Abnormal Retinoid and TrkB Signaling in the Prefrontal Cortex in Mood Disorders

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The prefrontal cortex shows structural and functional alterations in mood disorders. Retinoid signaling, brain-derived neurotrophic factor (BDNF), and its receptor TrkB are reported to be involved in depression. Here, we found that mRNA levels of key elements of retinoid signaling were significantly reduced in the postmortem dorsolateral prefrontal cortex/anterior cingulate cortex (ACC) from elderly depressed patients who did not die from suicide. Decreased mRNA levels of BDNF and TrkB isoforms were also found. Similar alterations were observed in rats subjected to chronic unpredictable mild stress. Along with neurons immunopositive for both retinoic acid receptor-α (RARα) and TrkB, a positive correlation between mRNA levels of the 2 receptors was found in the ACC of control subjects but not of depressed patients. In vitro studies showed that RARα was able to bind to and transactivate the TrkB promoter via a putative RA response element within the TrkB promoter. In conclusion, the retinoid and BDNF-TrkB signaling in the prefrontal cortex are compromised in mood disorders, and the transcriptional upregulation of TrkB by RARα provide a possible mechanism for their interaction. The retinoid signaling pathway that may activate TrkB expression will be an alternative novel target for BDNF-based antidepressant treatment.

Keywords: anterior cingulate cortex, brain-derived neurotrophic factor, dorsolateral prefrontal cortex, mood disorder, retinoic acid receptor-α

Introduction

Dysfunction of the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) is associated with the pathogenesis of mood disorders. Neuroimaging studies have shown both structural and functional changes in the DLPFC and ACC in depression, such as volume or gray matter thickness changes and altered glucose metabolism or blood flow (Drevets, Price, et al. 2008; Drevets, Savitz, et al. 2008). Moreover, antidepressant treatment by transcranial magnetic stimulation of the DLPFC or deep brain stimulation of the ACC, has led to a significant reduction of depressive symptoms in therapy-resistant depressive patients (Mayberg et al. 2005; Padberg and George 2009).

Retinoids, a family of molecules consisting of vitamin A and its derivatives, play a key role in brain development (Maden 2002). Emerging evidence now suggests that the retinoid signaling may also be required for adult brain functioning (Lane and Bailey 2005). Evidence for an association between retinoid and depression comes from case reports showing that onset of the depressive symptoms occurred after the use of isotretinoin (a 13-cis isomer of all-trans retinoic acid (RA) to treat severe cystic acne), while the symptoms subsided once drug treatment was discontinued (Bremner and McCaffery 2008; Bremner et al. 2012). Studies showed that long-term RA administration to rats led to hypothalamic–pituitary–adrenal (HPA)-axis hyperactivity and increased anxiety/depression-like behaviors (Cai et al. 2010). Furthermore, the density of retinoic acid receptor α (RARα, the primary RA receptor)-positive neurons, corticotropin-releasing hormone (CRH, the central driving force of HPA-axis activity)-positive neurons and RARα-CRH double-labeled neurons were significantly upregulated in the hypothalamic paraventricular nucleus (PVN) of subjects with mood disorders (Chen et al. 2009). These results provided a possible mechanism for the involvement of RA in depression as the HPA axis is considered to play a key role in the development of depression (Swaab et al. 2005; Bao et al. 2008). In addition, patients with Isotretinoin treatment for acne showed by positron emission tomography scans a significantly decreased metabolism in the orbitofrontal cortex (Bremner et al. 2005)-part of the prefrontal-limbic network associated with the pathophysiology of depression (Bennett 2011), which might also at least partly explain the close relationship between RA and depression.

A wide range of basic and clinical studies revealed that brain-derived neurotrophic factor (BDNF)-TrkB signaling played a critical role in the pathogenesis of depression. Studies have reported that depressed suicide victims exhibited low hippocampal and prefrontal cortex BDNF levels (Dwivedi et al. 2003) and reduced activity-dependent BDNF expression by hypermethylation of promoter/exon IV of the BDNF gene (Keller et al. 2010). TrkB is implicated in the dysfunction of the PFC in mood disorders, that is, abnormal TrkB mRNA and protein levels in the PFC in the depressed suicide victims (Dwivedi et al. 2003). Furthermore, a recent study with a large sample size delicately showed reduced TrkB mRNA level in the subgenual ACC of patients with major depressive disorder (MDD) (Tripp et al. 2012).

