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RNA Interference: A New Hope in Understanding and Treatment of Psychiatric Disorders

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ÖZET:

RNA interferansı: Psikiyatrik hastalıkların tedavisinde yeni bir umut

Psikiyatrik bozukluklar, toplumda çok sık görülen ve yol açtıkları işgücü kaybı nedeniyle hem bireye hem de topluma ağır yük getiren rahatsızlıklardır. Tıptaki diğer hastalıkların aksine, bu bozuklukların tanımlamaları hala etiyolojik olarak değil, fenomenolojik olarak yapılmaktadır. Dolayısıyla da etiyolojiye yönelik tedavi yapılamamaktadır. Mevcut tedavilerin etki etmesi vakit almakta, hastaların önemli bir bölümünde tedavi direnci ciddi sorun teşkil etmektedir. Psikiyatrik rahatsızlıkların altta yatan nedenlerinin ve psikopatolojik süreçlerinin anlaşılması yeni tedavi stratejileri geliştirmek açısından son derece önem taşımaktadır. Psikiyatrik bozuklukların nörobiyolojisini anlamaya yönelik çalışmalar birçok koldan yürütülmektedir. Bu alanda son yıllarda gündeme gelen yeni bir teknik, RNA interferansı, önceki çalışmalarda var olan bazı kısıtlılıkları ortadan kaldırması ve hızlı ve kolay uygulanabilir olması nedeniyle heyecan yaratmış, 2006 Nobel Tıp Ödülü'ne layık görülmüştür. RNA interferansı hücrede ifade edilen bir genin ifadesini mRNA düzeyinde azaltmayı sağlayan bir yöntemdir. Bunun için ilgilenilen genin mRNA dizilimine tamamlayıcı olan çift zincirli kısa RNA moleküllerinden faydalanılır. Bu RNA molekülleri hücreye dışarıdan değişik şekillerde uygulanabilir, örneğin bir plazmid içinde veya transfeksiyon ajanı yardımıyla verilebilir. RNA interferansı, araştırma amaçlı ekzojen kullanımının yanı sıra, virüsleri ve hücredeki mobil genetik elemanları kontrol altında tutmak vb işlevler için organizmanın endojen olarak kullandığı bir yöntemdir. RNA interferansı, tıbbın diğer alanlarıyla birlikte psikiyatrik araştırmalarda da tercih edilen bir yöntem haline gelmiş; duygudurum ve anksiyete bozuklukları, madde kullanım bozuklukları gibi çok sayıda ruhsal bozuklukta rol oynayan moleküllerin anlaşılması ve yeni tedavi hedefleri belirlenmesinde yol gösterici nitelik almıştır. Bu teknikle önceki çalışmalarda tespit edilen ve psikiyatrik bozuklukların nörobiyolojisinde önemli olabileceği düşünülen aday genlerin hızla değerlendirilmesi imkanlı hale gelmiştir. Bu gözden geçirmede, RNA interferansı ve bu tekniği duygudurum, anksiyete ve madde kullanım bozukluklarını araştırmak amacıyla kullanan çalışmalar özetlenecektir.

Anahtar sözcükler: RNA interferansı, mizaç bozuklukları, anksiyete bozuklukları, madde kullanım bozuklukları

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ABSTRACT:

RNA interference: a new hope in understanding and treatment of psychiatric disorders

