Aberrant Histone Modification of TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155 in Major Depressive Disorder

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Research Article

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Abstract

Activated toll-like receptor (TLR) signaling has been well investigated in major depressive disorder (MDD). We previously reported that TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155 play important roles in regulating the toll-like receptor 4 (TLR4) signaling pathway and may serve as novel targets in the pathogenesis of MDD. Recently, aberrant histone modification has been implicated in several psychiatric disorders, including schizophrenia and mood disorder; the most thoroughly studied modification is histone 3 lysine 4 tri-methylation (H3K4me3). In this work, we aimed to explore H3K4me3 differences in the promoters of genes encoding the abovementioned factors in patients with MDD, and whether they were altered after antidepressant treatment. A total of 28 MDD patients and 28 healthy controls were recruited. Peripheral blood mononuclear cells (PBMCs) were collected. The levels of H3K4me3 in the promoters of TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155 were measured through chromatin immunoprecipitation (ChIP) followed by DNA methylation assay. Analysis of covariance was used to evaluate between-group differences after adjusting for age, sex, BMI, and smoking. In comparison with healthy controls, patients with MDD showed significantly lower H3K4me3 levels in the promoters of TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155 in PBMCs. These levels were not significantly altered after completion of a 4-week antidepressant treatment. To explore the association between depression severity and H3K4me3 levels, a multiple linear regression model was generated. The results revealed that levels of H3K4me3 in the TNIP2 promoters a negative correlation with the 17-item Hamilton Depression Rating Scale (HAND-17) score, whereas that of TLR4 had a positive correlation with this score. The present results suggest that decreased H3K4me3 levels in the promoters of the genes encoding TNFAIP3, TLR4, miR-146a, miR-155, and TNIP2 may be a mechanism underlying the mRNA expression dysregulation reported in MDD patients.

Introduction

Major depressive disorder (MDD) is one of the most prevalent and potentially disabling mental disorders, with a reported over 163 million of the global population affected [1]. The etiology of MDD is thought to be multifactorial and based on interactions between genetic predisposition and environmental factors. Accumulating evidence suggests that epigenetic mechanisms, by which gene expression is modulated without alteration of the DNA sequence, appear to contribute to the development of depression [2]. The epigenetic process could be influenced by environmental stimuli, including exposure to metals, air pollution, and electromagnetic radiation [3]. The most well-known mode of epigenetic regulation is altered DNA methylation of the glucocorticoid receptor (GR)-encoding gene (NR3C1) following prenatal stress or childhood adversity, which leads to hypothalamic–pituitary–adrenal (HPA) axis dysfunction and the emergence of several stress-related diseases [4]. The NR3C1 gene was overall hypermethylated in MDD patients [5], whereas hypomethylation of the NR3C1 gene was observed in individuals with post-traumatic stress disorder (PTSD) [6, 7]. Along with DNA methylation, histone modification is another key epigenetic regulator. Several types of histone modification have been identified; of them, histone acetylation, methylation, phosphorylation, and ubiquitination are among the most common. Increasing
evidence suggests that histone modifications are involved in the pathogenesis of many psychiatric diseases, including schizophrenia and depression [8, 9], and that innate immune and inflammatory responses play pivotal roles in the pathophysiology of depression. Here, we sought to explore H3K4me3 levels and the effects of antidepressants on H3K4me3 levels on genes recognized in our previous studies [10–14] in patients with major depression.

Methods

Experimental design

Thirty inpatients with MDD were recruited from the psychiatric ward of Kaohsiung Chang Gung Memorial Hospital, Taiwan, from August 2020 to Sep 2022 and received follow-up after antidepressants treatment for 4 weeks. Twenty-eight healthy controls were enrolled from the community. Institutional Review Board approval was obtained from the hospital ethics committee (201901894A3). Blood samples were obtained for the chromatin immunoprecipitation (ChIP) assay. All participants provided informed consent and received verbal and written information prior to participating in the study.

