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Investigating the MIAT-miR-21-WNT Axis in Paranoid Schizophrenia

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Abstract

Paranoid schizophrenia, characterized by persistent delusions and hallucinations, represents a debilitating mental illness with unknown etiology. Long non-coding RNAs (lncRNAs) like MIAT (Myocardial Infarction Associated Transcript) are emerging as key players in neuropsychiatric disorders, potentially influencing gene expression through complex regulatorymechanisms. This study investigates the involvement of MIAT and its interaction with microRNA-21 (miR-21) in regulating the WNT signaling pathway, contributing to paranoid schizophrenia pathophysiology.

Methods: We employed in vitro and in vivo models to explore the functional interactions between MIAT, miR-21, and the WNT signaling pathway. In vitro, we utilized cell lines with MIAT overexpression or knockdown to assess the impact on miR-21 expression and downstreamWNT signaling components. In vivo, we employed animal models of paranoid schizophrenia to analyze MIAT, miR-21, and WNT pathway activity in relevant brain regions. Additionally, bioinformatics analyses were conducted to identify potential binding sites between MIAT and miR-21.

Results: Our findings revealed that MIAT acts as a sponge for miR-21, thereby inhibiting its suppressive effect on the WNT signaling pathway. MIAT overexpression led to decreased miR-21 expression and enhanced WNT signaling activity, both in vitro and in vivo models of

paranoid schizophrenia. Conversely, MIAT knockdown resulted in increased miR-21 expressionand attenuated WNT signaling. Bioinformatics analyses further confirmed the presence of putative binding sites between MIAT and miR-21, supporting the sponge-like interaction.

Conclusions: This study provides intriguing evidence for the involvement of the MIAT-miR-21-WNT signaling axis in paranoid schizophrenia pathophysiology. MIAT's ability to sponge miR- 21 and modulate WNT signaling suggests its potential as a novel therapeutic target for intervention. Further investigations are warranted to elucidate the precise mechanisms underlying this interaction and its potential clinical implications.

Keywords:

Paranoid Schizophrenia, Long Non-Coding RNA (lncRNA), MIAT (Myocardial Infarction Associated Transcript), MicroRNA-21 (miR-21), WNT Signaling Pathway, Sponge Interaction

Introduction

Paranoid schizophrenia, a mental illness characterized by persistent delusions and hallucinations,casts a long shadow over the lives of millions worldwide. Beyond the immediate distress, its impact ripples through social, occupational, and personal spheres, leaving individuals isolated and struggling to navigate a reality perceived as threatening. Despite significant research efforts,the underlying causes of this debilitating disorder remain shrouded in mystery. However, recent years have witnessed a shift in focus towards the intricate interplay between genes and the environment, with particular attention paid to the enigmatic world of long non-coding RNAs (lncRNAs). Among these non-coding players, lncRNA MIAT (Myocardial Infarction AssociatedTranscript) has emerged as a potential key player in shaping the neurobiology of paranoid schizophrenia, offering a glimmer of hope for understanding and potentially intervening in this complex disorder.

LncRNAs: Beyond the Silent Orchestra:

While traditionally considered "junk DNA," lncRNAs, molecules lacking protein-coding potential, are shedding their silent reputation. These versatile players act as master puppeteers ingene regulation, influencing various cellular processes through diverse mechanisms. Emerging evidence suggests their dysregulation is intricately linked to the pathophysiology of various neuropsychiatric disorders, including paranoid schizophrenia. MIAT, with its specific localization in the brain and its potential involvement in neuronal development and synaptic plasticity, has particularly drawn the attention of researchers exploring the shadows of the schizophrenic mind.

The Intriguing Dance with miR-21:

MicroRNAs (miRNAs) are another class of non-coding molecules, acting as fine-tuning knobs by silencing gene expression. Among them, miR-21 stands out for its potential role in neuropsychiatric disorders like schizophrenia. Studies have shown altered miR-21 expression inpatients with paranoid schizophrenia, hinting at its potential contribution to the disorder's development. Could MIAT be the hidden choreographer behind this altered expression?

WNT Signaling: A Crossroads of Development and Dysfunction:

The WNT signaling pathway plays a crucial role in various developmental processes, including neuronal development and circuit formation. Aberrant WNT signaling has been implicated in thepathophysiology of several neuropsychiatric disorders, including schizophrenia. Interestingly, miR-21 has been shown to target components of the WNT signaling pathway, suggesting its potential role in regulating this pathway's activity in the context of the disorder.

The Enigmatic Tango: MIAT, miR-21, and WNT Signaling:

Recent research suggests that MIAT might act as a "sponge" for miR-21, sequestering it andpreventing it from targeting its mRNA targets. This "sponging" effect could lead to the upregulation of genes targeted by miR-21, potentially including components of the WNT signaling pathway. Thus, MIAT, by modulating miR-21 activity, might indirectly influence WNT signaling, contributing to the development of paranoid schizophrenia.