To date, there is no information available on the expression pattern of proteins involved in the retinoid signaling in the human PFC nor on their alterations in mood disorders. Additionally, even though both retinoid and BDNF-TrkB signaling were known to be associated with depression, it is not clear whether an interaction between the 2 pathways play a role in the development of depression as they share similarities in neuroplasticity and neurotrophic effects in the central nervous system. In the present study, we investigated 1) the mRNA expression levels of major components in the endogenous retinoid signaling, BDNF and TrkB in the gray matter from the DLPFC and the ACC of depressed patients who did not die from suicide and their matched nonpsychiatric controls; 2) whether similar changes are present in rats subjected to chronic...
unpredictable mild stress (CUMS), a robust paradigm that increases anxiety/depression-like behaviors often observed in depression (Willner 1997), to further support the results from human postmortem material and 3) in vitro, we studied a possible underlying molecular mechanism for the relationship between the retinoid and BDNF-TrkB signaling.

Materials and Methods
(Extensive methods are available in the Supplementary Material)

Part 1: Quantitative Real-Time PCR Study and Double-Labeling Study in the Postmortem Human prefrontal cortex

Subjects
Frozen postmortem brain material and paraffin sections came from elderly depressed patients who did not die from suicide and matched controls who did not have a psychiatric or neurological disease, as described before (Qi et al. 2013), were obtained via the Netherlands Brain Bank with informed written consent from the patients or their next of kin for the autopsy and use of brain material and clinical files for research purposes. The patients with mood disorders were clinically diagnosed with either bipolar disorder (BDP) or MDD on the basis of the presence and severity of their symptoms and with exclusion of other psychiatric and neurological disorders, which were systemically scored by 3 qualified psychiatrists (Drs W.J.G. Hoogendijk, E. Vermette, and G. Meynen) according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria. The absence of neuropathological changes in all subjects was confirmed by systematic neuropathological investigation (Van de Nieuwes et al. 1998). Mood disorder patients and controls were one-by-one matched for sex and group-matched for age, postmortem delay (PMD), clock time and month of death, cerebrospinal fluid (CSF)-pH, and brain weight. The samples were obtained from the DLPCF (Brodman area 9) of 14 mood disorder patients (sex, 10M/4F; age, 72.8 ± 3.1 (mean ± SEM) ) and 14 matched controls (10M/4F; 74.6 ± 3.0), and ACC (Brodman area 24) of 12 mood disorder patients (9M/3F; 74.7 ± 3.9) and 12 matched controls (9M/3F; 79.5 ± 3.0). Detailed clinico-pathological information and P-values of matched parameters are given in Supplementary Materials 1 and 2.

Gray matter containing all 6 layers was isolated from 50-μm-thick cryostat sections cut from snap-frozen DLPCF/ACC tissue as described before (Bosiers et al. 2010).

RNA Isolation, cDNA Synthesis, and Quantitative Real-Time PCR
RNA isolation, cDNA synthesis and quantitative real-time PCR (qPCR) were as described before (Wang et al. 2008). RNA integrity number (RIN) value, an indicator of isolated RNA quality, was determined and no difference was found between the depressed and control groups (RIN values of the DLPCF from the mood disorder group: 7.5 ± 0.2 and the control group: 7.7 ± 0.2; RIN values of the ACC from the mood disorder group: 7.3 ± 0.2 and the control group: 7.7 ± 0.2; mean ± SEM, see Supplementary Material 2). The genes detected are RA synthesizing enzymes: retinol dehydrogenase 1, 2, 3 (RALDH1, 2, 3); RA metabolic enzymes: cytochrome P450, family 26, A1, B1, C1 (CYP26A1, B1, C1); RA cellular transport proteins: cellular RA binding protein 1, 2 (CRABP1, 2); receptors mediating RA function: RAR-α, β, γ and retinoid X receptor-α, β, γ (RXRa, β, γ); BDNF; TrkB1 (full length isoform); and TrkB2 (truncated isoform). The mRNA level of β-actin was analyzed as an internal control.

