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The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder

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Abstract

Recent studies demonstrate that rapid antidepressant response to ketamine is mediated by activation of the mammalian target of rapamycin (mTOR) signaling pathway, leading to increased synaptic proteins in the prefrontal cortex (PFC) of rats. Our postmortem studies indicate robust deficits in prominent postsynaptic proteins including N-methyl-D-aspartate (NMDA) receptor subunits (NR2A, NR2B), metabotropic glutamate receptor subtype 5 (mGluR5) and postsynaptic density protein 95 kDa (PSD-95) in the PFC in major depressive disorder (MDD). We hypothesize that deficits in the mTOR-dependent translation initiation pathway contribute to the molecular pathology seen in the PFC of MDD subjects, and that a rapid reversal of these abnormalities may underlie antidepressant activity. The majority of known translational regulation occurs at the level of initiation. mTOR regulates translation initiation *via* its downstream components: p70-kDa ribosomal protein S6 kinase (p70S6K), and eukaryotic initiation factors 4E and 4B (eIF4E, eIF4B). In this study, we examined the expression of mTOR and its core downstream signaling targets: p70S6K, eIF4E, eIF4B in the PFC of 12 depressed subjects and 12 psychiatrically healthy controls using Western blot. Levels of eIF4E phosphorylated at serine 209 (p-eIF4E-Ser209) and eIF4B phosphorylated at serine 504 (p-eIF4B-Ser504) were also examined. Adjacent cortical tissue samples from both cohorts of subjects were used in our previous postmortem analyses. There was a significant reduction in mTOR, p70S6K, eIF4B and p-eIF4B protein expression in MDD subjects relative to controls. No group differences were observed in eIF4E, p-eIF4E or actin levels. Our findings show deficits in mTOR-dependent translation initiation in MDD particularly via the p70S6K/eIF4B pathway, and indicate a potential association between marked deficits in synaptic proteins and dysregulation of mTOR signaling in MDD.

Keywords

prefrontal cortex; translation initiation pathway; major depressive disorder; postmortem

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1. Introduction

A major limitation of established antidepressants is the delayed onset of therapeutic response, resulting in non-compliance and dramatically increased risk for suicidal behavior. Of particular relevance is the demonstration that a single dose of ketamine, a glutamate N-methyl-D-aspartate (NMDA) receptor antagonist, induced a rapid (within hours), long lasting (up to 1 week), and robust antidepressant effect in treatment-resistant cases of MDD (Berman et al., 2000; Zarate et al., 2006). Recent animal studies indicate that the fast antidepressant response to NMDA receptor antagonists (ketamine and Ro 25-6981) is mediated by rapid activation of the mammalian target of rapamycin (mTOR) pathway leading to an increase in synaptic signaling proteins and increased number and function of new spine synapses in the prefrontal cortex (PFC) of rats (Li et al., 2010). Moreover, it has been demonstrated that a single dose of these antagonists rapidly reversed the chronic stress induced behavioral and synaptic deficits in an mTOR-dependent manner (Li et al., 2011). Our recent postmortem studies show significant reductions in the expression of prominent postsynaptic proteins involved in glutamate neurotransmission, including NMDA receptor subunits (NR2A, NR2B), metabotropic glutamate receptor subtype 5 (mGluR5) and postsynaptic density 95kDa (PSD-95) in the PFC from depressed subjects (Deschwanden et al., 2011; Feyissa et al., 2009). These studies may indicate an association between marked deficits in synaptic proteins and dysregulation of mTOR signaling in MDD (Karolewicz et al, 2011).

Traditionally, it was thought that the change in the proteome is caused by transcriptional activity. Now it is known that regulation of translation is another way of altering protein production (Nilsson et al., 2004). Protein synthesis is a highly regulated process that can be separated into three general phases: initiation, elongation and termination (Hoeffer and Klann et al., 2010; Klann et al., 2004). The rate-limiting step in the process of protein synthesis is translation initiation (Hoeffer and Klann et al., 2010; Holz et al., 2005). The activity of mTOR, an ubiquitously expressed serine/threonine kinase, is central to the regulation of translation initiation and, consequently, protein synthesis required for long-term potentiation and new synaptic connections (Hashimoto, 2011; Hoeffer and Klann et al., 2010; Klann et al., 2004; Tang et al., 2002; Tang and Schuman, 2002).

