Let-7e Differentiates Stress-Resilient from Susceptible

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Definition

Three strains of mice with various susceptibilities to restraint stress (RS), i.e., mice with a knocked out norepinephrine transporter gene (NET-KO), SWR/J and C57BL/6J (WT) mice were shown to serve as a good model to study the molecular mechanisms underlying different stress-coping strategies. We identified 14 miRNAs that were altered by RS in the PFC of these mice in a genotype-dependent manner, where the most interesting was let-7e. Further in silico analysis of its potential targets allowed us to identify five mRNAs (Bcl2l11, Foxo1, Pik3r1, Gab1 and Map2k4), and their level alterations were experimentally confirmed. A next-generation sequencing (NGS) approach, which was employed to find transcripts differentially expressed in the PFC of NET-KO and WT mice, showed that, among others, two additional mRNAs were regulated by mmu-let-7e, i.e., mRNAs that encode Kmt2d and Inf2. Since an increase in Bcl2l11 and Pik3r1 mRNAs upon RS in the PFC of WT mice resulted from the decrease in mmu-let-7e and mmu-miR-484 regulations, we postulated that MAPK, FoxO and PI3K-Akt signaling pathways were associated with stress resilience, although via different, genotype-dependent regulation of various mRNAs by let-7e and miR-484. However, a higher level of Kmt2d mRNA (regulated by let-7e) that was found with NGS analysis in the PFC of NET-KO mice indicated that histone methylation was also important for stress resilience.

1. Introduction

Mood disorders, including major depressive disorder (MDD), are still a considerable burden for modern society. Despite the availability of approx. 50 different drugs, the treatment of MDD is far from ideal, nor is the understanding of the mechanism of action of antidepressant drugs that are administered repeatedly (which is necessary to achieve clinical efficacy). Most of the numerous behavioral, biochemical and molecular preclinical studies focused on various kinds of stress since it is regarded as the main factor contributing to mood disorders. Stress is defined as a condition that seriously perturbs the psychological and physiological balance of an individual. Serious stress, however, does not affect everyone in the same manner; some susceptible individuals (not only humans but also animals) adapt poorly to stressors and express inappropriate responses that can become persistent states of stress themselves. On the other hand, some resilient individuals can perceive adversity and develop adaptive psychological and physiological responses. The underlying mechanisms of these responses are not fully understood, although they are known to depend on a combination of genetic and non-genetic factors that interact in complex ways. Coping strategies are essential to minimize the impact of stress and determine the degree of resilience and susceptibility; however, as the biological basis of the stress response is not clearly defined, the same is true for coping strategies [1].

Stress susceptibility and resilience in pre-clinical rodent models are often studied in relation to MDD [2][3]. Three strains of mice with various susceptibility to restraint stress (RS), i.e., mice with a knocked-out gene that encodes the norepinephrine transporter (NET-KO), as well as C57BL/6J (WT) and SWR/J mice, were shown to serve as a good model to study the mechanisms underlying different stress-coping strategies. In our recent study, we were able to show alterations at the level of microRNAs (miRNAs) in the serum of these three genotypes, both between strains and regarding their response to RS [4][5]. MicroRNA molecules present in the blood may serve as potential biomarkers for brain pathologies, including depressive disorders [6][7], but their presence and possible alterations in the specific brain regions are even more interesting. In the present study, we chose the prefrontal cortex (PFC) since it is widely accepted that this brain region is involved in the adaptive control of behavior. Acute stress influences the activity of PFC, which in turn results in long-term neurobiological alterations [8]. The molecular

mechanisms underlying these alterations, especially mechanisms that differentiate stress responses, namely, resilience and susceptibility, are not fully understood, but the increasing number of experimental data indicates that phenomena of epigenetic regulation of gene expression by microRNA have gained great interest. MicroRNA molecules, which are widely expressed in eukaryotes, are small (17–24 nucleotides), non-coding RNA transcripts that play an important role in the post-transcriptional regulation of many genes; therefore, studying alterations at miRNA level one can allow for gaining deeper insight into the complex network and mutual relationships of various transcripts that are targeted by a given miRNA.