The prevalence and global burden of mental disorders are high. The number of years lost due to disability they cause, has major impact both on the individual and the society. Unlike the classification of other medical problems that are based on the etiology, psychiatric disorders are defined phenomenologically, thus treatments do not address the etiology of the disorders. Moreover the onset of action of the present treatment options takes time and treatment resistance is a major issue in the management of psychiatric patients. Understanding the etiology and psychopathological processes underlying the mental disorders would help to develop new treatment strategies and options for these disorders. There are several studies investigating the neurobiology of mental disorders from various aspects. The discovery of RNA interference, a new technique, has led to excitement and innovating scientist was awarded by Nobel Prize in Medicine in 2006, as it has overcome the limitations of previous techniques. RNA interference technique is used to decrease the mRNA expression of target genes by means of double stranded small RNA molecules complimentary to the target gene. These small RNA molecules can be administered into the cells exogenously by different ways, for instance they can be coupled to plasmids or transfection reagents. Besides its exogenous use as a research tool to suppress gene expression, RNA interference is an endogenous process that the organisms use for functions like the control of viruses and mobile genetic elements. Besides being used for research in other medical disciplines, RNA interference has become the preferred method in psychiatric research. It yields to understanding of many molecules that play role in the neurobiology of mood, anxiety, and substance use disorders and results in identification of new treatment targets. This technique enables researchers to evaluate candidate genes quickly that were identifed in previous studies and were thought to be important in the neurobiology of psychiatric disorders. In this paper, we review RNA interference and the studies that used this technique to investigate the neurobiology of mood, anxiety, and substance use disorders.

Key words: RNA interference, mood disorders, anxiety disorders, substance use disorders

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Due to the lack of knowledge regarding their neuropathological processes, psychiatric disorders are defined phenomenologically in current practice. Although several neurobiological studies have been conducted to overcome this problem, methodological issues limit the pace of these studies. For instance, genetic modification of the target gene is a valid method to investigate the role of that gene in disease processes; however, the methodological difficulties and the need for specialized personnel limit its use to specific laboratories. Moreover developmental adaptations complicate the interpretations of the results of these genetic manipulations (1). Ribonucleic acid interference (RNAi), which is a relatively new concept, has provided important contributions to overcome the above mentioned limitations. In addition to being easier and less time consuming to develop in any laboratory than the traditional methods, RNAi can be used to decrease target gene expression at any time point and in any brain region of interest; therefore, RNAi is becoming a preferred technique in many studies designed to understand pathophysiological processes underlying mental disorders in recent years.

In the first part of this paper, we will review RNAi and its mechanisms. In the second part, we will summarize the findings of the studies that have used RNAi as a means to investigate the etiopathogenesis of mood, anxiety and substance use disorders, and to identify new therapeutic targets for these disorders.

1. RNA interference

RNAi is a method to selectively and efficiently knockdown the expression of a targeted messenger RNA (mRNA). RNAi is also an endogenous process that regulates several cellular functions. Specifically RNAi is reported to function in the cellular defense against viral infections, in controlling the expression and mobility of transposons, which are mobile genetic elements, and in the regulation of endogenous gene expression (2).

The mechanisms of RNAi have been described by many studies in the last decade. Briefly, once double stranded RNA (dsRNA) molecules enter the cytoplasm, they are cleaved into 21-28 nucleotide long small interfering RNAs (siRNA) by an RNAase called "Dicer."

