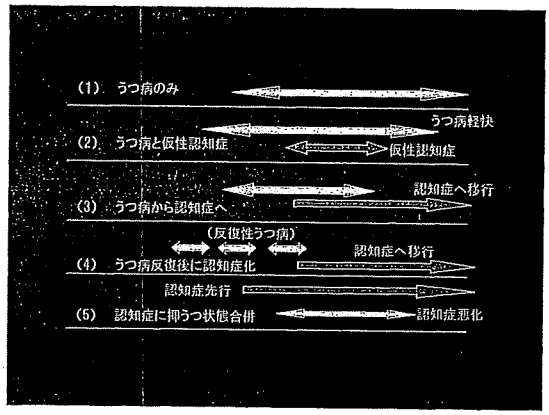

図2　うつ病/認知症の関係の類型

性認知症）を呈することがある．一方，認知症のごく初期では，明らかな知的機能障害がさほど目立たないうちから，興味・関心の喪失や意欲低下など自発性低下が認められることがある．このため，老年期におけるうつ病とごく初期の認知症，特にADとの鑑別は横断的にはしばしば困難となる．また，うつ病に認知機能低下が併存すると，それぞれの症状が相互に影響しあうこともしばしばみられる[12]．日常臨床で，老年期うつ病と認知症が併存する症例や連続性のスペクトラムとして考えられる症例を多数経験する（図1）．縦断的調査では，うつ病性仮性認知症を呈する患者は認知症に移行する可能性が高いという報告が多い．うつ病はADの独立した危険因子であること[14]，およびうつ病既往が老人斑や神経原線維変化の増加と関連し，同既往歴があると認知機能低下がより急激に起こること[15]も報告されている．単極性うつ病よりも双極性感情障害患者で認知症への移行が多いという報告[3]もある．また，うつ病に向精神薬に対する感受性の亢進やパーキンソン症状が加わった場合にはLewy小体型認知症へ移行の可能性が高い．したがって，診断学的には，一時点における横断的観察ばかりでなく，縦断的な経過観察が重要となる．

表1　認知症に合併する抑うつ状態の特徴

①	悲哀感の乏しさ
②	深刻感の欠如
③	病態無関心〜否認
④	促せば行動するが，放置すれば何もしない
⑤	症状の動揺があまりみられない〜遷延化
⑥	抗うつ薬が奏効しにくい〜難治化

文献13）を一部改変

筆者が作成したうつ病/認知症の関係の類型を図示する（図2）．

老年期におけるうつ病と認知症に伴う自発性低下状態との鑑別は横断的には難しいが，認知症患者に見られる抑うつ状態の特徴を表示する（表1）[14]．

3. 総　括

老年期うつ病の発症には，病苦・経済苦そして離別（配偶者との死別など）による孤独感などのストレスが誘因として大きいが，生物学的要因（脳・身体要因）も大きく関与する．脳血管病変やその危険因子はうつ病発症に関与し，さらに，脳血管性病変の危険因子と考えられる基礎疾患（糖尿病，高脂血症，高血圧など）がAD併存とも関連し，その治療薬（スタチン類や降圧薬など）がADの症状悪化の抑制や予防に対する効果が期待できるという報告も存在する[16][17][6]．したがって，ストレス解消，生活習慣（ライフスタイル）の是正，運動や身体機能の維持等により生活習慣病の治療や予防が中高年期のうつ病，血管性病変および

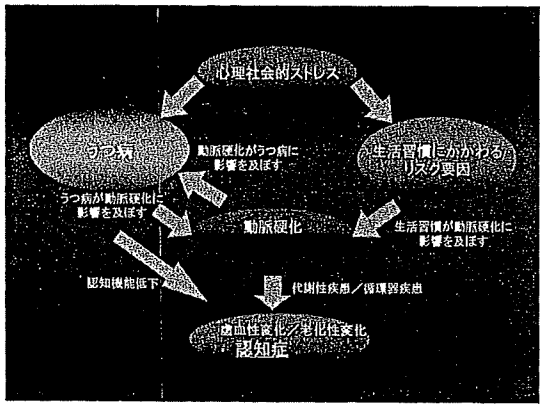

図3 ストレスはうつ病/認知症の発症に関与する

AD予防や認知症の症状進行抑制に重要である(図3).

