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Microarray Analysis of VHL-gene-transferred Neural Stem Cells Around Transcriptional Signaling Pathways of Neuronal Differentiation

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Abstract

Previously, we reported that the von Hippel-Lindau (VHL) tumor suppressor gene induces neural stem cell (NSC) differentiation to neurons, and that VHL's involvement in differentiating NSCs or mesenchymal stem cells (MSCs) into neurons is related to the inhibition of the JAK/STAT pathway through the proteasomal degradation of proteins following their ubiquitination by the VHL/elonginBC/Cul2 complex. However, the relationship between VHL and other pathways and factors around VHL-mediated neuronal differentiation is still unclear. In this study, the VHL gene was transferred to adult rat NSCs, and the gene expression profiles of the VHL-gene-transferred and control NSCs were analyzed using cDNA arrays. Then, the genes of the transcriptional signaling pathways around the VHL gene were examined. In particular, we examined the expression profiles of VHL-downstream genes, neurogenesis genes, hormonal genes, cytokine genes, and ubiquitin/proteasome genes. VHL-transfected NSCs showed increased expression of most neurochemical and neurogenesis genes, hormonal genes, homeobox genes, and cytokine genes. However, they showed decreased expression of VHL-downstream genes (HIF/VEGF/EPO, JAK/STAT/Smad), neurogenesis-associated genes (Wnt, Notch, and GFAP), and ubiquitin/proteasome genes. Meanwhile, the expression of neurotrophic factors was either increased or decreased. cDNA microarray analysis revealed that VHL not only regulates neuronal differentiation and the HIF/VEGF/EPO and JAK/STAT/Smad pathways but also development, hormones, and cytokines. Understanding the transcriptional signaling pathways around VHL that induce neuronal differentiation is very important in neuronal regenerative medicine.

Keywords: cDNA Microarray; VHL; Neural Stem Cells; Neuronal Differentiation; Transcriptional Signaling Pathway

Introduction

Neural stem cells can divide and proliferate. They can be repeatedly cultured for transplants and have the potential to differentiate into any cell type in the nervous system. These cells have been found in both the embryonic and adult brains and can be isolated and used as donors for regenerative therapy transplants for neurological diseases [1, 2]. There are two theories about the origin of neural stem cells: that they are ependymal cells of the lateral ventricles or astrocytes under the ependymis. Neural stem cells eventually differentiate into neurons or glia and can also differentiate into parenchymal brain tumors, such as astrocytic and neurocytic tumors. The transcriptional signaling pathways of neuronal differentiation of neural stem cells have not yet been fully clarified. Neural and mesenchymal stem cells have been used as donor cells in neuronal regenerative medicine. Implanted neural or mesenchymal stem cells can differentiate into neuronal cells in the nervous system. The neuronal differentiation of these stem cells inside the body is related to numerous factors.

Previously, we reported that the von Hippel-Lindau (VHL) tumor suppressor protein induces neural stem cell (NSC) differentiation to neurons without neurotrophic factors, such as basic fibroblast growth factor (bFGF). Conversely, VHL antisense inhibits NSC differentiation [3]. In addition,

we have reported that VHL's involvement in the differentiation of NSCs or mesenchymal stem cells (MSCs) into neurons is related to the inhibition of the JAK/STAT pathway through the proteasomal degradation of proteins following their ubiquitination by the VHL/elonginBC/Cul2 (VBC) complex [4]. STAT3 contributes to the maintenance of the stem cell nature of neural stem cells; however, anti-STAT3 antibody induces the neuronal differentiation of neural stem cells [5]. On the other hand, the VHL-HIF pathway is a key axis of the VHL pathway. At normoxia, the VBC complex degrades HIF. Degradation of HIF leads to cAMP upregulation [6]. followed by neuronal differentiation of neural stem cells. Additionally, the VBC complex degrades β-catenin at normoxia, which leads to the upregulation of Wnt [7]. Here, we analyzed VHL-gene-transferred neural stem cells with a cDNA microarray. Next, we discuss the neuronal differentiation mechanism of neural stem cells.