It has been reported that neuronal mTOR function is influenced by the activity of growth factors, insulin, cytokines, as well as glutamate activity *via* NMDA receptors and metabotropic glutamate receptors (mGluR) (Antion et al., 2008; Gong et al., 2006; Hay and Sonenberg, 2004; Hoeffer and Klann, 2010) (Fig. 1). Activated mTOR phosphorylates p70-kDa ribosomal protein S6 kinase (p70S6K) followed by p70S6K-induced phosphorylation of eukaryotic initiation factor 4B (eIF4B) which promotes the initiation of protein translation (Raught et al., 2004). mTOR also phosphorylates and inactivates eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), reducing its affinity for eIF4E and releasing eIF4E to facilitate translation initiation. Activated translation initiation factors, particularly eIF4E and eIF4B, are responsible for ribosome recruitment to the 5′ end of mRNA. The 5′ end of all nuclear-transcribed mRNAs possess a cap structure (m^7GpppN, in which “m” represents a methyl group and “N”, any nucleotide) that is specifically recognized by eIF4E. Thus, eIF4E guides the ribosome to an mRNA 5′ end and facilitates its binding. On the other hand, eIF4B potentiates ribosome recruitment by stimulating the helicase activity of the eukaryotic initiation factor 4A (eIF4A), to unwind mRNA secondary structure for efficient translation (Gingras et al., 1999; Hay and Sonenberg, 2004; Holz et al., 2005; Rogers et al., 2002). Thus, mTOR controls the efficiency of protein translation within cells via its critical downstream targets (Fig. 1).

There is abundant evidence linking mTOR signaling to synaptic plasticity, memory, neurological disorders, and cancer (Gong et al., 2006; Hay and Sonenberg, 2004; Hoeffer and Klann et al., 2010). To date there are no studies that implicate the mTOR signaling pathway in the pathology of MDD. We hypothesize that deficits in the mTOR-dependent translation initiation pathway contribute to the molecular pathology seen in the PFC in MDD. Therefore, the goal of this study is to examine MDD-related changes in the protein level of mTOR and its downstream signaling targets: p70S6K, eIF4E, eIF4B in cortical tissue (PFC BA10) from the same MDD subjects as those used in our previous postmortem studies (Deschwanden et al., 2011; Feyissa et al., 2009). Additionally, levels of eIF4E phosphorylated at serine 209 (p-eIF4E Ser209) and eIF4B phosphorylated at serine 504 (p-eIF4B Ser504) were examined.

2. Methods

2.1. Human Subjects

Postmortem brain samples were collected at autopsy at the Cuyahoga County Coroner's Office in Cleveland, OH. Informed written consent was obtained from the legal next-of-kin of all subjects. Next-of-kin were interviewed and retrospective psychiatric assessments were conducted in accordance with Institutional Review Board policies at Case Western Reserve University and the University of Mississippi Medical Center. A trained interviewer administered the Schedule for Affective Disorders and Schizophrenia: lifetime version (SADS-L) or the Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID-IV) to knowledgeable next-of-kin to subjects in the study approximately three months after death to determine current and lifetime Axis I psychopathology (First et al., 1996; Spitzer and Endicott, 1978). Diagnoses for Axis I disorders were assessed independently by a clinical psychologist and a psychiatrist. Consensus diagnosis was reached in conference, using information from knowledgeable informants, The Cuyahoga County Coroner's Office, and all available inpatient and outpatient medical records. Twelve subjects met criteria for major depressive disorder and twelve subjects did not meet criteria for an Axis I disorder (termed psychiatrically healthy controls) except for nicotine and alcohol dependence based on the Diagnostic and Statistic Manual of Mental Disorders-Revised DSM-IV (Table 1). Among the twelve depressed subjects, 10 were suicide victims. Blood and urine samples from all subjects were examined by the coroner's office for psychotropic medications and substances of abuse, including ethanol (Table 1 and 2). The average duration of depression was 9.6 (± 3.6) years. Depressed subjects and psychiatrically healthy controls were matched as closely as possible for age, gender, post-mortem interval (PMI), tissue pH, and storage time in freezer (Table 1 and 2).

