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Effects of quetiapine on DNA methylation in neuroblastoma cells

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ABSTRACT

Epigenetic regulation may be involved in the pathophysiology of mental disorders, such as schizophrenia and bipolar disorder, and in the pharmacological action of drugs. Characterizing the epigenetic effects of drugs is an important step to optimal treatment. We performed comprehensive and gene-specific DNA methylation analyses of quetiapine using human neuroblastoma cells. Human neuroblastoma cells were cultured with quetiapine for 8 days, and DNA methylation analysis was performed using Infinium HumanMethylation27 BeadChip. A total of 1173 genes showed altered DNA methylation. Altered DNA methylation predominantly occurred as hypomethylation within the CpG island compared to DNA isolated from non-treated cells. Gene ontology analysis revealed that these genes were related to the cellular process of intracellular protein binding. There was no common effect of quetiapine with three mood stabilizers (lithium, valproate, and carbamazepine). However, common DNA methylation changes in eight genes, including *ADRA1A*, which encodes adrenoceptor alpha 1A, were found with quetiapine and lithium treatments. Finally, bisulfite-sequencing analysis revealed that quetiapine decreased the DNA methylation level of the promoter region of *SLC6A4*, where hypermethylation with bipolar disorder and hypomethylation with mood stabilizers have been reported.

1. Introduction

Elucidating the pathophysiology of major mental disorders such as schizophrenia and bipolar disorder (BD) is important for developing an optimal therapeutic strategy. Numerous studies have been performed to detect genetic factors; however, no specific gene with a significant effect has been identified so far, suggesting that a complex gene–environment interaction may contribute to the etiology and pathophysiology of the diseases.

Epigenetics is the study of heritable and stable changes in gene expressions that are not caused by changes in the DNA sequences but are caused by modifications of genomic DNA and proteins, such as DNA methylation and histone modifications (Bird, 2002). Genetic and environmental factors affect the epigenetic status of tissues, including brain, and altered epigenetic status has been proposed to be involved in the pathophysiology of major mental disorders (Dempster et al., 2013; Nishioka et al., 2012).

We performed comprehensive DNA methylation analyses to detect epigenetic factors of BD by using lymphoblastoid cell lines derived from monozygotic twins discordant for BD. We found hypermethylation of the promoter region of the serotonin transporter, *SLC6A4*, in the twins. This difference was also observed in peripheral tissue and postmortem brains in case–control studies (Sugawara et al., 2011). The DNA methylation level of the promoter region of *SLC6A4* was decreased in human neuroblastoma cells treated with mood stabilizers such as lithium, valproate, and carbamazepine (Asai et al., 2013). These results suggest that mood stabilizers may have therapeutic efficacy by altering the DNA methylation status of *SLC6A4* to some extent.

Accumulating evidence also suggests that mood stabilizers and antipsychotic drugs affect the epigenetic status of brain cells (Boyadjieva and Varadinova, 2012; Dong et al., 2010, 2008; Kurita et al., 2012; Popkie et al., 2010). Therefore, examining the epigenetic effects of each drug is the first step in understanding the pathophysiology of diseases and possible pharmacological actions of drugs.

We have been systematically analyzing the epigenetic effects of antipsychotic drugs and mood stabilizers using human neuroblastoma cells. In this study, we examined the effect of quetiapine (QTP) on DNA methylation of human neuroblastoma cells. QTP, which is an

Abbreviations: BD, bipolar disorder; SLC6A4, solute carrier family 6 (neurotransmitter transporter), member 4; QTP, quetiapine; QC, quality control; PCR, polymerase chain reaction; CT, control; CD44, CD44 molecule (Indian blood group); DSP, desmoplakin; ADRA1A, adrenoceptor alpha 1A; CD164L2, CD164 sialomucin-like 2; CSEN, calsenilin; EFEMP2, EGF containing fibulin-like extracellular matrix protein 2; GABRG3, gamma-aminobutyric acid (GABA) A receptor, gamma 3; RAB32, RAB32, member RAS oncogene family; ALX4, aristaless-like homeobox 4; WT1, Wilms tumor 1; FDR, false discovery rate; TSS, transcription start site.

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atypical antipsychotic drug, has been reported as being effective for treatment of acute depression in BD (Calabrese et al., 2005; Chiesa et al., 2012; Thase et al., 2006), and it is recommended as a first-choice drug for the treatment of bipolar depression (Vieta et al., 2010). QTP has also been reported to be effective in the treatment of acute mania (Smith et al., 2007; Yildiz et al., 2011) and as maintenance therapy for BD (Weisler et al., 2011), suggesting that QTP is useful as a mood stabilizer. The aims of this study were to evaluate the effect of QTP on DNA methylation status of neuroblastoma cells, to evaluate the similarities of QTP and mood stabilizers regarding epigenetic action, and to evaluate the effect of QTP on DNA methylation level in the promoter region of *SLC6A4*.

2. Methods

2.1. Cell culture and DNA extraction

Cell culture was performed following the same protocol of our previous report (Asai et al., 2013). In brief, the human neuroblastoma cell line SK-N-SH (American Type Culture Collection, Manassas, VA) was cultured for 8 days in Eagle's minimal essential medium containing 10% fetal bovine serum. QTP Hemifumarate (Tronto Research Chemicals, Tronto, Canada) was dissolved in 100% dimethyl sulfoxide (30 mM). 30 μl of this solution was added to the 30 ml of the medium (30 μM). The final concentration of QTP was decided according to the previous reports (Schmidt et al., 2009, 2010). The cell culture was independently performed in triplicate. The medium was changed on days 2, 5, and 8 by QTP-containing media. On day 9, we retrieved the cells and extracted DNA using the standard phenol–chloroform protocol. The data of control cell cultures have been reported in our previous report (Asai et al., 2013).