Materials and Methods

Cell Culture

E12 embryos were obtained from anesthetized pregnant Sprague-Dawley rats (CLEA Japan, Inc., Japan). The forebrain and hindbrain tissues were dissected and digested with 0.05% trypsin and 0.02% ethylenediamine tetra-acetic acid at 37°C for 10 minutes. The tissues were then dissociated by gentle trituration in DMEM with 10% FCS and filtered through a sterile 60-mesh membrane. The cells were plated onto poly-L-ornithine-coated coverslips in DMEM supplemented with 10% FCS at a concentration of 10^6 cells per 35-mm culture dish. After 24 hours, the medium was changed to serum-free DMEM/F12 (1:1; Life Technologies, Inc.) supplemented with glucose (0.6%), glutamine (2 mM), $NaHCO_3$ (3 mM), insulin (25 μg/mL), transferrin (100 μg/mL), progesterone (20 μM), putrescine (60 μM), selenium chloride (30 μM), and HEPES buffer (pH 7.4). EGF and bFGF were added alone or together at concentrations of 10^{-10} and 10^{-9} M, respectively. The serum-free medium was replaced after 24 hours to determine the conditions that promote optimal cell survival and differentiation.

VHL Gene Transfer

VHL cDNA was amplified using the PCR method.

The primers used were as follows:

Forward: 59 - C T G A A T T C A C C A T G G A G G C C G G G C G G C C G - 39

Backward: 59 - G A G A A T T C T C A A T C T C C C A T C C G T T G A T G - 39

A defective herpes simplex virus vector expressing pVHL (dvHSV/VHL) was generated as previously described [8]. The VHL gene was driven by the cytomegalovirus immediate-early promoter. Amino acids 54-213 of pVHL were expressed upon transfection with dvHSV/VHL. VHL gene transfer into rodent progenitor cells was performed using plaque-forming units of dvHSV/VHL on day 1. The control vector was a defective herpes simplex virus vector containing the bacterial LacZ gene, with the same plaque-forming units.

RNA Isolation and Rat cDNA Microarray Analysis

Neural stem cells (NSCs) derived from Sprague-Dawley rats were used for cDNA microarray detection. The cells were rinsed twice with PBS, and total RNA was extracted using the SV Total RNA Isolation System (Promega, Madison, WI), following the manufacturer's instructions. The RNA was treated with DNase I (Promega) to eliminate genomic DNA contamination. RNA sample purification was monitored by formaldehyde/agarose gel electrophoresis. RNA concentration was determined by taking repeated OD measurements of aliquots at a wavelength of 260 nm. cDNA array data were normalized to reference values before they were compared with data obtained from another array. The average expression of all genes was used as the reference value. The sVol of each array spot was calculated by subtracting the background volume value from the radioactive volume value. The nVol was presented as the sVol of each spot divided by the mean sVol of all spots. The ratio value was calculated by dividing the nVol of each spot by the nVol of the corresponding NSC spot. Micrograms of total RNA were used to synthesize [^{32}P]-dATP-radiolabeled cDNA probes for hybridization to rat 1.2 Atlas cDNA Expression Arrays containing 1,176 genes (Clontech, Palo Alto, CA). Probe labeling and hybridization were performed as described in the Atlas cDNA Expression Arrays User's Manual (PT3140-1) from Clontech. Reverse transcriptase transcribed RNA in the presence of 35 Ci [$^{-32}$P]dATP (NEN, Boston, MA) and specific primers for genes represented on the Broad Coverage Atlas Rat cDNA Expression Array. The Atlas Array membranes were processed in parallel for the RNA samples. The membranes were prehybridized at 68°C for 30 minutes in Express Hyb solution (Clontech), which contained 100 μg/ml of denatured salmon testis DNA. Then, the membranes were hybridized overnight at 68°C with radiolabeled cDNA probes (final concentration of radiolabeled cDNA of 0.5–2 × 10^6 cpm/ml). The next day, the arrays were washed three times with prewarmed 2x SSC/1% SDS at 68°C and twice with prewarmed 0.1x SSC/0.5% SDS at 68°C. The array membranes were exposed to an imaging screen for varying durations at room temperature. The hybridization signals were scanned with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and analyzed with ArrayVision 5.1 software. The signal intensity for the reference genes was adjusted so that the mean intensity for each membrane was equal. 0.05 was used as the criterion for determining whether the differential expression of a gene was significant.