2.2. Immunoblotting

Tissue samples were dissected from the anterior region of the prefrontal cortex (PFC) containing Brodmann's area 10 (BA10). Frozen blocks were cut into 50 um-thick sections and tissue punches containing all six cortical layers of the gray matter were collected and used. Western blot experiments were performed as described previously (Feyissa et al., 2009; Feyissa et al., 2010; Deschwanden et al., 2011). Immunoreactivities of mTOR, p70S6K, eIF4E, p-eIF4E (Ser209), eIF4B, and p-eIF4B (Ser504) were investigated in twelve depressed subjects and twelve psychiatrically healthy controls. Immunoblots of six pairs of subjects were on the same gel with duplicates on separate gels. All mTOR signaling pathway components were detected using rabbit monoclonal antibodies (1:1000, Epitomics Burlingame, CA, USA) and secondary anti-rabbit antibody (1:3000, Amersham Biosciences, Piscataway, NJ, USA). As a control for transfer and loading, actin was detected on each blot using mouse anti-actin primary antibody (1:10,000; Millipore, Temecula, CA, USA) and anti-mouse secondary antibody (1:5,000; Amersham Biosciences). In order to ensure that

actin is not affected by depression, amounts of actin immunoreactivity from depressed subjects were compared to amounts of actin immunoreactivity of matched controls. Actin immunoreactivity detected in depressed subjects was not changed compared to controls (t=1.09; df=11, p=0.299, not shown). The unchanged level of actin between study groups supports the suitability of this protein as internal control.

2.3. Data Analysis

Immunoreactive bands were analyzed using MCID Elite 7.0 (Imaging Research, St. Catherines, ON, Canada). To control for accuracy of tissue loading and efficiency of transfer, data were normalized to actin detected on the same blots. The final data are expressed as a ratio of the relative optical density (ROD) of protein of interest to ROD of actin. For each protein, statistical analyses compared expression values in MDD vs. matched controls. A maximum-likelihood mixed-models test was used to estimate parameters of the models, assuming pairs and subjects within pairs were random components (SAS; Version 9.1, SAS Institute Inc., Cary, NC, USA). As a first step, unadjusted models were fit to compare depressives versus controls without adjusting for potential confounders. Adjusted models included the main effect for comparing depressives versus controls and covariates for age, PMI, and tissue pH. Interactions between the main effect, depressives versus controls, and each of the potentially confounding covariates were investigated and dropped from the model. The covariate adjusted analyses produced similar results; the results for the unadjusted model are reported for simplicity. Results for the depressed and control groups are reported as mean ± SEM based on the mixed model. In order to adjust for multiple comparisons (six signaling proteins analyzed) the *p* value of 0.05 was adjusted to <0.0083 (threshold for significance).

3. Results

Amounts of mTOR, p70S6K, eIF4E, eIF4B, p-eIF4E (Ser209), and p-eIF4B (Ser504) were analyzed in the PFC BA10 from 12 pairs of subjects with MDD and matched healthy controls. Fig. 2 shows representative immunoblots from 3 pairs of subjects used in the analysis. The amount of mTOR immunoreactivity from depressed subjects (0.42±0.06) was significantly lower compared to controls (0.616±0.055; t=4.13 df=11, p=0.0017, Fig.3). Similarly, there was a robust reduction in the level of p70S6K in depressed subjects (0.37±0.04) compared to control subjects (0.54±0.046; t=4.42 df=11, p=0.001, Fig.3). The amount of eIF4B immunoreactivity from depressed subjects (0.26±0.10) was also significantly lower compared to control subjects (0.78±0.14; t=3.64 df=11, p=0.0039, Fig.3). The amount of p-eIF4B (Ser504) from depressed subjects (1.18±0.32) was significantly lower compared to control subjects (2.18±0.36; t=5.09 df=11, p=0.0004, Fig.4). However, there were no changes in the expression of eIF4E or p-eIF4E (Ser209) between the two groups (t=2.66 df=11, p=0.022 and t=0.161 df=10, p=0.87, respectively, Fig. 3 and Fig.4).