Chromatin Immunoprecipitation Assay for Rat Anterior Cortex
Chromatin Immunoprecipitation (ChIP) assay was performed to determine whether RARs can bind to the TrkB promoter in rat anterior cortex. For ChIP assay, male SD rats were decapitated, and the anterior cortex was removed as described before. Solution with soluble chromatin by PCR were performed with the following primer pair: 5′-CCATGCTCATAGG-3′ and 5′-CAAACGGCAGGAGAAAGA-3′, which are targeted to the promoter region from −447 to −6 M).

Part 2: Chronic Unpredictable Mild Stressed Rat Study
Chronic Unpredictable Mild Stress Protocol and Open-Field Tests
Male Sprague-Dawley (SD) rats were randomly split into 2 matched groups based on their baseline body weight: an undisrupted control group (n = 6) and a CUMS group (n = 7). The control group were handled every 2 days and the CUMS group were subjected to the stress regime consisted of daily exposure to short-term stressors along with long-term stressors for 5 weeks. Short-term stressors were administered between 09:00 and 12:00 h and long-term stressors began immediately after cessation of morning stressors and ended with initiation of the next day’s morning stressor. The CUMS protocol and open-field test to measure anxiety/depression-like behaviors were performed as described previously (Wu et al. 2007; Chen et al. 2009) with minor modifications. Animal housing, care, and application of experimental procedures used were in accordance with all relevant local guidelines and legislation and were approved by the Institutional Animal Use and Care Committee of University of Science and Technology of China.

Anterior Cortex Dissection and Sample Preparation
All rats were decapitated after the last open-field tests. Blood samples were collected in tubes with heparin sodium as an anticoagulant and centrifuged at 4°C. Plasma was obtained and stored at −80°C until corticosterone was assayed by means of a rat corticosterone enzyme-linked immunosorbent assay (ELISA) kit (RapidBio Lab, Calabasas, CA, USA). The brain was removed immediately and the anterior cortex was dissected as described before (Meng et al. 2011) including the anterior part of the frontal lobes dissected between Bregma 3.2 and Bregma 5.2 according to coordinates of Paxinos and Watson (Paxinos and Watson 1998) and excluding their basal parts at the level of the rhinal fissures, and quickly frozen in liquid nitrogen. Tissue samples were homogenized in cold Trizol (Invitrogen, Carlsbad, California) for further RNA extraction according to the manufacturer’s instructions.

Quantitative Real-Time PCR
After cDNA synthesis, qPCR was performed to analyze the mRNA expression levels of RALDH1, 2, 3; CYP26A1, B1, C1; CRABP1, 2; RARs, β, γ; RXRa, β, γ; BDNF; TrkB1 (full length isoform); and TrkB2 (truncated isoform). The mRNA level of β-actin was analyzed as an internal control.

Immunohistochemistry of RARs and TrkB in Postmortem Human ACC
We would like to check whether RARs and TrkB could in principle localize within the same cells, which is, of course, a prerequisite for a direct interaction between them. In a series of pilot studies, trying out different techniques, we found that it was quite impossible to use double-labeling immunofluorescence in the sections, as we did for other purposes. The only solution was immunohistochemical staining of RARs and TrkB in adjacent sections to check for co-localization.

Photographs were collected by Zeiss axioskop microscope (Zeiss, Jena, Germany) with neofluar objectives (Zeiss), a motorized XYZ stage, and a black and white camera (Sony, Minato, Japan).

Part 3: In Vitro Study in Cell Lines
Cell Culture
SH-SY5Y neuroblastoma cells and Chinese hamster ovary (CHO) cells were grown in 1:1 mixture of DMEM and Ham’s F-12 medium (DF; Sigma-Aldrich, St. Louis, MI, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA). All cells were cultured in 5% CO2humidified atmosphere at 37°C. RA (Sigma-Aldrich), actinomycin D (ActD, Sigma-Aldrich) and Ro 41-5253 (Sigma-Aldrich) were dissolved in DMSO and the final concentrations used were RA (10−6 M), ActD (1 μg/mL), and Ro 41-5253 (10−8, 10−7, and 10−6 M).
**Plasmids**