Results

Quality of mRNA

The quality of mRNA in both control and VHL-transferred neural stem cells was examined. The 260 nm/280 nm ratios were 1.60 and 1.62 for the control and VHL-transferred neural stem cells (NSCs), respectively. Additionally, the rRNA 28S/18S ratios were 1.8 and 1.8, respectively. Agarose gel electrophoresis revealed no degraded mRNA (Figure 1).

cDNA Microarray Analysis

We analyzed the cDNAs of rat neural stem cells, both VHL-transferred and control vector-transfected, using the Atlas Glass Rat 3.8 cDNA microarray (Clontech). A total of 7,466 cDNAs from 3,733 control and VHL-transferred neural stem cells were analyzed, and their expression levels were compared (Figure 2, 3). Then, genes with low expression levels were excluded. The remaining genes were classified as either 1,044 upregulated or 2,205 downregulated following VHL gene transfection.

Upregulated Genes

According to the ratio of VHL-transferred neural stem cells to control neural stem cells, the 1,044 upregulated genes were distributed as follows: ratio >8.0: 1 gene; ratio 7.0 to 8.0: 7 genes; ratio 6.0 to 7.0: 17 genes; ratio 5.0 to 6.0: 19 genes; ratio 4.0 to 5.0: 44 genes; ratio

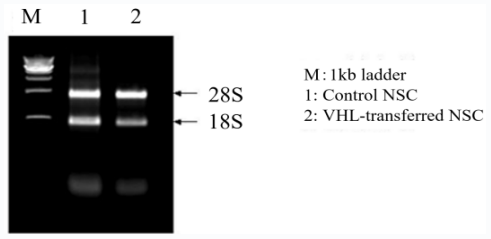

Figure 1: Control *vs*. VHL-gene-transferred neural stem cells derived RNA. Two µg of control RNA (1) and VHL-gene-transferred RNA (2) were run beside markers (M) on a 1.5% denaturing agarose gel. The 18S and 28S ribosomal RNA bands are clearly visible in the both RNA samples.

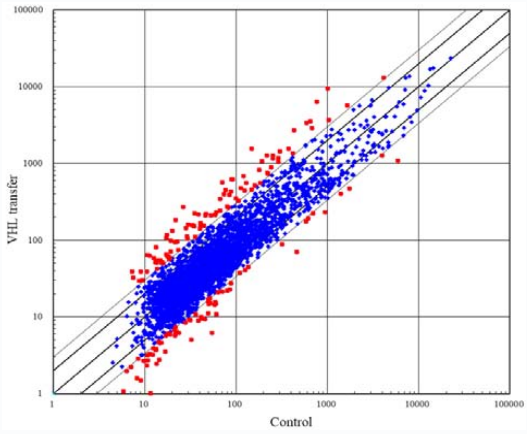

Figure 2: Scatter plot of the microarray data. The data from the microarray are graphed on a scatter plot to visualize variations in gene expression between arrays. The values on the X (control) and Y (VHL-transfer) axes of the scatter plot are the normalized signal values for the samples (log2 scaled). The green lines are fold-change lines (the default fold-change value is 2.0). The expression of the genes above the top green line or below the bottom green line differed more than two-fold between the VHL-transfer and control samples.