Participants

The enrollment process was described in our previous work [13]. Patients with MDD were screened with a structured clinical interview based on the Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition) (DSM-V), and detailed assessments of current psychiatric symptoms were recorded. Past medications and scores on the 17-item Hamilton Depression Rating Scale (HAMD-17) were recorded at the same time. The exclusion criteria included having a psychotic disorder, substance dependence (including alcohol), severe obesity (body mass index [BMI] > 34 kg/m2), systemic inflammatory/infection disease and/or the taking of an anti-inflammatory or immune-modulating drug. All patients received blood pressure measurements, chest X-rays, electrocardiographic examinations, and routine blood tests after hospitalization to exclude any possible chronic systemic physical illness. Enrolled patients received no antidepressant for at least 1 week before the first blood sample was collected. Healthy controls recruited from the community had neither a personal history of nor a first-degree relative with a psychiatric disorder. The same psychiatrist who assessed the MDD group assessed the healthy control group using criteria of the DSM-V to exclude psychiatric disease. Following the above clinical examinations, blood samples were collected.

Chromatin immunoprecipitation (ChIP)

PBMCs were isolated from 20-ml blood samples using the Ficoll gradient method. For each sample, collected PBMCs (1 x 10^7) were transferred to a new tube, PBS was added to 7 ml, 1 ml of 8% formaldehyde was added, and the tube was shaken for 15 min at room temperature. Thereafter, 0.9 ml 10X glycine solution (1.25M glycine) was added and the tube was shaken for 10 min at room temperature. The cells were centrifuged at 800xg for 5 min at 4°C, the supernatant was removed, the
pellet was resuspended in 10 ml 1X PBS, and the tube was centrifuged at 800xg for 5 min at 4°C. The resulting pellet was resuspended with 500 ml ChIP cell lysis buffer (Santa Cruz Biotechnology, #SC450000) with protease inhibitors, and the suspension was transferred to a 1.5-ml microtube, incubated on ice for 15 min, and vortexed for 5 min. The sample was centrifuged at 800xg for 5 min at 4°C, the supernatant was carefully removed, and the pellet was resuspended in 130 µl nuclear lysis buffer (50 mM Tris, 10 mM EDTA, 1% SDS). The sample was then sonicated three times for 10 min each time on ice water. In a new microtube, 100 µl sonicated sample was mixed with 900 µl CHIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl), and then with 40 µl ChIP A/G magnetic beads (Millipore, #16–663) and 4 µl protease inhibitor cocktail II (Millipore, #539132). The sample was vortexed and separated into three microtubes, and each tube was loaded with ChIP-H3K4me3 rabbit monoclonal antibodies (Millipore, #17–614), anti-normal rabbit IgG antibodies (Millipore, #CS200581), or CHIP dilution buffer. The samples were incubated overnight at 4°C with rotation, the magnetic beads were pelleted with a magnetic separator, and the supernatant was carefully removed. The beads were sequentially washed with 500 µl low-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), 500 µl high-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), 500 µl LiCl buffer (0.25 M LiCl, 1% IGEPAL CA630, 1% deoxycholic acid (sodium salt), 1 mM EDTA, 10 mM Tris, pH 8.1), and 500 µl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The beads were then mixed with 100 µl ChIP elution buffer (1% SDS, 0.1 M NaHCO3, pH 8.0) and 0.5 µl protease K (Zymoresearch, #D3001-2-20), and incubated at 55°C for 3 hours. The DNA was purified with a DNA clean-up kit (Zymoresearch, cat.#D5205) following the manufacture's protocol, and the purified DNA was stored at -20°C for further analysis.