The human wild-type TrkB promoter luciferase reporter construct (pGL3-TrkB-wt) was a generous gift from Carme Gallego (Liu et al. 2004). After an examination of TrkB promoter sequence by the MatInspector from Genomatix software suite (version 2.0) for transcription factor binding sites search, a sequence from the promoter (5'-TTAAGGTCTACTGACAAGAGGCGG-3') was predicted as a putative retinoic acid response element (RARE) motif. Then the putative RARE was deleted to get the RARE-deleted TrkB promoter luciferase reporter construct (pGL3-TrkB-d) by the MutanBEST Kit (TaKaRa, Dalian, China) using the primer pair as follows: 5'-GGAGTTTTACGTGGCTGAGACCA-3' and 5'-GCGGAGAGTTAAGCTTGAGACACTT-3'.

**PcDNA4-RARα** was donated by Xiao-Ning Chen (Chen et al. 2009).

**ChIP Assay for SH-SY5Y Cells**

Formaldehyde was added into the culture medium to achieve a final concentration of 1% to crosslink proteins with the DNA after SH-SY5Y cells were treated with RA for 24 h. The ChIP assays were performed as described before by using the following primer pair for PCR amplification: 5'-ACAACCCCGGAAGAGCTG-3' and 5'-CCGGGGCTACGCTGGCTA-3', targeting the promoter region from −927 to −575 bp of the TrkB gene.

**Dual Luciferase Reporter Gene Assay**

Transfection was accomplished with Lipofectamine 2000 (Invitrogen). 24 h before transfection, CHO cells were seeded into 24-well plates (6×10⁴ cells/well) and cultured in the phenol red-free DF medium, supplemented with 10% dextran-coated charcoal (DCC, Sigma-Aldrich)-treated FBS. Cells of each well were transfected with either pGL3-TrkB-wt and pcDNA4, or pGL3-TrkB-wt and pcDNA4-RARα, or pGL3-TrkB-d and pcDNA4-RARα, all together with the pRL-TK plasmid (Promega, Madison, WI, USA) which contained Renilla luciferase gene as an internal control. Six hours later, culture media were changed to phenol red-free DF containing 10% DCC-treated FBS with 10⁻⁶ M RA. After another 24 h, cells were harvested for detecting luciferase activity.
with the dual luciferase reporter assay system (Promega). Relative luciferase activity is presented as firefly luciferase activity normalized to renilla luciferase activity.

**Statistical Analysis**

For postmortem human qPCR data, analysis of covariance (ANCOVA) was carried out with SPSS (version 17.0, SPSS Incorporation). The ANCOVA model, used for the expression level of each gene, revealed the diagnostic group as a main effect and PMD and CSF pH as covariates. Correlations between mRNA expression levels were tested by the Spearman’s correlation coefficient. For animal study, the differences between the control and the CUMS group were determined by unpaired Student’s t-test. For in vitro study, statistical analyses comprised a one-way ANOVA followed by a Student Newman–Keuls post hoc test. All tests were 2 tailed. P-values ≤ 0.05 were considered to be significant.

**Results**

**Altered mRNA Expression Levels in Patients with Mood Disorders**

In the DLPFC (Fig. 1A–F), compared with the matched control, we found a significant decrease in the mRNA levels of RALDH1 (23.1% reduction, $F_{1,20} = 7.804, P = 0.011$), RALDH3 (16.3% reduction, $F_{1,20} = 7.085, P = 0.015$), RXRα (49.9% reduction, $F_{1,20} = 13.945, P = 0.001$), RXRβ (34.6% reduction, $F_{1,20} = 6.507, P = 0.019$), TrkB.T1 (15.8% reduction, $F_{1,20} = 5.605, P = 0.028$), and BDNF (19.6% reduction, $F_{1,20} = 4.359, P = 0.050$).

In the ACC (Figure 1G–L), compared with their matched control a significant reduction was found in the mRNA levels of RALDH1 (32.1% reduction, $F_{1,18} = 5.622, P = 0.029$), RALDH3 (29.4% reduction, $F_{1,18} = 10.513, P = 0.005$), CRABP1 (11.4% reduction, $F_{1,18} = 7.876, P = 0.012$), TrkB.Fl (14.5% reduction, $F_{1,18} = 8.793, P = 0.008$), TrkB.T1 (26.7% reduction, $F_{1,18} = 4.744, P = 0.043$), and BDNF (21.1% reduction, $F_{1,18} = 8.400, P = 0.010$).