さらに，中高年のうつ病患者で自殺者が多い現実があるため，自殺防止の観点から，人生各時期における生き甲斐・趣味活動の充実，目標の転換，思考の柔軟性，価値観の多様化を念頭に置くことが重要であろう．そして，各人が価値を見出せる生活，喪失するものがあっても互いに支え合い，補充し合える社会の構築がうつ病および認知症の予防や対応に必要と考える．

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Coexistence and continuity of geriatric depression and dementia

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Biological (brain/physical) factors as well as psycho-social factors (stress such as pain of illness, economic problems and feeling isolated by separation) contribute to the onset of geriatric depression.

Cerebral vascular lesions and their risk factors contribute considerably to the onset of depression, and there are cases in which depression and dementia symptoms coexist and also cases in which they are regarded as a continuous spectrum.

For diagnostics, longitudinal follow-up becomes important, as well as cross-sectional observation at one point. Risk factors of cerebral vascular lesions (hyperlipidemia, hypertension, diabetes mellitus) are associated with coexistence and progression of Alzheimer disease (AD), and curatives for them (statins or antihypertensives) can be expected to suppress the advance of AD symptoms and also to prevent the onset of AD.

Therefore, treatment and prevention of life-style related diseases, by correction of life-style habit, exercise and maintenance of a physical function, may be important in prevention and repression of the progress of symptoms not only for geriatric depression but also for dementia.

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Geriat. Med. 47(11)：1427～1430, 2009

特集 総説 高齢者のうつ病をめぐって

KEY WORD ㊀うつ病 ㊁認知症 ㊂脳器質病変 ㊃身体疾患 ㊄老人病学

2．高齢者のうつ病の症候学と診断学

山口　登

SUMMARY

うつ病患者では，精神症状と身体症状(自律神経系症状が中心)が出現する．特に高齢者のうつ病の症状は多彩であり，不安・心気症状，仮性認知症，妄想，そして昏迷などの緊張病症状および身体機能低下などを呈する．高齢者うつ病の発症には，高齢者に特徴的な社会・心理的要因(心理社会的縮小/喪失体験)とともに，身体要因(脳器質病変・身体疾患)が大きく関連する．特に，脳器質病変(脳血管性病変や脳変性疾患)はうつ状態発症に関与し，うつ病と認知機能の低下が併存する症例や，うつ病から認知症へ連続性のスペクトラムとして考えられる症例などがみられる．したがって，診断学的には一時点における横断的観察ばかりでなく，縦断的な経過観察が重要となる．

症候学的特徴

うつ病では精神症状と身体症状(自律神経系症状が中心)が出現する．精神症状として，抑うつ気分，意欲・興味の喪失，作業能率の低下，不安・取り越し苦労など，身体症状として，頭痛，肩こり，疲労・倦怠感，睡眠障害，胃腸症状(食欲不振・便秘)などがある．特に老年期のうつ病の症状は多彩であり，不安・心気症状，仮性認知症，妄想，そして昏迷などの緊張病症状，さらには身体機能の低下などを呈することがある．薬物治療抵抗性・不耐性のため遷延化することがあり，生きる喜びの消失(無価値感，希死感)につながるおそれがある．高齢者のうつ病の症候論的特徴を表示する(表1)．

また，高齢者のうつ病では身体疾患の併存例が多い．代謝性疾患(糖尿病，脂質異常症など)，循環器性疾患(虚血性心疾患，高血圧など)，がん疾患などが抑うつ状態を合併しやすく，さらにその身体疾患の転帰にうつ状態が悪影響を与えることがある[1,2]．つまり，上記疾患患者でうつ病合併例では，生命的予後が悪化する．これら身体疾患時には，社会心理的要因(病苦・経済苦など)とともに生物学的変化が影響し，うつ病に対するresilienceの低下が引き起こされること，そしてうつ病が併存すると身体疾患とうつ病が相互に重症化(悪循環)することが推測される．