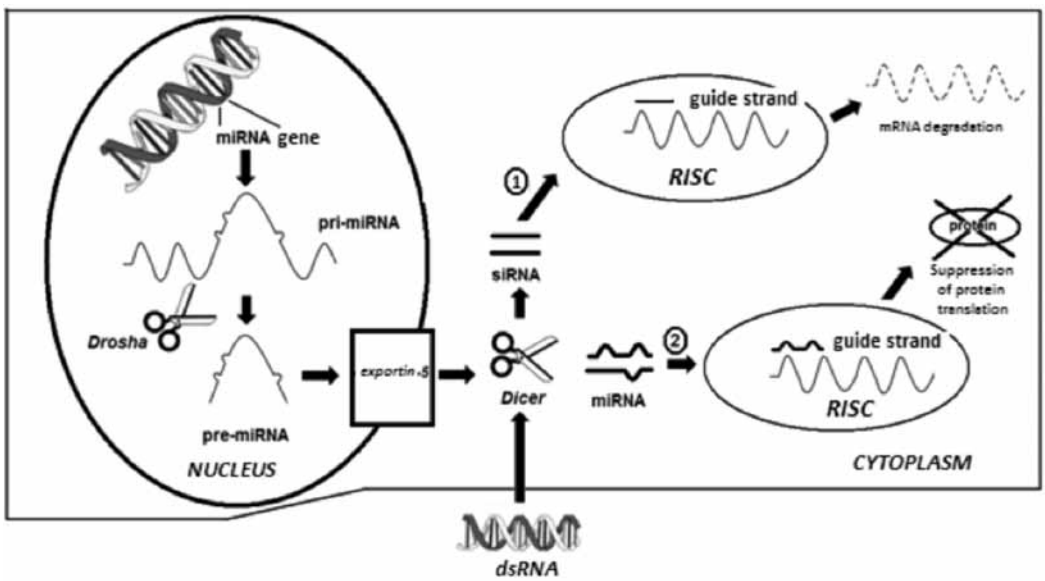

Figure 1: Mechanism of action of RNA interference: Double stranded RNA (dsRNA) molecules can be endogenously expressed from microRNA (miRNA) genes or exogenosly introduced by means of viruses, plasmids or directly in the form of dsRNA or siRNA. Once dsRNA molecules enter the cytoplasm, they are cleaved into 21-28 nucleotide long small interfering RNAs (siRNA) by an RNAase III called "Dicer". These siRNAs are then incorporated into RNA induced silencing complex (RISC) and the double helix of siRNA unwinds. Then the guide strand binds the target mRNA, which is cleaved by an RNAase H in RISC (Indicated by arrow 1 in the figure). This process is somewhat different for the miRNA: miRNA's are first expressed as primary miRNA transcripts (primiRNA), cut in the nucleus by an RNAase III called Drosha, the resulting premiRNA transcripts (premiRNA) are then exported into the cytoplasm by a nuclear membrane transporter exportin-5. In the cytoplasm "Dicer" cleaves premiRNA leading to miRNA. miRNA then enters a similar path to siRNA. Since the complementarity between miRNA and target mRNA is not complete, it suppresses protein expression not by mRNA degradation but with stopping the translation (Indicated by arrow 2 in the figure).

These siRNAs are then incorporated into a complex of proteins and nucleases, which is called "RNA induced silencing complex (RISC)." After incorporation into the RISC, the double helix unwinds and the guide strand binds the target mRNA, which is cleaved by an RNAase in the RISC called "Argonaute" (Figure 1) (2-4).

This process is somewhat different for the microRNAs (miRNA), the endogenous post-transcriptional regulators of gene expression. MicroRNAs are first copied from DNA as primary miRNA transcripts (primiRNA) in the nucleus. "Drosha," which is an RNAase located in the nucleus, cuts the primiRNAs into premiRNA, which is then exported into the cytoplasm by a nuclear membrane transporter exportin-5. In the cytoplasm "Dicer" cleaves premiRNA leading to miRNA. For the endogenous processes miRNA is incorporated into the RISC complex, unwinding the double helix. Contrary to siRNAs, the base sequences of the guide strand of miRNA does not have an exact complementarity to that of the target mRNA, thus miRNA controls protein expression by stopping translation of the target mRNA rather than degrading it (Figure 1) (2-4).

Because of its potential to facilitate understanding the roles of different molecules in the pathophysiology of disease and to develop new treatment strategies, the discovery of RNAi caused a great deal of excitement in the scientific world. The first applications in mammals, however, resulted in disappointment due to the interferon response induced by the introduction of dsRNA's into cells. Fortunately this obstacle was overcome by the use of either siRNAs or short-hairpin RNAs (shRNA) instead of long dsRNA molecules (5-6).

2. Use of RNAi in Psychiatric Research

Owing to the ease and pace of its application, RNAi makes region and time specific screening of many candidate genes possible. Although it has not been long since its discovery, the number of studies that have used this technique in mental health research has increased over the past 5 years, most of which are published in high impact journals.