2.2. Bisulfite modification

For the comprehensive assay, we performed sodium bisulfite modification using an EZ DNA methylation kit (Zymo Research, Irvine, CA). We used an Infinium HumanMethylation27 BeadChip (Illumina, San Diego, CA) according to the instructions of the manufacturer, which quantified DNA methylation levels of 27,578 CpG sites in promoter regions (1 kb upstream and 500 bases downstream of transcription start sites of 14,475 consensus coding sequences genes based on NCBI database (Bibikova et al., 2009)). In this platform, the CpG sites predefined by the manufacture were annotated whether each CpG is located within or outside of CpG island. The definition of CpG island is based on the widely used criteria developed by Gardiner-Garden and Frommer (1987). For cloning and sequencing analyses, we performed bisulfite modification using an Epitect bisulfite kit (Qiagen, Venlo, the Netherlands).

2.3. Statistical analysis

In the Infinium platform, the array experiment was performed according to the previous report (Bibikova et al., 2009). We obtained the DNA methylation level as a beta value ranging from 0 (no methylation) to 1 (complete methylation). For initial quality control (QC), we extracted the CpG sites with $p < 0.05$ in detection p value, which show the specificity of signal level. We calculated the difference in beta values for QTP-treated cell cultures and for the control cell cultures for every probe by independent *t* test. Significance was set at $p < 0.05$. Using CLC genomics workbench (CLC bio Japan, Tokyo, Japan), we performed unbiased clustering analysis for the probes that passed initial QC tests, and biased clustering analysis for the probes showing significant differences in independent *t* test to reveal the relative similarity of samples. Dendrograms were made by calculating pairwise distances using Euclidian distance. Gene ontology analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery software (Huang da et al., 2009). Using false discovery rate for correction, significance was set at $p < 0.05$.

2.4. Bisulfite sequencing

After sodium bisulfite modification, we performed polymerase chain reaction (PCR) according to previous studies (Asai et al., 2013; Sugawara et al., 2011) using the primer set for the promoter region of *SLC6A4*. We confirmed that each amplified product was a single band by agarose gel electrophoresis. The PCR products were excised from the gel, purified by a MinElute Gel Extraction kit (Qiagen) and cloned using a TOPO TA cloning kit (Life Technologies, Carlsbad, CA). Single bacterial colonies were used, and 20 colonies per one experimental condition were used for colony PCR. The colony PCR products were then subjected to DNA sequencing. Cloning and sequencing analyses were performed twice using independently cultured cells.

3. Results

3.1. Comprehensive DNA methylation analysis of QTP

After 8 days in human neuroblastoma cell line culture with a therapeutic concentration of QTP, we extracted genomic DNA and performed DNA methylation analysis using the Infinium HumanMethylation27 BeadChip (Illumina), which can measure DNA methylation levels at approximately 27,000 CpG sites. Three cell lines in control (CT) and QTP were not clustered separately in an unbiased hierarchical clustering analysis using the 27,562 CpG sites that passed the initial QC test (Fig. 1a). Among the 27,562 CpG sites, we identified 1216 differentially methylated CpG sites between QTP and control groups by *t* test ($p < 0.05$). Clustering analysis using DNA methylation levels of these 1216 CpG sites successfully separated control and QTP-treated groups (Fig. 1b). These 1216 CpG sites corresponded to the promoter regions of 1173 genes. Among these 1173 genes, the number of hypomethylated genes (807 genes) was larger than the number of hypermethylated genes (366 genes). We examined whether these CpG sites were located within or outside of CpG island (Gardiner-Garden and Frommer, 1987). The hypomethylation was more frequently found in the probes located within CpG island (hypomethylated genes, 666; hypermethylated genes, 228) than in the probes located outside of CpG island (hypomethylated genes, 141; hypermethylated genes, 138). Gene ontology analysis of these 1173 genes revealed that cellular process, protein binding, and intracellular part were enriched (Table 1 and Supplementary Table 1).

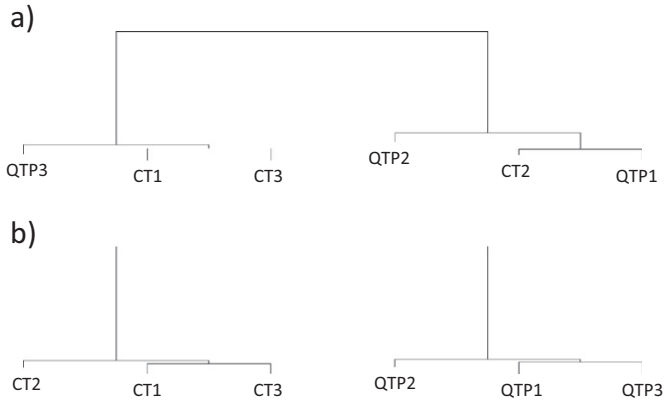

Fig. 1. Hierarchical clustering analysis. a) Unbiased hierarchical clustering analysis using the probes that passed the initial quality control test (N = 27,562 probes). b) Biased hierarchical clustering analysis using the probes that detected significant differences between CT and QTP by independent *t* test (N = 1216 probes). Experiments were independently performed in triplicate (CT1, CT2 and CT3 for cultures without quetiapine, and QTP1, QTP2, and QTP3 for cultures with quetiapine).