2. Current Insight on miRNA

In the previous study, we were able to identify numerous miRNAs that are present in the mouse serum, which differentiated three strains of mice depending on their response to restraint stress (RS) [4][5]. These miRNAs might be regarded as biomarkers of stress resilience. Our present study aimed to examine whether these miRNAs are also altered in the mouse brain. We chose the prefrontal cortex (PFC) as the brain area associated with stress and depression. Out of the 192 miRNAs that were selected based on our previous research, we found 14 that changed in the PFC upon RS, and these changes were dependent on the genotype: we observed a decrease in the level of the majority of identified miRNAs in the PFC of WT mice subjected to RS, and the lower level of the same miRNAs in the PFC of other two genotypes, which were regarded as resilient to RS. The stress-induced decrease of a majority of identified miRNAs in the PFC of mice subjected to RS can be regarded as an analogy to the results obtained by Smalheiser et al. [9], who showed a decreased level of miRNAs in the PFC of humans experiencing prolonged stressful conditions. Taken together, these results indicate that lower levels of certain miRNAs enable an organism to better cope with stress, probably via reduction of the main effect of miRNAs, i.e., the inhibition of translation processes. On the other hand, our previous studies [4][5] showed that the effect of RS was different in the mouse serum, i.e., more miRNAs were downregulated (10) than upregulated (4) in the PFC, while in the serum, the relations were the opposite (15 miRNAs were upregulated vs. 10 that were downregulated). Among these miRNAs were mmu-miR-99a-5p and mmu-miR-23a-3p, which were also detected in the PFC in the present study.

In contrast, the expression of mmu-let-7b-5p, mmu-let 7c-5p and mmu-let-7g-5p decreased similarly in the blood and PFC of WT mice under stress conditions. Among the miRNAs identified in the PFC, some were also identified in the serum, and they were associated with stress resilience, e.g., mmu-miR-19b-3p, the expression of which increased upon RS, both in the PFC and serum of WT mice, and remained unchanged in NET-KO and SWR/J mice [4][5].

One of the miRNAs that were upregulated in the PFC of NET-KO and SWR/J mice compared to WT mice, namely, miR-484, was also increased in the PFC of these genotypes under stress conditions, indicating its involvement in stress resilience. As was shown by Wingo et al. [10] that predicted targets of miR-484 were enriched in the protein co-expression module involved in synaptic transmission and regulation of long-term synaptic plasticity. Wingo et al. [10] also found a significantly lower level of miR-484 in the PFC (obtained postmortem) of humans who had been subjected to a longitudinal assessment for late-life depressive symptoms. Such results may indicate that acute stress, such as RS in our studies, led to increased expression of miR-484, while long-term stressful conditions led to downregulation of this species. Additionally, this miRNA showed a negative correlation with Map2k4 and Pik3r1, which indicated that MAPK, FoxO and PI3K-Akt signaling pathways could be connected with stress resilience. Another miRNA that was increased in the PFC of WT mice following RS, namely, miR-29c, was associated with the effect of social defeat stress in mice [11] and also with the effect of stress and bipolar disorder in humans [12]. Similarly interesting is miR-19b, the expression of which was increased in the PFC of WT mice following RS. It was shown that this miRNA was increased in the amygdala of mice that were subjected to chronic mild stress, and it affected the regulation of mRNA encoding of the adrenergic receptor β-1 (Adrb1) [13]. In the present study, we showed a higher expression of miR-19b in the PFC of NET-KO mice, which can be regarded as a stress-resilient genotype. However, we did not find any alterations in the level of mRNA that encodes Adrb1. Nevertheless, it is worth noting that the level of adrenergic receptor β-

1 protein was significantly lower in the PFC of NET-KO mice [14], which indicates that the relationship between the level of specific miRNA, its target mRNA and the protein encoded by this mRNA is not always straightforward.

It is worth noting that a decrease in mmu-miR-129-3p expression upon RS was found in the present study, similarly to findings provided by Buran et al. [15], who showed a decrease in this miRNA in the PFC of BALB/c mice subjected to chronic mild stress. On the other hand, we were able to find new stress-related miRNAs that varied with genotype and could be related to stress resilience. They included mmu-miR-1839-5p, mmu-miR-672-5p and mmu-miR-676-3p.

An interesting similarity between the effects of RS on miRNAs in the serum and the PFC was the RS-induced downregulation of the whole group of miRNAs encoding the let-7 family—mmu-let-7b-5p, mmu-let-7c-5p, mmu-let-7d-5p, mmu-let-7g-5p and mmu-let-7e-5p in WT mice—and significantly lower levels of these miRNAs in the PFC of NET-KO and SWR/J mice, which were not altered by RS in these mice. The decrease in miR let-7b expression was described in the blood of students under exam-induced stress conditions [16], similarly to patients diagnosed with major depression, where the expression of miR let-7c was also decreased in the blood of these patients [17]. Recently, Maurel et al. [18] showed a decrease in mmu-let-7d-5p expression in the PFC of mice that were subjected to RS (24 h), however with no correlation with the level of its mRNA targets.

Out of this miRNA family, let-7e seems to be the most interesting since its lower level was described in both the blood of major depressive disorder patients and in the PFC of the genetic rat model of depression, which is associated with an increase in a pro-inflammatory marker of depression (interleukin-6) [19][20]. The results obtained in the present study show that the expression of mmu-let-7e-5p decreased following RS in WT mice, and was lower and did not change following RS in the PFC of stress-resilient genotypes (NET-KO and SWR/J).