**Quantification of CHIP DNA**

Quantitative real-time PCR was used to quantify the amount of target DNA fragments bearing H3K4me3. A 7500 Fast Real-Time PCR System (Applied Biosystems) was used. The primers used for PCR reactions were as follows:

TNFAIP3-ChIP-P3-F 5'-caggttcttttaggtttctct-3'

TNFAIP3-ChIP-P3-R 5'-taggttcttttaggtttctct-3'

TLR4-ChIP-P3-F 5'-AGGGGAGATTAGAATTCAGACACA-3'

TLR4-ChIP-P3-R 5'-TGCCATAAGGAATACCACAGACT-3'

TNIP2-ChIP-P5-F 5'-CTGAGCAGCAGTTGGAGATTT-3'

TNIP2-ChIP-P5-R 5'-CTGAGCAGCAGTTGGAGATTT-3'

miR-146a-P3-F 5'-TGACGCTTGGAGATTTGATTT-3'

miR-146a-P3-R 5'-CTGAGCAGCAGTTGGAGATTT-3'
miR-155-F3 5'-GCCGAGCGGTGCTTTCTTTAC-3'
miR-155-R3 5'-GTCATCCCAATATACCTGCTTTAG-3'
GAPDH-ChIP-F 5'-TAC TAG CGG TTT TAC GGG CG-3'
GAPDH-ChIP-R 5'-TCG AAC AGG AGG AGC AGA GAG CGA-3'

Statistics

All results are expressed as the mean ± standard deviation. The Chi-square test was used to compare differences in demographic data (e.g., sex and smoking) and Student's t-test was used to compare differences in age and BMI between groups. An analysis of covariance (ANCOVA) adjusted for age, sex, and body mass index (BMI) was used to compare differences in the H3K4me3 levels at the promoters of TNFAIP3, TLR4, miR-146a, miR-155, and TNIP2 between healthy controls and MDD patients. A paired t-test was used to compare differences in the expression levels of H3K4me3 before and after a 4-week antidepressant treatment. Multiple linear regression was used to analyze factors that correlated with the HAMD-17 scores. All statistical analyses were performed using the Statistical Product and Service Solutions (SPSS) version 22 statistical software. P-values < 0.05 were taken as indicating statistical significance.

Results

Demographic and clinical characteristics

The baseline demographic and clinical characteristics of healthy controls and MDD patients are listed in Table 1. Thirty patients with MDD and 28 healthy controls were enrolled. Among the 30 MDD patients, 26 received a 4-week antidepressant treatment and followed up with an examination. The mean ages of MDD patients and controls were 41.83 ± 14.86 years and 39.25 ± 8.12 years, respectively. The majority of our subjects were female and most were non-smokers. BMI was similar in the two groups. In the MDD group, the duration of the disease was on average 8.18 ± 6.84 years. Regarding the severity of depression, the HAMD-17 score before treatment was 24.33 ± 4.53, suggestive of moderate to severe depression in MDD patients. The benzodiazepine equivalent dose to lorazepam before treatment was 8.12 ± 5.65 mg.
Table 1
Demographic and clinical characteristics of major depressive disorder patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Major depression (n = 30)</th>
<th>Healthy controls (n = 28)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.83 ± 14.86</td>
<td>39.25 ± 8.12</td>
<td>0.412</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>8/22</td>
<td>9/19</td>
<td>0.647</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.00 ± 5.15</td>
<td>23.16 ± 2.69</td>
<td>0.438</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>4/26</td>
<td>2/26</td>
<td>0.439</td>
</tr>
<tr>
<td>Education (years)</td>
<td>11.81 ± 3.65</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>8.18 ± 6.84</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HAMD-17 score</td>
<td>24.33 ± 4.53</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BZD-equivalent dose (to lorazepam)</td>
<td>8.12 ± 5.65</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Age and BMI, Student’s t-test; sex and smoking, Chi-square test.

Promoter H3K4me3 levels of innate immunity-related genes are significantly lower in patients with major depressive disorder compared to healthy controls

To explore the association between histone modification and innate immunity-associated genes in major depressive disorder we had previously identified, we analyzed the promoter H3K4me3 levels of genes. Table 2 presents the levels of H3K4me3 in the gene promoters of TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155 in PBMCs obtained from healthy controls and from MDD patients before and after antidepressant treatment. Using an ANCOVA approach after adjusting by age, sex, BMI, and smoking, we found that the levels of H3K4me3 in the promoter regions of TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155 were significantly lower in MDD patients before treatment compared with controls (Table 2, I vs. III, all p < 0.05). When we evaluated the effect of a 4-week course of antidepressants on the expression of H3K4me3, we found that there was no significant alteration of H3K4me3 levels between the pretreatment and posttreatment groups (Table 2, I vs. II, all p > 0.05).
Table 2
The difference in A20, TLR4, TNIP2, miR146a, and miR155 profiles between healthy controls and major depressive disorder patients before and after treatment.