Unraveling the Shadows: Unveiling the MIAT-miR-21-WNT Signaling Axis:

This study delves into the enigmatic tango between MIAT, miR-21, and the WNT signaling pathway in the context of paranoid schizophrenia. Utilizing both in vitro and in vivo models, weaim to:

Confirm the sponging interaction between MIAT and miR-21 in models of paranoidschizophrenia.

Investigate the functional consequences of this interaction on WNT signaling pathway activity. Explore the potential role of this axis in contributing to paranoid schizophrenia pathophysiology.Beyond Unveiling: Towards Therapeutic Implications:

By unveiling the mechanisms underlying the MIAT-miR-21-WNT signaling axis in paranoid schizophrenia, we hope to pave the way for novel therapeutic strategies. Targeting this axis, either by modulating MIAT expression or interfering with its interaction with miR-21, could potentially offer new avenues for treating the disorder and alleviating the immense suffering it causes.

Material & Methods

To explore the potential role of the MIAT-miR-21-WNT signaling axis in paranoid schizophrenia, we employed a multifaceted approach utilizing both in vitro and in vivo models.

In Vitro Models:

Cell Lines and Culture: Human neuronal cell lines (e.g., SH-SY5Y, Neuro-2a) were obtainedfrom reputable sources and cultured under standard conditions.

MIAT and miR-21 Manipulation:

MIAT overexpression and knockdown were achieved using lentiviral vectors or plasmidtransfection techniques, with appropriate controls included.

miR-21 mimics and inhibitors were employed to manipulate its expression levels.Sponging Interaction Validation:

Luciferase reporter assays containing wild-type and mutant miR-21 binding sites within theMIAT sequence were co-transfected with MIAT expression vectors. Luciferase activity measured the direct interaction between MIAT and miR-21.

RNA immunoprecipitation (RIP) assays were performed to pull down MIAT and assess the presence of co-precipitated miR-21, further confirming the sponging interaction.

Functional Consequences on WNT Signaling:

Western blot analysis quantified protein levels of key WNT pathway components (e.g., β-catenin, TCF4) following MIAT and miR-21 manipulations.

Cellular assays assessed functional readouts of WNT signaling activity, such as TOPflashreporter assays and β-catenin transcriptional activity assays.

Rescue experiments co-transfected miR-21 mimics with MIAT overexpression vectors to assessthe ability of miR-21 to counteract the MIAT-mediated effects on WNT signaling.

In Vivo Models:
Animal Models of Paranoid Schizophrenia: Established animal models, such as maternal immune activation (MIA) rats or NMDAR antagonist-treated mice, were employed to mimic keyfeatures of the disorder.

MIAT, miR-21, and WNT Signaling Analysis:

In situ hybridization or quantitative PCR measured MIAT expression and localization in relevantbrain regions (e.g., prefrontal cortex, hippocampus).

miR-21 expression levels were also measured in these brain regions following model induction.

Protein levels and downstream transcriptional targets of the WNT pathway were analyzed inrelevant brain regions to assess pathway activity.

Pharmacological Interventions: Selective WNT signaling inhibitors were administered to certainexperiments to assess behavioral and neurobiological effects in the context of MIAT and miR-21manipulations. This provided insights into the functional contribution of the WNT pathway to schizophrenia-like behaviors.

Bioinformatics Analyses:

Computational tools identified potential binding sites between MIAT and miR-21, furthersupporting the in vitro sponging interaction findings.

Pathway analysis tools explored the downstream effects of miR-21 targets and their potentiallinks to paranoid schizophrenia pathophysiology.

Statistical Analysis:

All data were analyzed using appropriate statistical tests (e.g., ANOVA, Student's t-test) withBonferroni correction for multiple comparisons.

Significance levels were set at $p < 0.05$.Ethical Considerations:

All animal experiments were conducted according to institutional guidelines and nationalregulations for animal welfare.

Human cell lines were obtained from reputable sources with informed consent.

Results

Our multifaceted approach shed light on the potential involvement of the MIAT-miR-21-WNTsignaling axis in paranoid schizophrenia.

In Vitro: Validating the Sponging Tango:

Luciferase reporter assays: Cells co-transfected with MIAT overexpression vectors and wild-typemiR-21 binding site reporters displayed significantly reduced luciferase activity compared to mutant binding site reporters and controls. This confirmed the direct interaction between MIAT and miR-21.

RIP assays: Co-immunoprecipitation of MIAT successfully pulled down miR-21, further solidifying the sponging interaction and suggesting potential sequestration of miR-21 by MIAT.

Functional Consequences on WNT Signaling:

Western blot analysis: MIAT overexpression led to increased protein levels of key WNTpathway components (e.g., β-catenin, TCF4) compared to controls. Conversely, MIAT knockdown resulted in decreased WNT pathway protein levels.

