Glucocorticoid Receptor Gene (*NR3C1*) Expression in the Pathogenesis of Depression in Cancer

ABSTRACT

Background: This study aimed to compare the *NR3C1* expression among cancer patients with major depressive disorder (cancer depression), cancer patients without major depressive disorder (cancer non-depression), and major depressive disorder patients without cancer (general depression), as a preliminary investigation of epigenetic changes in the glucocorticoid receptor gene.

Methods: From May 2019 to November 2019, patients were recruited from the Department of Psychiatry, Cancer Center in Busan, Korea. For gene expression studies, primers were designed using the Primer3 web tool (http://frodo.wi.mit.edu/primer3), and amplification reactions were performed.

Results: Expression levels of *NR3C1* were lower in cancer depression and general depression than in cancer non-depression group. Given that we observed downregulation of the *NR3C1* gene expression in depressive patients regardless of cancer status, it appears that methylation changes in *NR3C1* may contribute to the pathophysiology of depression.

Conclusion: The results of this study imply that the expression of *NR3C1* may be decreased in major depressive disorder.

Keywords: Cancer, depression, gene expression, glucocorticoid receptor, NR3C1

Introduction

Epigenetic changes in glucocorticoid signaling genes have been suggested to play a major role in the pathophysiological changes seen in the hypothalamic–pituitary–adrenal (HPA) axis-mediated stress response during the development of depression.[1,2] Oberlander et al[3] reported that the methylation status of the glucocorticoid receptor gene (*NR3C1*) in newborns was sensitive to prenatal maternal mood and may represent an epigenetic process linking HPA stress reactivity during infancy with depression. Hypothalamic–pituitary–adrenal axis gene (*NR3C1*, *CRH*, *CRHR1*, and *CRHR2*) DNA methylation levels in saliva samples among adolescent girls were associated with major depressive disorder (MDD).[4] Furthermore, in a cross-sectional study, *NR3C1* hypermethylation was associated with internalizing psychopathology and social environmental stressors, such as being bullied or lacking friends during adolescence.[5]

Especially for cancer patients, complex changes in bidirectional communication between the HPA axis and inflammatory process, resulting in hyperactivation of the HPA axis, hypercortisolemia, glucocorticoid resistance, and a surge of proinflammatory immunological factors, are a hallmark of depression.[6] Chronic stressors in the tumor itself (e.g., proinflammatory cytokines), or that onset during the course of tumor treatment, can disrupt homeostasis of the HPA axis in cancer patients.[6] The nutritional insecurity and lifestyle risk factors related to *NR3C1* DNA methylation can act as a mediator of depressive symptoms, especially in cancer patients.[7,8] Epigenetic changes in glucocorticoid signaling, associated with genetic predisposition or environmental stressors, may also contribute to the pathophysiology of depression in cancer patients.[9,10]

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However, research aimed at understanding the roles of the glucocorticoid receptor gene NR3C1 in depression in cancer patients is lacking.

This study aimed to compare the NR3C1 expression among cancer patients with MDD, cancer patients without MDD, and depressive patients without cancer, as a preliminary investigation of epigenetic changes in the glucocorticoid receptor gene.

Methods

Patient Enrollment

From May 2019 to November 2019, patients were recruited from the Department of Psychiatry, Cancer Center in Busan, Korea. The inclusion criteria were as follows: (1) patients aged > 19 years; (2) cancer patients with a clinical diagnosis of MDD ["cancer depression" (CD) group; Hamilton Depression Rating Scale-17 (HAMD-17) score ≥14] or without MDD ["cancer non-depression" (CND) group; HAMD-17 score ≤ 7]; and (3) patients able to read and write Korean. Patients with MDD recruited from the general population (general depression; GD group) were recruited as controls. They had a HAMD-17 score ≥ 14 and no Axis I disorders other than MDD. The exclusion criteria for this study were uncontrolled or unstable physical condition, such as poor functioning of the musculoskeletal system or bedridden status; pregnant or breastfeeding; and any of the following comorbid neuropsychiatric conditions: schizophrenia, bipolar disorder, dementia, severe cognitive disorders, or organic brain disease.

A total of 20 patients participated in this study (CD group, n = 7; GD group, n = 9; CND group, n = 4).

Assessments

Major depressive disorder was diagnosed via clinical interviews, based on the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 diagnostic criteria, using the HAMD-17. Patients were diagnosed with MDD if they had depressed mood, loss of interest, or both, accompanied by at least 4 other symptoms of depression over a 2-week period. All psychiatric interviews and psychometric tests were performed by the same experienced psychiatrist.

Data on patients' age, sex, type of residence, occupation, education level, psychiatric history, and smoking and alcohol drinking status were obtained. Medical charts were consulted for clinical data related to cancer site, stage, surgical treatment, and chemotherapy and radiation treatment status. Depression severity was measured using the HAMD-17, Hospital Anxiety and Depression Scale (HADS), and the Scale for Suicidal Ideation.