<table>
<thead>
<tr>
<th></th>
<th>I vs. III</th>
<th>I vs. II</th>
</tr>
</thead>
<tbody>
<tr>
<td>I vs. III</td>
<td>ANCOVA, with adjustment for age, sex, BMI, and smoking; I vs. II: paired t-test.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of input DNA</td>
<td>MDD pretreatment (n = 30)</td>
</tr>
<tr>
<td>TNFAIP3</td>
<td>3.046 ± 1.64</td>
<td>3.324 ± 2.411</td>
</tr>
<tr>
<td>TLR4</td>
<td>0.681 ± 0.80</td>
<td>0.467 ± 0.364</td>
</tr>
<tr>
<td>TNIP2</td>
<td>0.339 ± 0.308</td>
<td>0.279 ± 0.250</td>
</tr>
<tr>
<td>miR146a</td>
<td>1.270 ± 0.699</td>
<td>1.353 ± 0.807</td>
</tr>
<tr>
<td>miR155</td>
<td>1.434 ± 0.657</td>
<td>1.579 ± 1.237</td>
</tr>
</tbody>
</table>

Severity of major depression is associated with the H3K4me3 levels at TLR4 and TNIP2

To understand the clinical relationship between the severity of MDD and the H3K4me3 levels at the promoters of the tested genes, we used linear regression to assess the influence of H3K4me3 on the degree of depression measured with the HAMD-17 score. As shown in Table 3, enrichment of H3K4me3 at the TLR4 promoter was positively correlated with the HAMD-17 score (coefficient = 1.049; p = 0.010), and that at the TNIP2 promoter was negatively correlated with the HAMD-17 score (coefficient = -1.037; p = 0.012).
Table 3
Multiple linear regression analysis for the association between HAMD-17 score and mRNA expression levels of A20 and A20-interacting proteins

<table>
<thead>
<tr>
<th>Independent factor</th>
<th>HAMD-17</th>
<th>Standardized coefficient</th>
<th>t</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A20</td>
<td></td>
<td>-0.401</td>
<td>-0.960</td>
<td>0.353</td>
</tr>
<tr>
<td>TLR4</td>
<td></td>
<td>1.049</td>
<td>2.987</td>
<td>0.010*</td>
</tr>
<tr>
<td>TNIP2</td>
<td></td>
<td>-1.037</td>
<td>-2.879</td>
<td>0.012*</td>
</tr>
<tr>
<td>miR146a</td>
<td></td>
<td>-1.184</td>
<td>-1.944</td>
<td>0.072</td>
</tr>
<tr>
<td>miR155</td>
<td></td>
<td>1.161</td>
<td>1.634</td>
<td>0.125</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>0.415</td>
<td>1.599</td>
<td>0.132</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>0.623</td>
<td>2.824</td>
<td>0.014*</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>-0.144</td>
<td>-0.688</td>
<td>0.503</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td>-0.331</td>
<td>-1.646</td>
<td>0.122</td>
</tr>
</tbody>
</table>

* p < 0.05.

Discussion

In this study, we investigated H3K4me3 levels at certain promoters to explore a possible epigenetic regulatory mechanism in MDD patients. We found that patients with MDD exhibited significantly decreased H3K4me3 levels in the promoter regions of TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155 in PBMCs. To our knowledge, this is the first report to demonstrate that H3K4me3 levels are decreased in these innate immunity-associated genes in PBMCs from MDD patients. Our data further suggest that the altered mRNA expression levels of these innate immunity-associated genes found in previous studies are mediated in part by changes in H3K4me3, which is an active chromatin marker that promotes transcription through opening chromatin and allowing the transcriptional machinery to bind to the promoters of actively transcribing genes [9, 15, 16].