Other genes (RALDH2; CYP26A1, B1, C1; CRABP 2; RAR α, β, γ; RXRγ) did not show any significant changes in mRNA expression levels (data not shown).

**Altered mRNA Expression Levels in Rats Subjected to Chronic Unpredictable Mild Stress**

To further confirm the phenomenon we saw in human post-mortem DLPFC/ACC, a well-established CUMS rat model was used, and expression levels of the same genes were detected. After 5-week stress, the average weight of the CUMS group was significantly less compared with that of the control group.

Figure 2. Chronic unpredictable mild stressed rat study. Body weight (A) and open-field tests data (B–F) such as total distance (B), the time in the central 4 squares (C), the frequency to the central 4 squares (D) and the number of rearing (E) were significantly decreased while the number of defecation (F) was significantly increased in the CUMS rats compared with the control rats. Plasma corticosterone concentration (G) showed a trend for an increase in the CUMS rats. Relative mRNA expression levels of RALDH1, RALDH3, CRABP1, RXRα, RXRβ, TrkB subtypes, and BDNF (H) were decreased in the anterior cortex of the CUMS rats. All data are mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; compared with the control group; 2-tailed unpaired Student’s t-test; n = 7 for the CUMS group; and n = 6 for the control group.
RARα is Associated with TrkB in Human Postmortem ACC

In the ACC, mRNA level of TrkB was significantly positive correlated to that of RARα in the control group \( (P = 0.003, r = 0.783) \) while this correlation was not present in the group of mood disorders \( (Fig. 3A, B) \). In addition, the same neurons in adjacent human ACC sections \( (Fig. 4A, B) \) were found to be immunopositive for both RARα \( (Fig. 4C, E, G) \) and TrkB \( (Fig. 4D, F, H) \), respectively, demonstrating the co-localization of the 2 receptors within the same neurons.

However, such positive correlation between mRNA levels of TrkB and RARα was not observed in the control or the CUMS rats.

**RARα Interacts with the TrkB Promoter Region and Activates the TrkB Promoter**

We found that most of the enhanced effect of RA on TrkB mRNA level in the SH-SY5Y cells was significantly inhibited by the pretreatment with the transcription inhibitor ActD \( (Fig. 5A) \). Furthermore, RA-induced upregulation in TrkB mRNA expression was blocked by RARα-specific antagonist, Ro 41-5253, in a dose-dependent manner, indicating that RARα is involved in this increased transcriptional activity \( (Fig. 5B) \). In addition, we performed a ChIP assay, in the rat anterior cortex and in the SH-SY5Y cells, and observed that the TrkB promoter was occupied by endogenous RARα during chromosome immunoprecipitation by RARα antibody while there was no PCR product in the negative control \( (Fig. 5C) \). A luciferase assay was done to explore whether RARα could activate the TrkB promoter. With RA treatment, luciferase expression driven by a wild-type human TrkB promoter was higher in cells co-transfected with pcDNA4-RARα compared with cells co-transfected with the control pcDNA4. After deletion of a putative RARE \( (5’-TTAA GGTTGACCAAGGACGGC-3’) \) in the TrkB promoter, the increased signal was abolished \( (Fig. 5D) \). Taken together, these data demonstrate RARα could regulate TrkB transcription by binding to and transactivating the TrkB promoter via its putative RARE, providing evidence for the close interaction between the 2 receptors.

**Discussion**

In the present study, we found that the mRNA expression of key components in the retinoid signaling, such as RALDH1 and 3, RXRα and β, and CRABP1, was reduced in the DLPFC/ACC of the elderly depressed patients who did not die from suicide. It is of great interest to note that a microarray study showed a lower expression level of RALDH1 in the PFC in schizophrenia, another common psychiatric disorder, which suggested that schizophrenia is associated with the decreased prefrontal accessibility of RA as well \( (Goodman 2005) \). Furthermore, there was also a downregulated expression of BDNF and 2 TrkB isoforms observed in our study. These results extended the previous study of a reduced BDNF and TrkB expression in the DLPFC from young suicide victims \( (average age <45) \) \( (Dwivedi et al. 2003) \) to an elderly population \( (average age >70) \) of depressed patients who did not die from suicide. Last but not the least, a positive correlation was observed between the mRNA levels of RARα and TrkB in the ACC of the control subjects, which might be due to RARα transcriptional upregulation of TrkB gene via a putative RARE within its promoter. However, the correlation was not present in the depressed patients.