診断上の問題点(うつ病と認知症)

うつ病の診断基準は，米国精神医学会による精神疾患の分類と診断の手引き第4版(Diagnostic and Statistical Manual of Mental Disorders, 4th edition：DSM-Ⅳ)[3]の大うつ病エピソードや，WHOによる国際疾病分類第10版(International Classification of Disease, 10th revision：ICD-10)[4]のうつ病エピソードが広く用いられている．これらは操作的診断基準であり，推定される原因(内因性，外因性，反応性，神経症性など)での分類をやめ，臨床症状に注目し，客観性および臨床や研究での診断一致度を高めることに重点を置いている．もちろん，うつ病の真の原因は不明であるが，高齢者のうつ病の発症には，病苦・経済苦そして離別(配偶者との死別など)による孤独感などの高年期特有の社会心理的要因(ストレス)とともに，生物学的要因(脳・身体要因)も大きく関与する．

老年期を代表する精神神経疾患として，うつ病ならびに認知症が挙げられる．うつ病は脳機

やまぐち　のぼる(聖マリアンナ医科大学神経精神科)

表1　高齢者のうつ病の症状学的特徴

1．心気性(身体症状の訴え：仮面うつ病)
　便秘，排尿困難，体(口腔内など)の違和感，疼痛など

2．不安・焦燥
　激越性と自殺念慮・自殺企図に関連

3．精神運動機能変化
　活動の減少，無言，緊張病症状(昏迷など)

4．体重減少と身体機能低下の合併

5．意識障害(せん妄)の合併
　身体合併症(脱水，低栄養など)
　脳の脆弱性(微小脳梗塞など)

6．仮性認知症
　精神運動抑制(注意・集中障害)

7．妄想(心気，貧困，罪業)
　時に被害妄想，不死/虚無妄想(コタール症候)

8．睡眠障害
　深睡眠の減少と中途覚醒，睡眠・覚醒リズム障害

9．遷延化，難治化

10．再燃化

11．向精神薬の副作用が出現しやすい

能性疾患として，そして一方，特に老年期に好発する認知症は脳器質性疾患として，両者は二分的・対比的に論じられてきた．うつ病患者は，記憶機能の低下および判断力や集中力の低下を訴えることが多く，認知症類似の状態(仮性認知症)を呈することがある．一方，認知症の初期では，明らかな知的機能障害がさほど目立たないうちから，興味・関心の喪失や意欲低下など自発性低下を主体とした状態が認められることがある．しかし，現実にはこの両者の鑑別は非常に困難なことがある．日常臨床で，うつ病から認知症に臨床診断が移行する(連続性のスペクトラムとして考えられる)症例，あるいは両者が併存する症例を経験する．このため，老年期におけるうつ病と初期の認知症(特にアルツハイマー型認知症：ADやレビー小体型認知症：DLB)との鑑別は，一断面的にはしばしば困難となる．

また，うつ病性仮性認知症は回復可能(可逆性)，一方，認知症(脳器質性変化によるもの)の情動・意欲の障害は回復困難(非可逆的)という考えが定説化されている．しかし縦断的調査では，うつ病性仮性認知症を呈する患者は認知症に移行する可能性が高いことが報告されており，この定説が見直されつつある．Devanandら[5]は，60歳以上の5年間の縦断的調査から，抑うつ気分はAD進展のリスクファクター(相対危険率2.94)であり，さらに，うつ病はADの独立した危険因子であること[6]，およびうつ病既往が老人斑や神経原線維変化の増加と関連し，同既往歴があると認知機能低下がより急激に起こること[7]など報告されている．単極性うつ病よりも双極性感情障害患者で認知症への移行が多いという報告[8]もある．したがって，診断学的には，一時点における横断的観察ばかりでなく，縦断的な経過観察が重要となる．また，高齢期において，うつ状態ばかりでなく，幻覚，妄想などの精神病症状や，その治療薬である抗精神病薬に対する感受性の亢進(錐体外路症状出現)，さらに歩行の不安定，転倒などの身体機能変化の合併症例ではDLBの可能性がある．