Gene Expression Analysis

A 5-mL peripheral blood sample was collected from each participant. The blood was treated with ethylenediaminetetraacetic acid

MAIN POINTS

- Expression levels of NR3C1 were lower in major depressive disorder with/without cancer than in cancer patients without major depressive disorder.
- We observed downregulation of NR3C1 gene expression in depressive patients regardless of cancer status.
- Methylation changes in NR3C1 may contribute to the pathophysiology of depression.

(EDTA) as an anticoagulant. Total RNA was isolated from the blood samples using TRI-Solution (BioScience Technology, Rockaway, NJ, USA) following the manufacturer's protocol. RNA quantity was measured using a NanoDrop 2000/2000c instrument (Thermo Scientific, Waltham, Mass, USA), and 1 µg of total RNA was reverse transcribed into cDNA using the iScript™ cDNA Synthesis kit (Bio-Rad, Hercules, Calif, USA). For gene expression studies, primers were designed using the Primer3 web tool (http://frodo.wi.mit.edu/primer3), and amplification reactions were performed in a total volume of 25 µL, which contained 200 ng of cDNA, primers, dNTPs, and 0.5 U Taq DNA polymerase. The polymerase chain reaction (PCR) conditions for NR3C1 and the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were as follows: 35 cycles at 95°C for 30 s, 54°C for 30 s, and 72°C for 30 s. The primers used were as follows: NR3C1, forward 5′-AGAACCCCAAGAGTTCAGCA-3′ and reverse 5′-GGGACCC AGAAGAAAACTC C-3′; GAPDH, forward 5′-AAGGTCGGAGTCAACGG ATTT-3′ and reverse 5′-GCAGTGA GGGTCTCTCTCCT-3′. The amplified products were resolved by 1% agarose gel electrophoresis, stained with ethidium bromide, and photographed under ultraviolet (UV) illumination.

Statistical Methods

All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) for Windows software v.18 (SPSS Inc.; Chicago, IL, USA). For the normality test, both the Kolmogorov–Smirnov test and the Shapiro–Wilk test were used. Also, since it was a small experiment with less than 10 people per group, it was not possible to assume a normal distribution. Therefore, the Fisher's exact test or the Fisher Freeman Halton test (for tables larger than 2×2) were used to analyze categorical variables; the Kruskal–Wallis test was used to analyze continuous variables and Mann–Whitney test was used as post-hoc comparison for $P < .05$. P-values $< .05$ were considered to indicate statistical significance.

Ethics and Patient Consent

The present study was conducted according to the Declaration of Helsinki. Approval to conduct the present study was obtained from Dongnam Institute of Radiological and Medical Sciences (D-1904-001-002). Informed written consent was obtained from all participants.

Results

Demographic and Clinical Characteristics by Cancer and Depression Status

A total of 20 patients participated in the present study. There were no significant differences among the groups in any demographic or clinical characteristics, except depression-related scale scores (Table 1). The HADS scores [total scores, 21.00 (15.00-28.00) and 23.00 (9.00-26.00) vs. 1.50 (1.00-7.00), $P = .015$; depression subscale scores, 10.00 (7.00-15.00) and 12.00 (3.00-14.00) vs. 1.50 (1.00-7.00), $P = .014$] and HAMD scores, 23.00 (19.00-30.00) and 20.00 (19.00-28.00) vs. 0 (0.00-2.00), $P = .007$] of the CD and GD groups were higher than those of the CND group, although there was no significant difference in scores between the CD and GD groups.

Gene Expression Data of the Cancer Depression, General Population, and Cancer Non-Depression Groups

Total RNA was isolated from the serum samples of patients, and reverse transcription (RT)-PCR analysis was performed to examine

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Table 1. Demographic and Clinical Characteristics of the Patients by Cancer and Depression Status

	Cancer Depression (n = 7)	General Depression (n = 9)	Cancer Non-depression (n = 4)	P	Post-Hoc Comparison
Age, median (min–max)	58.00 (43.00-66.00)	54.00 (33.00-65.00)	58.50 (44.00-69.00)	.555	
Sex [female, n (%)]	7 (100)	8 (88.9)	3 (75.0)	.668	
Type of residence [n (%)]					
Living alone	1 (14.3)	0	0	.550	
Occupation [n (%)]					
Yes	1 (14.3)	5 (55.6)	1 (25.0)	.311	
No	4 (57.1)	1 (11.1)	2 (50.0)		
Retirement/student/housewife	2 (28.6)	3 (33.3)	1 (25.0)		
Education, median (min–max)	9.00 (9.00-16.00)	14.00 (6.00-18.00)	12.00 (6.00-14.00)	.428	
Neuropsychiatric history [n (%)]	0	3 (33.3)	1 (25.0)	.280	
Smoking [n (%)]				.200	
Nonsmoking	7 (100)	9 (100)	3 (75.0)		
Recently quit	0	0	1 (25.0)		
Smoking	0	0	0		
Alcohol [n (%)]					
≤Once a week	7 (100)	8 (88.9)	4 (100)	>.999	
Cancer site [n (%)]				.606	
Head and neck	0		1 (25.0)		
Breast	3 (42.9)		3 (75.0)		
Lung	2 (28.6)		0		
OBGY	1 (14.3)		0		
GI	1 (14.3)		0		
Cancer stage [n (%)]				.591	
I	3 (42.9)		0		
II	1 (14.3)		2 (50.0)		
III	1 (14.3)		1 (25.0)		
IV	2 (28.6)		1 (25.0)		
Surgical treatment [n (%)]	5 (71.4)		3 (75.0)	.721	
Chemotherapy [n (%)]	4 (57.1)		4 (100)	.236	
Radiation treatment [n (%)]	5 (71.4)		2 (50.0)	.576	
HADS, median (min–max)	21.00 (15.00-28.00)	23.00 (9.00-26.00)	1.50 (1.00-7.00)	.015*	CD = GD > CN
HADS—depression	10.00 (7.00-15.00)	12.00 (3.00-14.00)	1.50 (1.00-7.00)	.014*	CD = GD > CN
HADS—anxiety	11.00 (5.00-13.00)	11.00 (4.00-13.00)	4.50 (0-7.00)	.087	
HAMD, median (min–max)	23.00 (19.00-30.00)	20.00 (19.00-28.00)	0 (0.00-2.00)	.007*	CD = GD > CN
Scales for suicide ideation, median (min–max)	4.00 (0-15.0)	1.00 (0-15.0)	0 (0-2.00)	.176	