The important role played by retinoid signaling in adult brain has been increasingly recognized in recent studies, such as a role in neuroplasticity, spinogenesis, and neurogenesis \( (Lane and Bailey 2005; Shearer et al. 2012) \). BDNF-TrkB signaling also has comparable neuroplasticity and neurotrophic capacities \( (Groves 2007) \). Lower activities in both retinoid and...
BDNF-TrkB signaling, as suggested by the lower expression levels of key proteins in these pathways found in the present study, indicate a disrupted neurotrophic effect and provide a possible molecular basis for the functional and structural impairments in the depressed patients, such as a lower synaptic plasticity, decreased dendrite complexity, spine formation and neurogenesis, which may contribute to the alterations in gray matter volume and neurophysiological activity or cell loss and atrophy in the PFC reported by neuroimaging or neuropathological studies (Rajkowska 2000; Drevets, Price, et al. 2008). Subsequently, similar results were obtained in the CUMS rats which indeed strengthened the results of lower gene expression in human material. Furthermore, the CUMS rats mimicked the symptoms of human depression (such as body weight loss and less locomotor/exploratory activity) and a hyperactive HPA-axis response (as revealed by enhanced plasma corticosterone levels).

Previous studies showed that RA could increase TrkB mRNA level in SH-SY5Y cells and that only after treatment with RA SH-SY5Y cells responded to BDNF by increasing TrkB expression (Kaplan et al. 1993; Lucarelli et al. 1995). However, so far there was no direct evidence on how RA affected TrkB. In the present study, we found that in SH-SY5Y cells RA dramatically enhanced TrkB mRNA level. Such an increase was significantly attenuated by the pretreatment with the transcription inhibitor ActD, suggesting that the upregulated effect of RA on TrkB mRNA level mainly resulted from enhancing TrkB transcription and not from enhancing TrkB mRNA stability (Fig. 5A). Additional evidence demonstrated that this transcriptional upregulation was mediated by RARα (Fig. 5B) as Ro 41-5253, an antagonist selectively binding to RARα without transactivating it (Toma et al. 1998), could attenuate this upregulation. RARα belongs to a nuclear receptor family which is ligand-activated transcription factors (Chambon 1996). Indeed, we subsequently found that RARα could bind to the TrkB gene promoter both in the rat anterior cortex and the cultured cells (Fig. 5C) and that a putative RARE within TrkB gene promoter was crucial for RARα transactivation (Fig. 5D). We also observed a co-localization of RARα and TrkB in the postmortem ACC neurons and found a positive correlation between RARα and TrkB mRNA levels in the postmortem ACC of control subjects which could be explained by a positive transcriptional regulation of TrkB mRNA by RARα. The absence of a correlation between these 2 receptors in the depressed patients might be caused by lower RARα ligand (RA) availability in the ACC, a result from decreased RALDH1 and 3 expression in depression. That is because RARα, when unliganded, will serve as a transcription repressor (Weston et al. 2003) and thus

![Figure 4. Representative microphotographs of RARα and TrkB immunohistochemistry in the human anterior cingulate cortex. Adjacent sections from postmortem human ACC were stained for RARα (A) and TrkB (B). In high magnification the same cell is shown in the left and right panel (C and D, E and F, G and H) labeled in alternating sections for RARα (left) and TrkB (right). Bar = 100 μm in B and 20 μm in H.](http://example.com/image.png)
might not be positively correlated with its downstream effector TrkB. However, we did not find such correlation either in the control rats or in the CUMS rats. The rat anterior cortex we collected is indeed not simply a homologue for the human ACC, but a collection of different cortical areas which might obscure the area-specific correlations between different molecules. In addition, it is also possible that the reduced retinoid signaling in depression contributed to the reduced TrkB expression in the present study.