また，Magnetic Resonance Imaging(MRI)の導入により，うつ病患者の中には脳虚血性病変を合併する患者の存在が明らかとなり，1997年Krishnanら[9]ならびにAlexopoulosら[10]はVascular Depression(VD)の概念を提唱した．これは，脳器質性変化の関与すなわち脳血管障害や脳血管障害危険因子が臨床上あるいは検査上認められるものをいう．VDは，脳血管障害の発作後にうつ病を発症したPost-stroke depressionと，うつ病と診断された患者にMRI画像上脳梗塞(無症候性)が検出されるMRI-defined

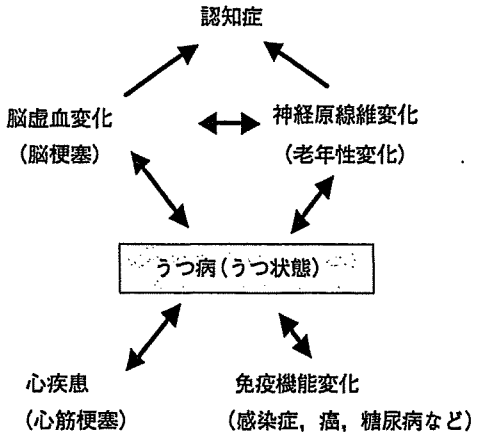

図1　高齢者のうつ病と脳器質性変化・身体疾患の関係

VDの2つに分類される．Coffeyら[11]によれば，老年期うつ病患者では大脳基底核と視床のMRI-T2強調画像での高信号が高率に認められ，神経症状を伴う患者では75%に，神経症状を伴わない患者でも40%にうつ症状が出現する．脳血管病変や脳血管性病変の危険因子と考えられる基礎疾患(糖尿病，脂質異常症，高血圧など)が高年期においてうつ病と関連し，さらには脳血管性認知症ばかりでなく，ADの併存とも関連し，その治療薬(スタチン類や降圧薬など)がADの症状悪化の抑制や予防に対する効果が期待できるという報告も存在する[12-14]．非認知症(認知症の前段階)である軽度認知機能障害(mild cognitive impairment：MCI)においても，うつ状態の発現率45%という報告もある[15]．また，高齢者のうつ病に認知機能低下の併存および心疾患など種々の身体疾患の併存が相互に影響し合うこともしばしばみられる．

高齢者のうつ病と脳器質性変化・身体疾患の関係を図示する(図1)．

このように，高齢者のうつ病は認知症の前駆症状として，そして認知症の経過中にも出現することがある．筆者は，老年期初発のうつ病症例および発症は中年期以前であっても，老年期においてうつ病の臨床像の変化が認められる症例においては，何らかの脳器質病変に基づく認知症疾患の潜在を念頭に置くことが重要であると考える．筆者が考える「認知症発症の可能性のある気分障害」を表示する(表2)．そして，本人ならびに家族にその可能性を説明しておくことは，本人ならびに家族による対応の準備・工夫につながり，長期的にはQOL維持に好ましいものと考える．

表2　高齢者のうつ病
—認知症発症の可能性のある気分障害—

1．65歳以降に初発の気分障害
2．65歳以降に病像変化のある気分障害
　(65歳未満の初発例であっても)
3．うつ病性仮性認知症
4．薬物治療抵抗性うつ病：ECTによってのみ軽快
5．頻回の再燃(再入院)
6．双極性障害：多数回のエピソード(うつ・躁・軽躁)歴

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