RNAi can be of use to mental research in different ways. First, RNAi can serve as a tool to understand the role of different candidate genes that have been implicated by previous studies in the neurobiology of a mental disorders. More specifically, the effects of decreasing the expression of a specific gene at a certain brain region by RNAi on different behaviors like depression- or anxiety-like behavior can be studied. Secondly, RNAi can be used as a therapeutic tool to decrease the expression of different molecules whose expressions are found to be increased in the disease process.

Despite its promising potential to illuminate disease processes, in vivo application of RNAi has raised methodological concerns. The rapid degradation of siRNAs by RNAases, the limited knowledge of their diffusion capacity from the injection site and potential immune response when injected into the brain have raised these concerns (7) which have all been addressed and successfully overcome. Thakker et al. (2004) have showed that siRNAs infused into the dorsal third ventricle can effectively knockdown the target gene at brain regions far from the injection site (8). The same group then confirmed the efficacy of in vivo application of siRNAs into the brain (9). They reported that infusion of siRNAs targeting the serotonin transporter gene for two weeks intracerebroventricularly had antidepressant effects comparable to those of a selective serotonin reuptake inhibitor, citalopram.

2.1. Substance Use Disorders

Owing to high rates of relapse due to cravings and to environmental cues associated with the substance, treatment of substance use disorders is very difficult (11). These issues should be addressed in order to develop new treatment strategies that are more successful than the current ones. This can only be possible by knowing the brain regions and molecules mediating the effects of different substances of abuse. In the following section, we will review the studies which used RNAi to improve our knowledge about the roles of different molecules in different aspects of substance use disorders.

CD81 is a protein whose expression is found to be increased following cocaine administration in gene-screening studies; thus an increase in its expression may be related to cocaine abuse. Interventions to decrease CD81 expression, therefore, may be of use in the treatment of cocaine abuse. To test this hypothesis, Bahi et al. (2005) injected shRNAs targeting CD81 via a lentivirus into the nucleus accumbens (NAc) or ventral tegmental area

(VTA). They recorded locomotor activity as it is a parameter to evaluate the psychomotor activating effect of psychostimulants (12). They found that decreasing CD81 expression in the NAc reversed the effects of chronic cocaine administration on locomotor activity (13) (Table 1). In another study, Bahi et al. (2008) injected lentiviruses expressing BDNF, trkB or shRNAs targeting trkB (LVtrkB-shRNA) into the NAc to investigate the role of BDNF and its receptor trkB in cocaine dependency (14). They found that over-expression of BDNF or trkB increased the locomotor response to cocaine, whereas trkB knockdown decreased sensitivity to the locomotor effects of cocaine. They also reported an increase in conditioned place preference for cocaine in BDNF or trkB over-expressing animals which is sustained for 5 weeks after the cessation of cocaine. Moreover extinction in the place preference test for cocaine was delayed in these animals and they showed an increased response to low dose cocaine after extinction (Table 1). Since there is an increase in tissue plasminogen activator (tPA) following psychostimulant administration and an increase in conditioned place preference for amphetamine in tPA over-expressing animals, Bahi et al. (2008) studied the therapeutic effect of decreasing tPA expression in NAc by RNAi (15). They found that knocking down endogenous tPA expression in NAc by LV-shRNA resulted in inhibition of the place preference response for amphetamine (Table 1). This finding points out tPA as an important candidate for the treatment of psychostimulant dependency.

Selective inhibition of metabotropic glutamate receptor 5 (mGluR5) on striatal D1-receptor expressing neurons by RNAi has been shown to have an important role in incentive learning in mice (16). In mGluR5 knockdown mice, interestingly, reinforcement of behavior linked to unconditioned stimuli, namely cocaine self-administration remained similar to controls, whereas reinforcement of behavior by conditioned (reward-paired) stimuli was not observed. In other words these mice had specific deficits in incentive learning processes that enabled a reward-paired stimulus to directly reinforce behavior (Table1). Extinction was seen at similar rates to those of control animals, where the responses to an active lever decreased when the lever was no longer associated with cocaine administration; however, there was a striking difference between the two groups on the presentation of cocaine-paired stimulus after extinction. Control animals increased lever press responses on the active lever whereas mGluR5 knockdown animals failed to increase their lever presses. In summary striatal mGluR5 was shown to mediate the process by which environmental cues acquire a motivational valence and become rewarding in the absence of the unconditioned stimulus. Therefore mGluR5 should be investigated further as a target molecule to prevent relapses due to environmental cues.