Further in silico analysis of potential targets of mmu-let-7e-5p allowed us to identify mRNAs that encode for Bcl2l11, Foxo1, Pik3r1, Gab1 and Map2k4 genes in the PFC. The alterations in the level of the above-mentioned transcripts were experimentally confirmed. These transcripts are associated with FoxO (forkhead box O), PI3K-Akt (phosphoinositide-3-kinase–protein kinase B/Akt), AMPK (5’AMP-activated protein kinase), ErbB (tyrosine kinases receptor) and MAPK (mitogen-activated protein kinase) signaling pathways. These pathways seem to be differently regulated, which in turn can result in either susceptibility or resilience to stress.

The results obtained in the present study are in line with other research that showed the involvement of these pathways in the stress response. Yang and coworkers demonstrated the association of PI3K-Akt and MAPK signaling pathways with resilience to psychological stress in the PFC of mice [21], and Liu et al. showed changes in Foxo1 expression under chronic unpredictable stress in the mouse PFC, which correlated with changes in Bdnf expression [22]. We also showed an RS-induced increase in the mRNA encoding Foxo1 in the PFC of WT mice, which correlated with the changes in the expression of Bdnf. The expression of Bdnf was increased following RS in the PFC of WT mice but did not change in the PFC of NET-KO and SWR/J mice. This is in line with the studies by Maurel and coworkers, who discovered an increase in the expression of mRNA encoding Bdnf in the PFC of stress-susceptible mice and no change in stress-resilient mice [18].

On the other hand, the results obtained in the present study showed no change in mTOR mRNA expression that would be dependent on the genotype or effect of RS. Similarly, stress-independent changes in the expression of mRNA encoding mTOR in the PFC of immobilized rats were reported in other studies [23].

To find additional mRNAs that differentiate the PFC of NET-KO mice from WT mice or are regulated by mmu-let-7e-5p, aside from those that were already studied, we used the NGS approach. Out of the

transcripts that differentiated these two genotypes, two mRNAs were regulated by mmu-let-7e, i.e., mRNAs encoding Kmt2d and Inf2. It is worth noting that a higher expression of these mRNAs in the PFC of NET-KO mice negatively correlated with the expression of mmu-let-7e. Kmt2d points to histone methyltransferase activity specific for H3-K4, and it is associated with histone lysine methylation. The appropriate correction of epigenetic factors related to environmental differences in cognitive abilities requires determining the mechanisms of chromatin modifications and variations in DNA methylation. Transposons representing stress-sensitive DNA elements appeared to mediate the environmental influence on epigenetic modifications [24]. This is interesting in the context of data showing that increased expression of the stress-responsive genes results from a lack of histone H3 lysine (K) 4 methylation [25]. Thus, an increased expression of mRNA encoding Kmt2d in the PFC of NET-KO mice can be associated with the stress resilience of these animals. On the other hand, the second mRNA, which encodes inverted formin-2 (Inf2), instead acts at the cellular level and it was shown that its expression changes under oxidative stress [26].

Additionally, we found that the level of transcripts that encode some neuropeptides and their receptors was higher in the PFC of NET-KO mice as compared to WT animals, which is especially interesting since Chmelova and colleagues [27] showed that rats isolation induced reduction in Vgf (VGF nerve growth factor inducible) gene expression and Farhang at al. [28] provided data indicating that the expression mRNA encoding Ntrk3 (neurotrophic tyrosine kinase, receptor, type 3) was significantly higher in the stress-resilient rats. Likewise, a higher level of cortistatin (Cort) decreased the expression of Pomc mRNA (encoding ACTH) in mice, which silenced the HPA (hypothalamic-pituitary-adrenal) axis [29]. Additionally, the expression of mRNA encoding Npy (neuropeptide Y) was increased after chronic mild stress in the PFC of females but not the males [30]. These studies indicate that the higher expression of mRNAs encoding neuropeptides and their receptors in the PFC of NET-KO mice might be related to their stress resilience.

Interestingly, the PI3K-Akt signaling pathway, which is described above as being regulated by mmu-let-7e-5p, was regulated by three (Gh, Spp1, Fn1) of the mRNAs that differed in the PFC of NET-KO mice relative to WT mice. This pathway was shown above to be related to the stress response. Therefore, one can assume that its regulation is associated with stress resilience.

Additionally, an increase in mRNAs that encode Bcl2l11 upon RS in the PFC of WT mice resulted from a decrease in mmu-let-7e, while the Pik3r1 decrease resulted from mmu-miR-484 regulations. Therefore, we postulate that mainly MAPK, FoxO and PI3K-Akt signaling pathways are associated with stress resilience, although via different, genotype-dependent regulation of various mRNAs via let-7e and miR-484. However, the differences in gene expression (Kmt2d) regulated by let-7e that were found with NGS analysis indicated that histone methylation was also important for stress resilience.

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Keywords

MicroRNA;NET-KO mice;SWR/J mice;restraint stress;stress resilient;stress susceptibility;signaling pathway;prefrontal cortex;next-generation sequencing;mRNA