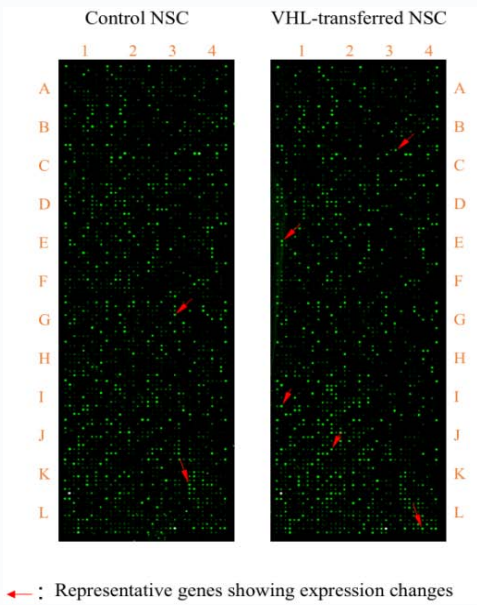

Figure 3: Planer images of microarray data. Green blots show expressions of cDNAs corresponding to cDNAs derived from genes of control neural stem cells (left) and genes of VHL-gene-transferred neural stem cells (right). Arrows show representative genes showing expression change.

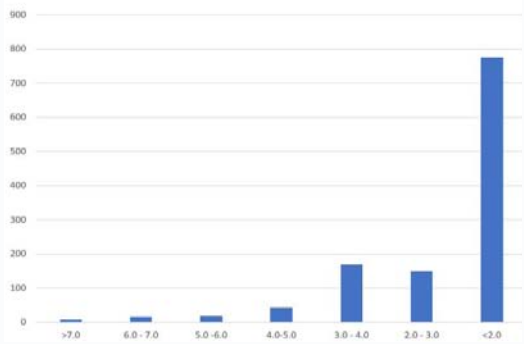

Figure 4: Expression ratios of upregulated genes. A graph showing gene expression ratios of VHL-transferred neural stem cells to control neural stem cells.

3.0 to 4.0: 170 genes; ratio 2.0 to 3.0: 150 genes; and ratio <2.0: 776 genes. Most of the upregulated genes belong to the ratio <2.0 category (Figure 4).

A total of 239 genes related to transcriptional signaling pathways around the VHL gene of 1,044 upregulated genes were selected. Additionally, the 36 selected representative genes are shown in Table 1. These representative genes include neuronal-specific proteins, neurotransmitter receptors, neurotrophic factors, hormone/hormone receptors, cytokine/cytokine receptors, homeobox proteins, SCF complex-associated proteins, and basic helix-loop-helix (bHLH) factors.

Downregulated Genes

According to the ratio of VHL-transferred neural stem cells to control neural stem cells, 1,366 down-regulated genes were distributed among four genes with a ratio of less than 0.2 and 17 genes with a ratio between 0.2 and 0.29; 52 genes, 0.30 to 0.39; 159 genes, 0.40 to 0.49; 237 genes, 0.50 to 0.59; 250 genes, 0.60 to 0.69; 273 genes, 0.70 to 0.79; 240 genes, 0.80 to 0.89; and 133 genes, 0.90 to 1.00 (Figure 5).

A total of 240 genes related to transcriptional signaling pathways around the VHL gene of 2,205 downregulated genes were selected. Additionally, the 34 selected representative downregulated genes related to VHL gene transcriptional signaling pathways are shown in Table 2. These representative genes include VHL downstream factors involved in the VHL/HIF, VHL/JAK/STAT, and VHL/β-catenin pathways; neuronal-specific proteins; basic helix-loop-helix (bHLH) transcription factors; neurotrophic factors and receptors;

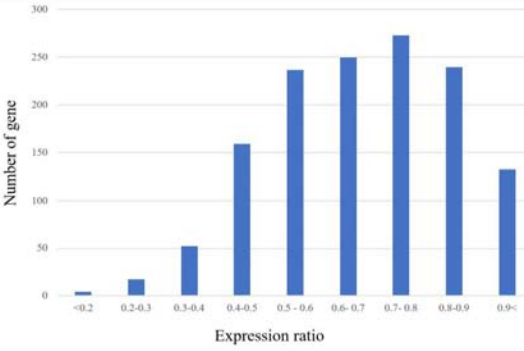

Figure 5: Expression ratios of downregulated genes. A graph showing gene expression ratios of VHL-transferred neural stem cells to control neural stem cells.