Studies investigating alcohol dependence also use RNAi as a technical tool. For instance, in order to study the role of mu opioid receptors (MOR) in alcohol consumption, Lasek et al. (2007), injected lentivirus expressing MOR shRNA into the ventral striatum. They showed that mice injected with MOR shRNA consumed less alcohol than the control shRNA injected group on the two-bottle choice test, especially at high alcohol concentrations (17) (Table 1). Since low activity of aldehyde dehydrogenase-2 (ALDH-2) results in aldehyde accumulation in the blood, which has dysphoric effects, decreasing the levels of ALDH-2 may protect against alcohol abuse or dependency. Thus Cortinez et al. (2009) transfected HEK-293 cells with shRNA's targeting ALDH-2 and reported a 50% decrease both in mRNA levels and activity of ALDH-2. However they did not test the in vivo effects of these shRNA's on alcohol consumption (18) (Table 1).

As a summary, treatment strategies which target CD81, tPA, BDNF, trkB or mGluR5 in mesolimbic dopaminergic system hold promise for the treatment of chronic cocaine use disorder. Likewise strategies to decrease MOR or ALDH-2 expressions in the treatment of alcohol dependency should be explored by further studies.

2.2. Mood and Anxiety Disorders

Mood and anxiety disorders are frequently seen and are disabling for both the individual and society, however knowledge on their etiopathogenesis is limited. Many studies to date have attempted to clarify the issue by different means, such as descriptive, genetic, etc. In this respect the serotonergic system, circadian rhythms, developmental and environmental factors have been reported to have a role in mood and anxiety disorders (19-29).

Dysregulation of the serotonergic system in mood disorders (MD) has long been known (26,27). P11 is a molecule which binds serotonin 1B (5HT-1B) and serotonin 4 (5HT-4) receptors and regulates their function

Table 1: Studies that used RNAi in psychiatric research.