4. Discussion

The present study is the first to analyze levels of the mTOR-dependent translation initiation factors in the PFC from subjects diagnosed with MDD. Significant reductions in the expression of mTOR, p70S6K, eIF4B, and p-eIF4B were observed in MDD subjects as compared to psychiatrically healthy controls. In contrast, levels of eIF4E and p-eIF4E were unchanged in depressed subjects. Previously, we reported marked deficits in prominent postsynaptic proteins involved in glutamate neurotransmission such as NMDA receptor subunits (NR2A, NR2B), mGluR5, and PSD-95 (Deschwanden et al., 2011; Feyissa et al., 2009) in the PFC from the same depressed subjects used in this study. Taken together, these findings support the hypothesis that deficits in the mTOR-dependent translation initiation pathway contribute to the molecular pathology seen in the PFC in MDD, and a rapid

reversal of these abnormalities may underlie antidepressant activity. Given that mTOR function is influenced by the activity of neuronal receptors including NMDA receptors, mGluR5 or tyrosine kinase (TrkB) a receptor for neurotrophic factors, additional studies will be required to elucidate whether deficits in these receptors, reported previously in MDD (Deschwanden et al., 2011; Feyissa et al., 2009, Thompson et al., 2011), are the reason for, or consequence of, mTOR signaling pathology.

It is generally accepted that mTOR acts as a node of convergence downstream of the aforementioned receptors and several signaling pathways, including phosphoinositide dependent kinase-1 (PDK1), phosphoinositide-3-kinase (PI3K), and Akt/protein kinase-B (Akt) (Hoeffer and Klann et al., 2010; Klann et al., 2004). A significant decrease in Akt1 activity has been previously reported in the PFC of suicide victims (Karege et al., 2007; confirmed by Dwivedi et al., 2010) and schizophrenics (Zhao et al., 2006), indicating an association between dysregulation of Akt/mTOR signaling and psychiatric disorders. Several core components of the mTOR signaling pathway are present in dendrites and enriched at postsynaptic sites (Tang et al., 2002), and the involvement of mTOR signaling in dendritic protein synthesis has been recently characterized (Gong et al., 2006).

In this study we have investigated eIF4B and eIF4E which are involved in translation initiation, the rate-limiting step of ribosome recruitment to the 5′ end of an mRNA (Gingras et al., 1999). In contrast to eIF4E, we have shown specific reductions in the levels of eIF4B (unphosphorylated and phosphorylated forms). Reduced level of phosphorylated eIF4B may indicate decreased eIF4B function. Given that eIF4B is phosphorylated/activated by p70S6K, reduced p-eIF4B would suggest reduced activity of p70S6K, indicating a dysfunction in mTOR/p70S6K/eIF4B pathway. Thus, based on our observation we hypothesize that the eIF4B-dependent steps in translation initiation are most likely impaired in depressed individuals.

Recent animal studies indicate that the fast antidepressant response to NMDA receptor antagonists (ketamine and Ro25-6981) is mediated by rapid activation of the mTOR pathway leading to an increase in synaptic signaling proteins and increased number and function of new spine synapses in the PFC of rats (Li et al., 2010). Moreover, blockade of mTOR signaling, using its specific inhibitor rapamycin, completely blocked ketamine-induced synaptogenesis and antidepressive effects in animal screening procedures (Li et al., 2010). Furthermore, it has been shown that a single dose of these antagonists rapidly reversed the chronic unpredictable stress-induced behavioral and synaptic deficits in an mTOR dependent manner (Li et al., 2011). The activation of mTOR and related proteins was also observed in rat cortical tissue after chronic treatment with NMDA receptor antagonist MK-801 (Yoon et al., 2008). Interestingly, chronic but not acute treatment with fluoxetine was shown to induce hyperphosphorylation of eIF4E, a key regulator of protein translation, suggesting that regulation of the translational machinery was involved in the mechanism of action of chronic fluoxetine administration (Dagestad et al., 2006). Therefore, the activation of the mTOR pathway may be related to the common effect of NMDA receptor antagonists and antidepressants, however, mechanisms underlying the induction of mTOR signaling are currently unclear. Therefore, further characterization of the mTOR signaling pathway in MDD and its involvement in antidepressant activity has the potential to identify novel therapeutic targets for antidepressant drug development.