Table 1: Representaive Upgenes around Neuronal Differentiation.

Classification	Expression Ratio
Neuronal-specific proteins:	
Microtubule-associated proteins 1a/2/5(MAP1a/2/5)	2.19/1.54/1.24
Neurofilament heavy polypeptide	1.45
Neural precursor cell expressed developmentally down-regulated gene 8 (NEDD8)	7.97
Rhodopsin (Retinitis Pigmentosa 4)	3.57
Amyloid beta (A4) precursor protein binding family A	3.79
S100 calcium-binding protein A8/A9/beta(neural)	4.53/1.51/1.23
Neurotransmitter receptors:	
Glutamate Receptor kainate 4/N-methyl D-aspartate	4.67/3.05
Neuronal Acetylcholine Receptor Protein Alpha 2 Subunit (NAChR2)	5.00
Serotonin receptor type 1B	1.38
Gamma-Aminobutyric Acid (GABA) Receptor Beta 3	3.44
Adrenergic Receptor Alpha 1c/1d	3.24/3.68
Dopamine Receptor D5	1.33
Neurotrophic factor/receptor:	
Brain-derived neurotrophic factor (BDNF)	2.31
Fibroblast growth factor 2/9/18 (FGF2/9/18)	1.16/1.06/2.49
Platelet-derived growth factor (PDGF)	1.81
Glial cell-derived neurotrophic factor (GDNF)	1.26
Neurotrophin-3 (NT-3)	1.38
Retinoid X receptor gamma	3.00
Hormone/Hormone Receptor:	
Corticotropin-Releasing Hormone	2.80
Prolactin	1.96
Androgen-Binding Protein	9.17
Thyroid stimulationg hormone beta subunit	2.79
Homeobox proteins:	
Ventral anterior homeobox 2	1.81
Homeobox gene Pem	1.87
Homeobox msh-like 1	2.07
Homeobox protein R3	3.59
Homeobox gene A4	1.29
SCF box-associated proteins:	
SRY-box-containing gene 10	1.92
Neural F-box protein (NFB42) elongin/proteasome	2.52
Translation elongation factor 1 delta subunit	2.26
Protease 26S subunit/28 subunit beta/subnit beta type3	1.68/1.24/1.23
Elongation factor SIII p15 subnit	1.22
Basic helix-loop-helix (bHLH) proteins:	
Noggin	1.90
Olig-1	1.51
Neurogenic differentiation 2/ 4(NeuroD2/D4)	1.74/2.13
Hairy and enhancer of split 1/ 2 (HES-1/2)	1.12/1.20

neurotransmitter receptors; the ubiquitin-proteasome system; and neuronal signaling factors interacting with Wnt1, 5a, and Notch3.

Discussion

A group of basic helix-loop-helix (bHLH)-type transcription factors of the proneural gene cluster plays a central role in the differentiation of neural stem and progenitor cells. The proneural genes involved in neural differentiation from the mammalian ectoderm are all bHLH-type transcription factors with a basic region and a subsequent helix-loop-helix domain, similar to the Drosophila

Table 2: Representative Downgenes around Neuronal Differentiation.