CD, cancer depression; GD, general depression; OBGY, Obstetric gynecology; GI, Gastro-Intestinal; CN, cancer non-depression; HADS, Hospital Anxiety and Depression Scale; HAMD, Hamilton Depression Rating Scale.
*P < .05.

the NR3C1 gene expression. As shown in Figure 1, the NR3C1 gene expression was lower in the CD and GD groups than in the CND group. In the latter group, the NR3C1 gene was expressed in all samples except sample 3. However, the NR3C1 gene was expressed in only 3 of the samples (4, 5, and 6; 42.9%) in the CD group and in only 4 samples (2, 4, 5, and 8; 44.4%) in the GD group.
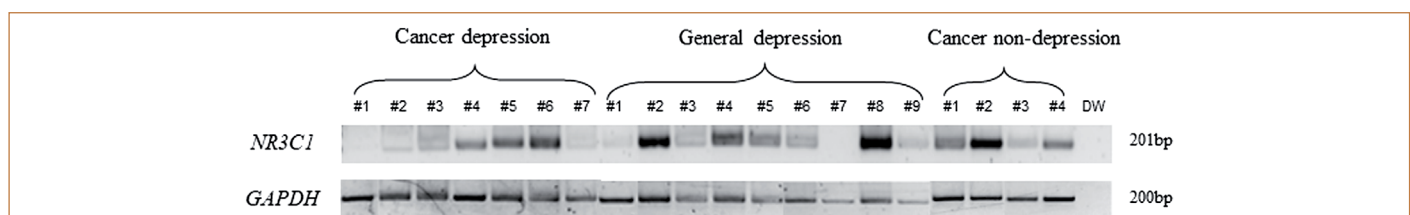

Figure 1. RT-PCR analysis of the NR3C1 gene expression in serum samples of depressive patients with and without cancer. GAPDH served as a control for RNA quality and loading. DW, water control (no cDNA addition); GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction.

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Discussion

Expression levels of *NR3C1* were lower in cancer patients with depression than in cancer patients without depression. In addition, levels of *NR3C1* gene expression in depressed patients without cancer were more similar to those of cancer patients with depression than to those of cancer patients without depression. These data imply that the *NR3C1* gene expression may be associated with depressive symptoms regardless of the presence of cancer. Given that we observed downregulation of the *NR3C1* gene expression in depressive patients regardless of cancer status (compared to non-depressive patients with cancer), it appears that methylation changes in *NR3C1* may contribute to the pathophysiology of depression.

Although the *NR3C1* gene is associated with methylation status in depression, the role of DNA methylation changes in *NR3C1* in depression remains controversial.[11] Melas et al[12] reported that *NR3C1* methylation in saliva samples was relatively high in a population with depression associated with childhood adversity versus a control group. Na et al[13] found that MDD patients had significantly lower *NR3C1* promotor methylation compared to healthy controls. Elsewhere, changes in HPA reactivity mediated by altered glucocorticoid receptor gene expression were studied in the prenatal and postnatal environment; *NR3C1* 1F promoter methylation was higher in infants who had been exposed to maternal depression in utero.[14] In contrast, Kim et al[15] reported weak associations between psychological factors and *NR3C1* gene methylation. Therefore, further studies are necessary to examine the association of *NR3C1* gene expression with promoter methylation in larger samples of individuals with depressive symptoms.

This study had several limitations. First, it was a preliminary investigation that included only a small sample size. Second, no healthy control group was included in the study. Third, we examined only gene expression; no analyses of underlying mechanisms, such as methylation, were conducted.

Taken together, the results of this study imply that the expression of *NR3C1* may be decreased in MDD. The greater severity of depression in cancer patients may be associated with downregulation of *NR3C1*. Further studies are necessary to examine the association of *NR3C1* gene expression with promoter methylation in larger samples of individuals with depressive symptoms.

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