Group	Animal	Target gene	Mode of application	Region and time of application	Results
Thakker et al. 2004	BALB/c mice	EGFP	Naked siRNA	icv/1-2 weeks	EGFP siRNA caused similar levels of knockdown in GP, SN and PFC after 1 week or 2 weeks of injection; the decrease in EGFP mRNA levels became evident in the hippocampus, striatum, NAc, thalamus and amygdala after 2 weeks of injection; knockdown effect appeared in the olfactory bulbus and hypothalamus only after the second week of injection
Thakker et al. 2005	BALB/c mice	SERT	Naked siRNA	icv/2 weeks	Antidepressant effect was observed
Salahpour et al. 2007	C57BL mice	DAT	With a transfection reagent	VTA/SN/14 days	There was a 30-50% decrease in locomotor activity after amphetamine injection when compared to controls
Babcock et al. 2005	CD rat	α-CaMKII	AAV-shRNA	Hippocampus/ 31 days	Habituation dissappeared Learning was disturbed in the water maze
Di Benedetto et al. 2009	C57BL mice	ERK2	AAV-shRNA	Lateral amygdala	There was a decrease in ERK2 expression 1 week after the injection
Bahi et al. 2005	Wistar rat	CD81	LV-shRNA	NAc or VTA	Reversed the effects of chronic cocaine administration on locomotion
Lasek et al. 2007	C57BL mice	Mu opioid receptor	LV-shRNA	Ventral striatum	Decreased the amount of alcohol consumption on two-bottle choice test
Cortinez et al. 2009	in vitro	ALDH-2	Plasmid-shRNA	in vitro (HEK-293 cells)	Decreased ALDH-2 expression and activity by 50%
Bahi et al. 2008	Wistar rat	trkB	LV-shRNA	NAc	Decreased the effects of cocaine on locomotion and conditioned place preference
Bahi et al. 2008	Wistar rat	tPA	LV-shRNA	NAc	Totally inhibited the conditioned place preference response for amphetamine
Novak et al. 2010	C57BL/6N mice	mGluR5	Transgenic miRNA	D1 receptor expressing neurons in the striatum	Behaviors related to unconditioned stimuli were similar to controls, whereas reward-related (conditioned) stimuli cannot acquire a motivational valence
Alexander et al. 2010	C57BL/6 mice	P11	AAV-shRNA	NAc or ACC	Decrease in P11 expression in NAc was depressogenic, but no effect was seen in ACG
Mukherjee et al. 2010	C57BL/6J mice	clock	AAV-shRNA	VTA	Decrease in anxiety, increase in depression-like behavior and locomotion
Bahi et al. 2009	C57BL/6J mice	Calcineurin A	AAV-shRNA	Amygdala	Anxiogenic and depressogenic effects were observed
Hovatta et al. 2004	C57BL/6J mice	Glyoxalase-1	LV-shRNA	Cingulate cortex	Decreased anxiety-like behavior
Spiteri et al. 2010	Wistar rat	ER- α	AAV-shRNA	Ventromedial nucleus of hypothalamus & Dorsolateral amygdala	Decrease in ER-α in dorsolateral amygdala disturbed social recognition and increased anxiety; ER- α knockdown in the ventromedial nucleus of hypothalamus had no such effects
Shishkina et al. 2004	Wistar rat (2-4 days old)	α-2R	Naked siRNA	Brain stem	Anxiolytic effects during adulthood
Sztainberg et al. 2010	C57BL/6J mice	CRFR1	LV-shRNA	Basolateral amygdala	Decreased anxiety similar to environmental enrichment
Lesscher et al. 2008	C57BL/6J mice	PKCε	LV-shRNA	Amygdala	Decreased CRF levels and anxiety-like behavior
Shin et al. 2009	C57BL/6J mice	PLC-β4	LV-shRNA	Medial septum	Increased anxiety-like behavior relative to controls
Eren-Koçak et al. 2011	Sprague Dawley rat	FGF-2	LV-shRNA	Dentate gyrus	Increased anxiety-like behavior relative to controls

AAV= Adeno-associated virus, ALDH= aldehyde dehydrogenase, α-2R= alpha adrenergic receptor-2, ACC= anterior cingulate cortex, CRFR= corticotrophine-releasing factor receptor, DAT= dopamine transporter, EGFP = Enhanced green florescent protein, ER-α = estrogen receptor-α, ERK = Extracellular signal regulated kinase, FGF-2 = fibroblast growth factor-2, GP = Globus pallidus, icv = intracerebroventricular, α-CaMK= Calcium calmodulin dependent protein kinase, LV= lentivirus, mGluR5= metabotropic glutamate receptor 5, miRNA= microRNA, NAc= nucleus accumbens, PFK= prefrontal corteks, PKCε= protein kinase C epsilon, PLC β4= phospholipase C-β4, SERT= serotonin transporter, shRNA= short hairpin RNA, siRNA= small interfering RNA, SN= substantia nigra, tPA= tissue plasminogen activator, trkB= tyrosine receptor kinase B, VTA= ventral tegmental area.

has become important in recent depression research. Knockout of p11 in mice has been reported to be depressogenic (28). This same study reported a decrease in p11 expression in postmortem analyses of the anterior cingulate cortex (ACC) of patients with major depression. Alexander et al. (2010) investigated the effect of p11 knockdown in NAc and ACC on depression-like behavior (30). shRNAs that target p11 were injected in adeno-associated virus (AAV) into the ACC or NAc, and knockdown of p11 only in the NAc was reported to

increase depression-like behavior (Table 1). In parallel with this finding, increasing p11 expression in the NAc in p11 knockout mice reversed the depression-like behavior. In postmortem analysis of depression patients, p11 expression was found to be decreased in the NAc. These findings point out a potential therapeutic effect of strategies which increase p11 levels in the NAc.