Classification	Expression Ratio
VHL downstream factors	
HIF/VEGF/EPO pathway:	
Hypoxia-inducible factor 1 alpha subunit (HIF1α)	0.50
Vascular endothelial growth factor receptor 2 (VEGFR2)	0.24
Erythropoietin (EPO)	0.55
JAK/STAT/Smad pathway:	
Janus kinase 2/3 (JAK2/3)	0.92/0.83
Signal transducer and activator of transcription 3/4/5a/6 (STAT3/4/5a/6)	0.72/0.66/0.47/0.80
Smad5 /8	0.91/0.46
β-catenin/nuclear factor kappa b (NFκb) pathway	
β-catenin	0.74
Nuclear factor kappa b (NFκb) p105 subnit	0.94
Basic helix-loop-helix (bHLH) factors:	
Hairy and enhancing split 5 (HES5)	0.46
Neurogenic differentiation 1/ 3 (NeuroD1/ D3)	0.94/ 0.60
Neurotrophic factor/neurotrophic factor receptors:	
Fibroblast growth factor 17(FGF17)	0.78
Ciliary neurotrophic factor (CNF)	0.63
Platelet-derived growth factor A (PDGFA)	0.6
Bone morphological protein 1/2/3/4(BMP1/2/3/4)	0.68/0.80/0.91/0.79
Nerve growth factor (NGF) receptor	0.38
Neuronal-specific protein :	
Microtubule-associated protein (MAP)1A/1B light chain 3	0.76
Neurofilament protein middle polypeptide / light polypeptide	0.75/ 0.68
Neuron-specific enolase (NSE)	0.54
Alpha-tubulin	0.73
S100 calcium-binding protein A4	0.75
Neuropeptide Y/receptor Y1/ Y5 receptor	0.74/ 0.63/ 0.77
Glial Fibrillary Acidic Protein(GFAP)	0.70
Amyloid-beta (A4) precursor protein-binding family A	0.59
Tau	0.57
Neurotransmitter receptor:	
Gamma-aminobutyric acid -A (GABA-A) receptor subnit alpha 1/2/4/5/6	0.76/ 0.68/0.62/0.70/ 0.75
Gamma-aminobutyric acid -A (GABA-A) receptor subnit delta/epilon/subnit rro 1,2	0.68/0.44/0.68, 0.83
Acetylcholine recptor beta 4	0.5
Ubiquitin/proteasome :	
Ubiquitin 1	0.66
Ubiquitin-cojugating enzyme/enzyme E21/emzyme E211kB/emzyme UBC7	0.82/0.42/0.69/0.64
Proteasome 28 subunit alpha/ 26S subunit ATPase 4/non-ATPase 10/ non-ATPase 4	0.65/ 0.81/ 0.70/0.70/0.96
Proteasome subunit alpha type 1/ 3/ 4/ 5/ 7	0.58/ 0.83 /0.70 /0.79 /0.52
Proteasome subunit beta type 1/ 4	0.85/ 0.73
Neuronal signaling factors: Wnt1,5a, Notch3	
Wnt-5a	0.78
Notch3	0.63

proneural gene group. Among these, neural stem cell maintenance is regulated by inhibitory bHLH factors among the pro-neural genes, while differentiation into neurons and glia is regulated by promotive bHLH factors [8]. In addition, these inhibitory and promotive bHLH factors are known to be closely related to the signaling pathway of Notch, a cell-penetrating protein that activates and binds to Notch

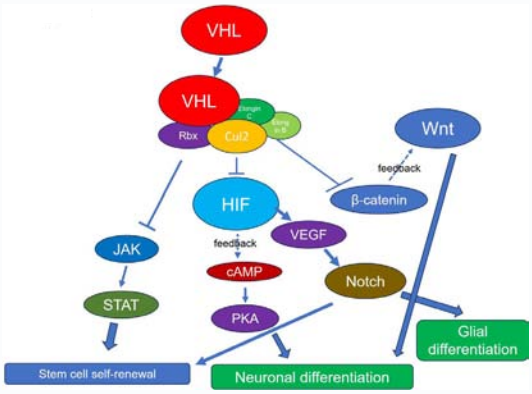

Figure 6: VHL-based neuronal differentiation transcription mechanism. VHL forms a complex with elonginB/C, Rbx, and Cul2, which ubiquitinates HIF, β-catenin, and JAK and degrades them in the proteasome. The HIF-repressed cAMP and Notch are released from their repression and activated to induce neuronal differentiation. The key transcription factors in this mechanism are VHL, HIF, β-catenin, JAK, and ZHX.

in surrounding cells when Notch ligand is expressed on the cell surface [9]. Upon activation, Notch cleaves the protein's periplasmic intracellular domain just below the plasma membrane and translocates into the nucleus, where it forms a complex with the nuclear protein RBP-J/Su(H) and activates transcription of Hes 1 and Hes 5, a type of inhibitory bHLH factor, which is thought to act to retain neural stem cells [10] or to promote glial differentiation of neural stem cells.