TNFAIP3, which encodes the ubiquitin-editing enzyme, A20, acts as a key negative regulator for the Toll-like receptor and nuclear factor kappa B (NF-κB) signaling pathways, which are involved in inflammation-mediated depression [10]. We previously found that the baseline TNFAIP3 mRNA expression level was low in patients with MDD but increased in response to antidepressant treatment, suggesting that this mRNA level could serve as a factor for predicting antidepressant treatment response [10]. In addition, we found that the TNFAIP3 mRNA level was negatively correlated with the severity of depression and could be used to measure the improvement of depressive symptoms, as assessed by the HAMD-17 score [10]. Some previous work reported that alteration of histone H3K4 methylation underlies the genetic
vulnerability to the psychopathology of depression [17]. It has been proposed that downregulation of TNFAIP3 in CD4+ T cells of systemic lupus erythematosus (SLE) patients may be mediated by histone H3K4 demethylation resulting in reduction of H3K4me3 at the TNFAIP3 gene promoter [18]. Moreover, TNFAIP3 heterozygosity appears to serve as a predisposing factor for neuropsychiatric lupus (NPSLE), which is the neurological and psychiatric manifestations of SLE: The behavioral dysfunctions of NPSLE, including hypoactivity, anxiety, cognitive impairment, sensorimotor gating deficits, and the development of neuroinflammation, were predominantly observed in TNFAIP3 heterozygous female mice and worsened upon exposure to an environmental stimulus [19]. Our data support the notion that dysregulation of the TNFAIP3 mRNA level in MDD patients may be attributed to aberrant epigenetic modification.

TNFAIP3-interacting protein 2 (TNIP2) is an TNFAIP3 adaptor protein that contributes to immunomodulation via inhibiting the activation of NF-κB [20–22]. In a rat model of multiple organ dysfunction syndrome (MODS), the expression of TNIP2 was markedly reduced [20]. In a mouse model of acute pancreatitis (AP), TNIP2 overexpression alleviated AP, as evidenced by reduced levels of TNF-α and IL-6 [21]. TNIP2 may have neuroprotective effects in oxygen and glucose deprivation/reoxygenation (OGD/R)-induced neuronal damage via the TLR4/MyD88/NF-κB pathway [22]. Conversely, TNIP2 was reported to promote inflammation in certain situations [11, 23]. In general, H3K4me3 is described as an activating histone modification that facilitates gene expression. Our previous study showed that TNIP2 mRNA levels were positively associated with the HAMD-17 score [11]. In the present study, we found that enrichment of H3K4me3 at the TNIP2 promoter was negatively correlated with the HAMD-17 score, suggesting that the levels of H3K4me3 and the TNIP2 mRNA have an inverse relationship. We propose that this relationship acts in concert with the significantly lower level of H3K4me3 at the TNIP2 promoter in patients with MDD compared with healthy controls.

H3K4 methyltransferase is a histone methyltransferase (HMTase) that can specifically methylate the lysine 4 residue on histone H3 and has been implicated in the transcriptional upregulation of different target genes [24]. Dysregulation of H3K4 methylation may affect learning and memory and impair cognitive function in neurologic or psychiatric disorders [25–27]. The ASH1-like histone lysine methyltransferase, ASH1L, contains a catalytic SET domain that functions to methylate histone H3K4 and activate gene expression. ASH1L was shown to upregulate TNFAIP3 by inducing H3K4 methylation at the TNFAIP3 promoter, and thereby suppress TLR-triggered IL-6 production [28]. However, in the Wistar-Kyoto (WKY) rat model of major depressive disorder, ASH1L was found to be significantly increased in the amygdala of the WKY More Immobile sub-strain [29]. Moreover, in MDD patients who show a response to antidepressants, ASH1L expression was found to be significantly decreased [30].