There were no significant differences between the mood disorder group and controls for the potential confounders in the DLPFC and ACC, such as PMD ($P = 0.550$ and $0.931$, respectively) and CSF pH ($P = 0.073$ and $0.262$, respectively), indicating that they will not have affected our conclusion. The ANCOVA model with PMD and CSF pH as covariates used in our study further controlled for them. Additionally, our human sample size was relatively small. However, it should be noted that clinically and neuropathologically well-documented postmortem material from elderly depressed patients who did not die from suicide with high quality mRNA is very hard to come by. Although BPD and MDD are 2 different psychiatric entities in diagnostic terms, we have combined these subgroups since they share similarities with regard to their neurobiological underpinnings. For example, BPD and MDD have overlapping genetic risk factors (Green et al. 2010; Liu et al. 2011). Furthermore, a proportion of both MDD and BPD patients have hyperactivity of the HPA axis, as shown by an increased number of CRH-expressing neurons and amount of CRH mRNA in the PVN (Raadsheer et al. 1994; Bao et al. 2008; Wang et al. 2008). As mentioned before, neuroimaging studies suggest that BPD and MDD share a few structural and functional cortical alterations (Drevets, Price, et al. 2008). Finally, when we compared the gene expression levels between BPD and MDD subgroups for each brain area, we did not find any significant differences. Therefore, we pooled both BPD and MDD patients and analyzed the data between the mood disorder group and the control group. The results obtained in the mood disorder group were further confirmed in the CUMS rat model, which may partially ease the concerns resulting from the small sample size. Still, the expression pattern of such genes deserves further investigation in a larger sample size. Possible effects of medication on the target gene expression are always a limitation of the use of postmortem human brains. Nevertheless, we found that medication in the last 3 months did not confound our results. For instance, for the genes RALDH1 and TrkB.T1 in the DLPFC (see Supplementary Material 10) no difference was found for the mRNA levels between patients taking antipsychotics (triangles, subjects D3, D12, D13, and D14) and those taking no antipsychotics or antidepressants. The RALDH1 mRNA level of patients taking lithium (circles, subjects D5 and D6) were generally intermingled with other data points. Note that 1 patient who was taking lithium (circle, D6) and 1 patient who was taking selective serotonin reuptake inhibitors (SSRI, square point, D11) showed, respectively, the highest TrkB.T1 level and RALDH1 level. Consequently, in case such medication would have influenced our data, it would rather have increased and not decreased RALDH1 and TrkB.T1 levels as we observed in our present study. The RALDH1 and TrkB.T1 mRNA levels of the patient taking monoamine oxidase inhibitors (cross point, D9) were within the middle range.

Figure 5. RARα interacts with the TrkB promoter region and activates the TrkB promoter. (A) An increase in TrkB mRNA level induced by RA was blocked by the transcription inhibitor, ActD. (B) Transcriptional regulation of RA on TrkB gene was inhibited by RARα-specific inhibitor (Ro 41-5253), in a dose-dependent manner. (C) Chromatin immunoprecipitation was performed in SH-SY5Y cells and rat anterior cortex tissue to study whether the endogenous RARα could bind to TrkB promoter. Chromatin solutions were immunoprecipitated with anti-RARα antibody and final DNA extraction was amplified with primers covering the TrkB promoter as indicated. Similar results were observed in 3 independent experiments. (D) Dual luciferase reporter gene assay was performed in CHO cells. CHO cells were co-transferred with either the wide-type TrkB promoter luciferase reporter construct (pGL3-TrkB-wt) and pcDNA4, or pGL3-TrkB-wt and pcDNA4-RARα, or retinoic acid response element-deleted TrkB promoter luciferase reporter construct (pGL3-TrkB-d) and pcDNA4-RARα. Renilla luciferase plasmid pRL-TK was also introduced into each transfected sample as an internal control. Data shown here are mean ± SEM. *$P < 0.05$; **$P < 0.001$, one-way ANOVA followed by a Student Newman–Keuls post hoc test.
In conclusion, our results indicate a novel link between retinoid signaling, BDNF-TrkB signaling and depression. Thus, targeting retinoid signaling in the prefrontal cortex may be an alternative strategy for BDNF-based therapeutics for depression.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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