We have previously shown that the expression of VHL protein in neural stem cells derived from fetal rat cerebrum is mainly found in the cytoplasm of cells that only express the neuronal marker MAP2, and that forced expression of VHL protein rapidly induces differentiation into neurons.[3] In addition, electrophysiologically, VHL-transferred neural stem cells showed an average maximum voltage-dependent sodium current of more than 4000 pA, indicating the electrophysiological characteristics of mature neurons [11]. Furthermore, *in vivo*, VHL transferred neural stem cells transplanted into Parkinson's disease model rats not only differentiated into TH-positive dopamine-producing cells in more than 50%, but also showed a marked reduction of apomorphine-induced rotation in behavioral analysis, with about 30% of model rats showing no induced rotation at all. This means that VHL-transferred neural stem cells function as dopamine-producing neurons in the transplanted rat brain and may contribute significantly to neuronal regenerative medicine for Parkinson's disease and other neurological disorders using neural stem cell donors [12]. However, it remains unclear why the VHL gene has the ability to induce neural differentiation, and the relationship with the bHLH-type transcription factor group, the Notch signaling pathway, and the STAT/Smad pathway is also unclear.

VHL proteins form a VBC-Cul 2 complex with their binding proteins, elongin B, C, and Cul2, to degrade hypoxia inducible factor (HIF) and regulate the transcriptional response of genes such as VEGF induced by HIF [13]. In order for VHL proteins to function normally and degrade HIF, normal oxygen pressure is required. Under hypoxic conditions, VHL proteins do not function normally, and VEGF and other proteins are induced without degradation of HIF. Therefore, we placed neural stem cells under hypoxic pressure and examined how neural stem cell differentiation proceeds under conditions in which VHL proteins do not function normally. The percentage of cells positive for the neuronal marker MAP decreased, but the percentage of GFAP-positive cells conversely increased, indicating that TUNEL-positive apoptotic cells are at the same time mostly MAP-positive and that differentiation into neurons is inhibited under hypoxia [14]. Since the VHL protein does not function under hypoxia, HIF is not degraded and a group of transcription factors downstream of it are induced, suggesting not only that HIF may be involved in the differentiation of neural stem cells to glia, but also that inhibition of HIF may induce neural stem cells to differentiate into neurons HIF may be involved in the differentiation of neural stem cells into glia, but it is possible to induce neural stem cells to differentiate into neurons by suppressing HIF. Furthermore, we demonstrated that the BC-box motif amino acid sequence [(A,P,S,T)LXXXC(A,C)XXX(A,I,L,V)] of VHL protein induces neural progenitor cells to differentiate into neurons [11]. BC-box motif of VHL with an additional protein transduction domain was transplanted locally into the brain and spinal cord of various rat mesenchymal stem cell models of neurological diseases (Parkinson's disease, spinal cord injury), and differentiation into neurons and functional improvement in the tissues have been demonstrated [15-19]. Additionally, we reported that the VHL-JAK-STAT pathway is involved in the transcription mechanism of neuronal differentiation of mesenchymal stem cells originating from this VHL,[5] and more recently, we reported that the VHL-ZHX2-NFκb pathway is involved in neuronal differentiation [20].