In summary, the data reported herein, in conjunction with recent animal studies, implicate the involvement of mTOR signaling particularly via p70S6K/eIF4B pathway in the pathophysiology of depression and antidepressive activity. Reduced activity of critical core components of mTOR signaling may underlie the synaptic deficits previously reported in the PFC in MDD. These findings further confirm the potential of targeting the mTOR signaling

cascade as an innovative and valuable strategy for the discovery of novel, fast-acting antidepressant medications.

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Abbreviations

MDD	major depressive disorder
PFC	prefrontal cortex
NMDA	N-methyl-D-aspartate
NR2A	NMDA receptor subunit 2A
NR2B	NMDA receptor subunit 2B
mGluR5	metabotropic glutamate receptor subtype 5
PSD-95	postsynaptic density-95 kDa
mTOR	mammalian target of rapamycin
p70S6K	70-kDa ribosomal protein S6 kinase
eIF4B	eukaryotic initiation factor 4B
eIF4E	eukaryotic initiation factor 4E
p-eIF4E (Ser209)	eukaryotic initiation factor 4E phosphorylated at serine 209
p-eIF4B (Ser504)	eukaryotic initiation factor 4B phosphorylated at serine 504

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*Highlights

- Depression is associated with deficits in synaptic proteins
- We investigated levels of mTOR-dependent translation initiation factors in depression
- Reductions in mTOR, p70S6K, eIF4B and p-eIF4B were indentified
- No differences seen in eIF4E, p-eIF4E or actin levels.
- An association between deficits in synaptic proteins in depression and dysregulation of mTOR/p70S6K/eIF4B signaling is evident

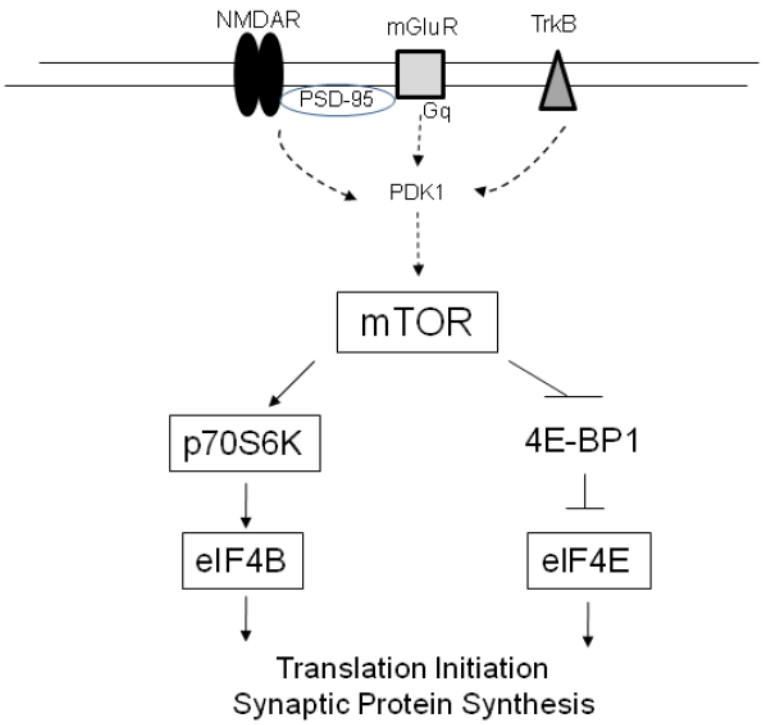

Figure 1.

Simplified diagram illustrating the mTOR signaling pathway. Neuronal receptors (NMDAR, mGluR, and TrkB) activate downstream signaling pathways, including PDK1, leading to mTOR activation. Activated mTOR phosphorylates p70S6K followed by p70S6K induced phosphorylation of eIF4B which promotes the initiation of protein translation. mTOR also phosphorylates and inactivates eukaryotic 4E-BP1 reducing its affinity for eIF4E and releasing eIF4E to facilitate translation initiation. Abbreviations: NMDAR, N-methyl-D-aspartate receptor; mGluR, metabotropic glutamate receptor; PSD-95, postsynaptic density-95 kDa; TrkB, tyrosine kinase B receptor; PDK1, phosphoinositide-dependent kinase 1; mTOR, mammalian target of rapamycin; p70S6K, p70 kDa ribosomal protein S6 kinase; eIF4B, eukaryotic initiation factor 4B; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; eIF4E, eukaryotic initiation factor 4E. Diagram drawn using information from Hoeffer and Klann (2010) and Klann et al. (2004).