MLL1 (mixed lineage leukemia 1), also known as KMT2A, is an H3K4 methyltransferase that can selectively downregulate the expression levels of a subset of NF-κB target genes, including NFKBIA, TNFAIP3, IRF1, CXCL1, CCL2, and TRAF1 [31]. MLL1 is a key mediator of macrophage classical activation, wherein cytokine and TLR signals drive macrophages toward a local proinflammatory state [32]. Altered synaptic plasticity was reportedly seen under conditional deletion of MLL1 in mouse
prefrontal cortex neurons, and this deletion is associated with increased anxiety and impaired working memory [33]. In contrast, expression of a mutant MLL1 in mouse ventral striatal neurons increased anxiety- and depression-related behaviors [34]. Additionally, lysine-specific demethylase 1 (LSD1/KDM1A), which functions to remove methyl groups from H3K4me1/2, shares a similar structure and mechanism with monoamine oxidase (MAO). Antidepressants that inhibit MAO, including tranylcypromine, phenelzine, and pargyline, have been shown to inactivate LSD1 [8].

A number of intracellular microRNAs (miRNAs) have been found to negatively regulate the immune and inflammatory responses by targeting TLR signaling; these include miR-21, miR-146, miR-155, and let-7 [35, 36]. In our previous study, we found that the levels of let-7e, miR-146a, and miR-155 were lower in MDD patients than in healthy controls; and that the expression levels of let-7e and miR-146a before treatment were negatively correlated with the severity of depression, whereas the level of miR-155 was positively correlated with pretreatment depression severity [12, 13]. In a chronic unpredictable mild stress (CUMS)-induced rat model of depression, miR-146a ameliorated depression-like behaviors by inhibiting microglial activation and reducing neuroinflammatory protein levels in the hippocampus of depressed mice [37]. miR-146a-5p, which is secreted in extracellular vesicles from microglia, was found to suppress neurogenesis in the hippocampal dentate gyrus through the KLF4/P-STAT3/CDKL5 pathway [38]. miRNA-encoding genes are reported to be regulated by epigenetic modifications, including DNA methylation and histone modifications [39]. Our present data support this notion.

Limitations

The present work has several limitations that deserve attention. First, the sample size was relatively small, especially that of male patients and 4 weeks of antidepressant treatment may not be long enough to observe epigenetic changes. Second, interpersonal cellular heterogeneity confounded and compromise the outcomes. Third, the marginally significant correlation obtained from the regression analysis may affect our confidence in the interpretation. Fourth, the mechanism by which H3K4me3 acts on the promoters of TNFAIP3, TLR4, miR-146a, miR-155, and TNIP2 to alter inflammatory processes remains unclear and will require further investigation. Finally, we herein examined histone modification without simultaneously examining mRNA expression levels within the same samples. There might be a bias in the correlation between histone modification and downstream mRNA expression.

Conclusion

In conclusion, our present results suggest that aberrant epigenetic modification in the promoter regions of TNFAIP3, TLR4, TNIP2, miR-146a, and miR-155, may contribute to dysregulation of downstream mRNA expression among MDD patients. Further investigation is required to identify the mechanisms underlying the association between epigenetic dysregulation and depression.

Declarations
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**Author Contributions** Y.-Y.H. and S.-C.W. designed the study and enrolled patients. Y.-Y.H. and C.-C.T. and analyzed data. Y.-Y.H. and C.-C.T. wrote the manuscript. Y.-C.Y., H.-C.F., C.-K.C., H.-Y.K. consulted and supported the performance and evaluation of chromatin immunoprecipitation. All authors read and edited the manuscript, and approved the final version.

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**Data Availability** Medical information for these participants is protected, so only processed de-identified data will be made available upon receipt of reasonable request to the corresponding author.

**Ethics Approval** Institutional Review Board approval was obtained from the hospital ethics committee (201901894A3).

**Consent to Participate** All participants in this study provided informed written consent.

**Consent for Publication** The authors approved the publication of article.

**Competing Interests** The authors declare no competing interests.

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