Many studies have reported a dysregulation in circadian rhythms in bipolar disorders; however, the causal relationship of this dysregulation in the disorder has not been studied (19,21,23,28). Mukherjee et al. (2010) decreased the expression of the Clock gene in the VTA by RNAi and studied the effects of this knockdown on behavior (31). Interestingly these animals displayed hyperactivity, decreased anxiety-like behavior but increased depression-like behavior (Table 1). The authors concluded that this phenotype resembles the mixed state of bipolar disorder and the clock gene is important for the regulation of depression- and mania-like behavior.

Owing to the increase in prevalence of MD in transplant patients who receive cyclosporine, a calcineurin inhibitor, Bahi et al. (2009) studied the effects of injecting shRNAs targeting calcineurin-A into the amygdala on depression- and anxiety-like behaviors in mice. They showed that knocking down calcineurin A in the amygdala had depressogenic and anxiogenic effects (32) (Table 1). Many frequently used drugs (steroids, chemotherapeutic agents, etc.) are known to cause an increase in the prevalence of MD. MD not only decreases the patient's quality of life but also increases morbidity and mortality due to the primary disease (33-35). For this reason, knowledge of how these drugs act and on which brain regions may help in the development of preventive strategies.

Hovatta et al. (2005) studied the causal relationship between two genes, glyoxalase-1 and glutathione reductase-1, which they had found to be related to anxiety-like behavior in their previous study (36). Glyoxalase-1 is a critical enzyme for the detoxification of methyl-glyoxal, which is formed in spontaneous cellular biochemical reactions. Similarly glutathione reductase-1 is an enzyme that protects cells against oxidative stress. Both enzymes are important for cell survival. Over-expression of glyoxalase-1 and glutathione reductase-1 in the cingulate cortex in mice has been showed to have anxiogenic effects. In parallel, knockdown of glyoxalase-1 gene expression in the cingulate cortex by RNAi has been reported to decrease anxiety-like behavior (Table 1). It is interesting that over-expression of molecules that protect cells against stress resulted in increased anxiety and requires further investigation in other brain regions.

Estrogen is another molecule that is a focus of interest in behavioral studies. The facts that mental disorders affect women more frequently and that some psychological symptoms may be triggered by menstruation, pregnancy, labor or menopause has attracted attention to the role of estrogen in mental disorders (37,38). Spiteri et al. (2010) has studied the effects of decreasing the expression of the estrogen receptor- α (ER- α) in the ventromedial nucleus of the hypothalamus and dorsolateral amygdala by RNAi on behavior (39). They reported that knockdown of ER- α only in the dorsolateral amygdala resulted in social recognition deficits and increased anxiety (Table 1).

Early environmental factors can affect the development of psychopathology in adulthood (22,24,25,29). One of the molecules thought to be important for this process is alpha adrenergic receptor-2 (α-2R). To test this hypothesis, Shishkina et al. (2004) injected siRNA targeting α-2R into the brain stems of 2-4 days old rats and found that α-2R knockdown in brain stem had anxiolytic effects during adulthood (40) (Table 1).

Environmental enrichment (EE) is a paradigm that decreases anxiety and is used to study the effects of positive environmental factors in animals (20). Sztainberg et al. (2010) have reported a decrease in corticotropin-releasing factor receptor type 1 (CRFR1) in the basolateral amygdala following EE (41). They showed that knock down of CRFR1 expression in the basolateral amygdala by means of RNAi had an anxiolytic effect similar to EE (Table 1). Lesscher et al. (2008) also studied the relationship of CRF with anxiety (42). In this study protein kinase C epsilon (PKCε) was knocked down by RNAi in mouse amygdala and this manipulation was reported to decrease both CRF levels and anxiety-like behavior (Table1). They concluded that PKCε regulates anxiety in the amygdala through CRF. These findings suggest that decreasing the expressions of α-2R in the brain stem, and CRFR1 and PKCε in the amygdala hold promise for the treatment of anxiety disorders.