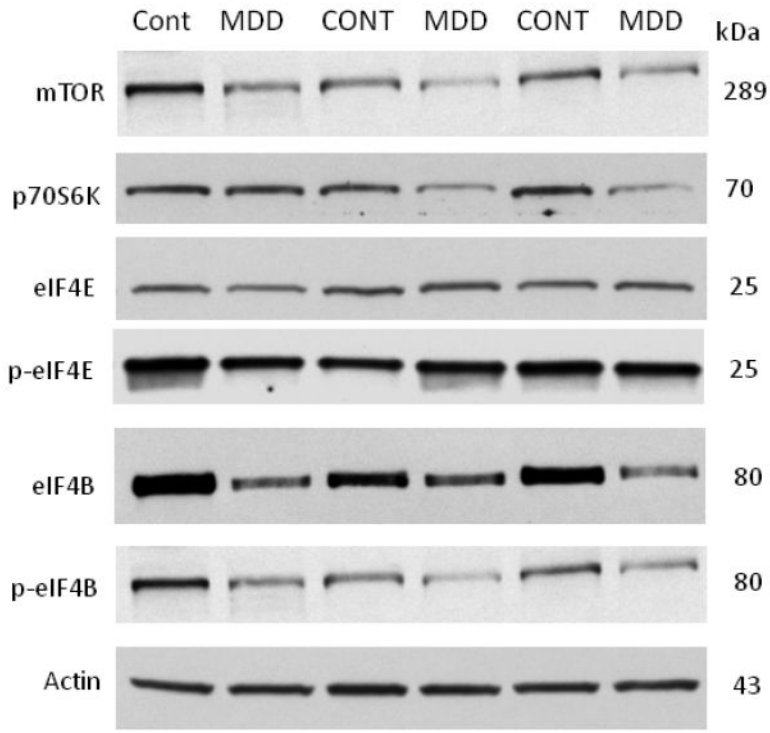

Figure 2.
Immunoblots of mTOR, p70S6K, eIF4E, p-eIF4E, eIF4B, p-eIF4B and actin from six representative subjects used in the analysis. Each well was loaded with 20ug of total protein. Cont, control; MDD, major depressive disorder.

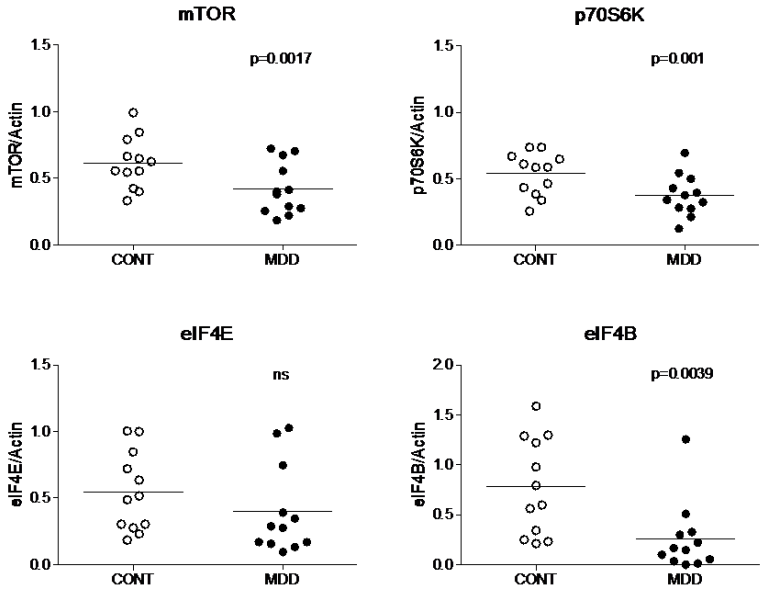

Figure 3.

Scatter plots of mTOR, p70S6K, eIF4E, and eIF4B protein levels normalized to actin. Significant reductions in mTOR, p70S6K, and eIF4B immunoreactivities were observed in depressed subjects (filled circles; n=12) as compared to controls (open circle; n=12). Normalized optical density values for the individual subjects and mean values (horizontal lines) are presented. A p-value <0.0083 was considered as a threshold for significance.