In this cDNA microarray analysis, we found that VHL-transfected neural stem cells showed a higher level of differentiation than control neural stem cells. This included the majority of neuron-specific proteins, various neuronal receptors, and neurotrophic factors. The expression of various hormone and hormone receptors, homeobox proteins, cytokines, zinc finger proteins, heat shock proteins, AKT, elongin B/C, and cyclic AMP mRNA was elevated. However, the expression of some neuron-specific proteins and mRNA of neuronal receptors decreased. Conversely, the factors whose mRNA expression decreased in VHL-transfected neural stem cells compared to controls were HIF/VEGF/EPO, JAK/STAT/Smad, ubiquitin/proteasome, Wnt, Notch, tau, amyloid precursor protein, glial fibrillary acidic protein (GFAP), neuropeptide Y, MAP1A/1B, β-catenin, and transforming growth factor (TGF). Upregulation of homeobox genes suggests that the VHL gene promotes various organ formation. Downregulation of GFAP and STAT suggests that VHL inhibits glial differentiation. Conversely, the expression of neurotrophic factors/receptors was either upregulated in FGFs, BDNF, GDNF, and NT-3, or downregulated in the NGF receptor, GDNF receptor, CNF, PDGF-A, and FGF17 receptor, suggesting that neurotrophic factors can be classified into two types: neuronal promotion and inhibitory neuronal promotion. The results of the cDNA microarray analysis of VHL-gene-transferred neural stem cells (NSCs) largely support the previously reported VHL transcriptional signaling pathways. Additionally, the results suggest that various factors are related to VHL-mediated neuronal differentiation. The expression of neuron-specific proteins is mostly upregulated, while the expression of glia-specific proteins is downregulated. The downregulation of VHL downstream factors suggests that they are related to the inhibition of neuronal differentiation and the promotion of glial differentiation. Similarly, the results indicate that some neurotrophic factors promote neuronal differentiation of neural stem cells (NSCs), while others promote glial differentiation or maintain NSCs' characteristics. Furthermore, the microarray results indicate that VHL regulates hormones [21] and cytokines [22] and is associated with various organ developments [23].

Based on these results, the transcription mechanism of neuronal differentiation centered on VHL is shown in Figure 6. VHL forms a complex with Elongin B/C, RBX, and Cul2. This complex ubiquitinates and degrades HIF, β-catenin, and JAK by the proteasome.[24] cAMP and Notch are released from their inhibitory state and activated when HIF is inhibited. This leads to neuronal differentiation. This is thought to induce neuronal differentiation. The key transcription factors in this mechanism are VHL, HIF, β-catenin, JAK, and ZHX. It is possible that the forced expression of VHL or the suppression of HIF, β-catenin, and JAK in neural stem cells could induce neuronal differentiation. Previous studies have shown that this neuronal differentiation mechanism is not only common to neural stem cells, but also to mesenchymal stem cells. We have also reported on the GABAergic differentiation of rodent mesenchymal stem cells and the behavioral improvement of animal models following the transplantation of neural-differentiated stem cells [19]. Additionally, we have recently reported on the cholinergic neuronal differentiation of human adipose tissue-derived mesenchymal stem cells using this mechanism [5]. Our previous studies suggest that neuronal regenerative therapy using neural-differentiated stem cells is more effective than therapy using non-treated stem cells [15-19, 25]. Therefore, it is expected that this neuronal differentiation mechanism can be used to develop regenerative medicine using somatic mesenchymal stem cells (Figure 6).

Conclusion

cDNA microarray analysis revealed that VHL regulates neuronal differentiation, as well as the VHL/HIF/VEGF/EPO and JAK/STAT/Smad pathways. Additionally, VHL plays a role in development, hormonal regulation, and cytokine regulation. Understanding the transcriptional signaling pathways around VHL inducing neuronal differentiation is very important for neuronal regenerative medicine. This neuronal differentiation mechanism is expected to be useful for developing regenerative medicine using neural or mesenchymal stem cells.

Acknowledgments

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Data Availability Statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Kimihiro Nakahara and Hiroshi Kanno played a leading role in the experimental design and execution and took primary responsibility form manuscript writing. Atsuhiko Kubo and Hidetoshi Murata contributed to the experimental design and execution. Akira Matsuno and Hidetoshi Murata contributed to the supervision and provision of research facilities.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section (Supporting Information).

Table S1. Data of cDNA microarray analysis for all genes.

Table S2. Upregulated genes.

Table S3. Downregulated genes.

References

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