In recent years the importance of growth factors for mood and anxiety disorders has been shown by several studies and a possible etiological role for growth factors in mood disorders is put forward as "the neurotropic

hypothesis of depression" (43). Studies in this context are increasingly using RNAi as a method to knockdown growth factors and molecules in the related pathways. For instance knockdown of fibroblast growth factor-2 (FGF-2), a growth factor known to be important for neuron survival and differentiation during development, in the dentate gyrus during adulthood was reported to have anxiogenic effects in rats (44) (Table 1). The relationship of signaling pathways secondary to growth factors with depression- and anxiety-like behavior is another focus of interest. For instance a study has reported that the expression of the active (phosphorylated) form of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) in the NAc was decreased following EE (45). In parallel with this finding, knockdown of CREB in the NAc resulted in decreased depression-like and increased anxiety-like behavior similar to that caused by EE. Interestingly knockdown of adenyl cyclase-5 (AC5), an enzyme that converts ATP into cAMP, in the NAc was reported to have anxiolytic effects (46).

A problem in diagnosing psychiatric disorders is the lack of selective markers to differentiate psychiatric diseases from each other. In an attempt to identify such a marker, the deficiency of theta activity seen in the EEGs of generalized anxiety disorder patients was modeled in animals (47). The origin of cholinergic neurons, the medial septum, was targeted, because of its role both in the generation of the theta rhythms and in the development of anxiety. The researchers decreased the expression of phospholipase C-β4 (PLC-β4), a molecule known to be important for the generation of cholinergic theta rhythms in the medial septum (48), and found a significant increase in the anxiety levels of these animals when compared to controls (Table 1). Moreover acetylcholinesterase inhibitors ameliorated cholinergic theta rhythms and decrease anxiety in PLC-β4 knockout mice by increasing cholinergic transmission. The authors suggest that their findings may help to identify patients with mild cognitive impairment who will proceed to Alzheimer's disease and to develop new strategies for the treatment of anxiety disorders.

3. Use of RNAi in human studies

As summarised above, RNAi holds promise for the treatment of many psychiatric disorder. The difficulties in introduction of siRNA's into the cells and the immunological responses they may cause are the main obstacles for the use of RNAi in clinical trials (7). For these reasons none of the siRNA's tested in animals that hold promise for treatment has yet been translated into human studies. Many promising studies, however are being conducted to overcome these obstacles. In a recent randomized, double-blind, placebo controlled study, the intranasal administration of siRNA's was reported to be effective against respiratory syncytial virus infection (49), which indicates the therapeutic potential of RNAi in human diseases. There is also a case report in which intradermal injection of siRNAs targeting the mutant gene of a rare autosomal dominant skin disorder resulted in the regression of the lesion (50). One of the main advantages of this method is that siRNAs only target the mutant gene, leaving the wild-type gene unaltered; therefore, the function of the normal gene is not affected by this method. In another recent study, injection of siRNA's targeting the ribonucleotide reductase M2 subunit (RRM2), a target molecule for cancer treatment, via nanoparticles into the bloodstream was found to decrease RRMr mRNA and protein levels in solid tumors and the authors provided evidence for an RNAi mechanism of action for this observed effect (51).

CONCLUSIONS

In summary RNAi is a method that can help to identify the vulnerability of genes underlying mental disorders, to determine which molecules mediate the action of environmental and developmental factors on behavior, to determine selective markers for different mental disorders and to develop new treatment strategies. It has several advantages. First siRNAs can be developed to suppress the expression of any gene. Secondly it can be designed and prepared rapidly and easily, and can be effective at picomolar concentrations. Finally, depending on the half-life of the target gene, its effect may last for several weeks. For all these reasons it is a method that holds promise for the treatment of mental disorders. The above mentioned obstacles for its use in the clinical trials, however, should be overcome. Important improvements in this area have been made in recent studies. In the light of these new improvements, siRNAs that were previously tested in animals and hold promise for the treatment of psychiatric disorders should be tested in clinical trials.

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