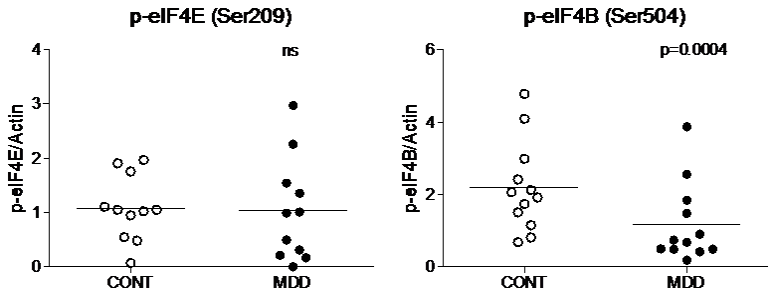

Figure 4.

Scatter plots of p-eIF4E, and p-eIF4B protein levels normalized to actin. Significant reduction in p-eIF4B immunoreactivity was observed in depressed subjects (filled circles; n=12) as compared to controls (open circle; n=12). Normalized optical density values for the individual subjects and mean values (horizontal lines) are presented. A p-value <0.0083 was considered as a threshold for significance.

Table 1

Demographic data of controls and major depressive subjects

Control	Age	Sex	PMI	Brain pH	Toxicology	Cause of death
1	37	F	13	5.93	Clean	Viral myocarditis
2	46	M	11	6.95	Clean	Heart disease
3	54	M	17	6.87	Brompheniramine	Heart disease
4	67	F	28	6.39	Clean	Heart disease
5	69	M	26	6.7	Clean	Heart disease
6	70	M	20	6.81	Clean	Heart disease
7	43	M	23	6.49	Propoxyphene, norpropoxyphene, oxycodone	Pulmonary thromboemboli
8	59	M	6	6.79	Lidocaine	Heart disease
9	34	M	24	6.61	Ethanol	Thrombophlebitis
10	54	M	19	6.52	Lidocaine	Heart disease
11	52	M	17	6.28	Clean	Heart disease
12	33	M	23	6.86	Clean	Heart Disease
MDD						
1	40	F	25	6.32	Morphine, codeine, hydrocodone, diphenhydramine	Heart disease
2	46	M	17	6.26	Clean	Homicide
3	54	M	23	6.24	Phenobarbital, phenytoin, CO	Suicide by CO poisoning
4	42	F	24	6.62	Acetaminophen, propoxyphene	Suicide by overdose of propoxyphene & acetaminophen
5	64	M	26	6.85	Ethanol	Suicide by gun shot to head
6	74	M	25	6.67	Diazepam, Acetaminophen	Suicide by gun shot to head
7	81	M	33	6.78	Clean	Suicide by drowning
8	60	M	20	6.31	Ethanol	Suicide by gun shot to chest
9	42	M	20	6.8	Clean	Suicide by gun shot to chest
10	52	M	17	6.48	CO	Suicide by CO poisoning)
11	48	M	21	6.9	Flurazepam	Suicide by gun shot to chest
12	65	M	30	6.24	Codeine	Suicide by gun shot to chest & slashed wrists

M, male; F, female; PMI, postmortem interval; MDD, major depressive disorder; CO, carbon monoxide.

Table 2

Summary of demographic characteristics of subjects

Parameter	Controls (n=12)	Major Depression (n=12)
Age*	51 (3.8) years	55 (3.8) years
Postmortem interval*	19 (1.9) hours	23 (1.4) hours
pH*	6.62 (0.09)	6.54 (0.07)
Gender (female/male)	2/10	2/10
Medication history [a]	none	Sertraline (n=2) Fluoxetine (n=1) Paroxetine, fluoxetine & amitriptyline (n=1)
Comorbid diagnosis	History of alcohol abuse (n=1) History of alcohol dependence (n=2)	Alcohol abuse (n=1) History of alcohol abuse (n=1) Dysthymia (n=1) Polysubstance dependence & bulimia nervosa (n=1)
Smoking	Smokers (n=2) History of smoking (n=2)	Smokers (n=3)
Suicide	none	n=10

* Mean (SEM)

[a] prescriptions for antidepressants within 4 weeks prior to death; none of the 12 depressed subjects had antidepressants present in their postmortem toxicology screening.