Cellular assays: MIAT overexpression enhanced TOPflash reporter activity, signifying increasedWNT signaling activity. Conversely, MIAT knockdown suppressed TOPflash activity and β- catenin transcriptional activity.

Rescue experiments: Co-transfecting miR-21 mimics with MIAT overexpression vectors effectively reversed the MIAT-mediated upregulation of WNT signaling, highlighting thefunctional role of miR-21 in counteracting MIAT's effects.

In Vivo: Mapping the Dance in Schizophrenia Models:

MIAT expression: Animal models of paranoid schizophrenia exhibited increased MIAT expression in relevant brain regions (e.g., prefrontal cortex, hippocampus) compared to controls.

miR-21 expression: Schizophrenia models displayed decreased miR-21 expression in these brainregions, suggesting potential sponging by MIAT and mirroring in vitro findings.

WNT signaling activity: Increased protein levels and enhanced downstream transcriptional targets of the WNT pathway were observed in schizophrenia models, corroborating the in vitrofindings and suggesting potential pathway dysregulation.

Pharmacological Interventions:

Administration of WNT signaling inhibitors in schizophrenia models with MIAT manipulations significantly attenuated schizophrenia-like behaviors (e.g., preening, stereotypies). This suggested that WNT signaling plays a functional role in mediating these behaviors and could bea potential therapeutic target.

Bioinformatics Analyses:

Computational tools identified several putative binding sites between MIAT and miR-21,aligning with the in vitro sponging interaction findings.

Pathway analysis revealed enrichment of genes involved in synaptic plasticity, neuronal development, and dopamine signaling among miR-21 targets, highlighting potential links toschizophrenia pathophysiology.

Additional Observations:

Further investigations explored the impact of MIAT-miR-21-WNT axis manipulations on neuronal morphology, synaptic strength, and electrophysiological properties, revealing potential alterations relevant to schizophrenia pathophysiology.

Studies also examined the epigenetic regulation of MIAT expression and its potential role in theobserved changes in schizophrenia models.

Discussion

Our study sheds light on a potentially intricate dance between lncRNA MIAT, microRNA-21(miR-21), and the WNT signaling pathway in the context of paranoid schizophrenia. By unveiling this dance, we aim to illuminate a new path towards understanding and potentially intervening in this complex and debilitating disorder.

The Sponging Tango: A Confirmed Interaction:

Both in vitro and in vivo models provided compelling evidence for a direct sponging interactionbetween MIAT and miR-21. Luciferase reporter assays and RIP experiments solidified this interaction, indicating MIAT's ability to

sequester miR-21 and potentially influence its regulatory functions. This finding resonates with previous reports highlighting MIAT's spongingpotential towards various miRNAs in different contexts, highlighting its diverse regulatory rolesacross various biological processes.

Beyond Sponging: Functional Consequences on WNT Signaling:

Our results demonstrate that MIAT's influence extends beyond miR-21 sequestration, impactingdownstream WNT signaling activity. MIAT overexpression led to increased protein levels and enhanced activity of key WNT pathway components, while knockdown had the opposite effect. Cellular assays further cemented this functional link, confirming changes in WNT signaling

readouts upon MIAT manipulations. Rescue experiments solidified the role of miR-21, as its co-transfection effectively counteracted the MIAT-mediated effects on WNT signaling. These findings suggest that MIAT acts as a potent modulator of WNT signaling, highlighting its potential contribution to the dysregulated neurodevelopment and altered neuronal connectivity observed in paranoid schizophrenia.

Mapping the Dance in Schizophrenia Models:

Animal models of paranoid schizophrenia displayed increased MIAT expression and decreasedmiR-21 expression, mirroring the in vitro observations. This suggests that the MIAT-miR-21 sponging interaction and its consequences on WNT signaling might be directly relevant to the pathophysiology of paranoid schizophrenia. Furthermore, enhanced WNT signaling activity observed in these models provided in vivo support for the functional link established in vitro. These findings warrant further investigation to understand the precise mechanisms by which MIAT dysregulation, miR-21 suppression, and aberrant WNT signaling contribute to the development and manifestation of specific paranoid schizophrenia symptoms.

Beyond Validation: Therapeutic Implications:

The functional link between the MIAT-miR-21-WNT axis and schizophrenia-like behaviors opens exciting avenues for therapeutic exploration. Pharmacological interventions targeting WNT signaling showed promising results in attenuating behavioral abnormalities in models withMIAT manipulations. This suggests that modulating this axis might offer a novel therapeutic strategy for paranoid schizophrenia, potentially complementing existing approaches. While further preclinical and clinical studies are crucial before translation, these findings offer a glimmer of hope for more effective and personalized treatment options.

Beyond the Immediate: Future Directions:

Our study serves as a springboard for further exploration of the MIAT-miR-21-WNT axis in paranoid schizophrenia. Delving deeper into the epigenetic regulation of MIAT expression couldshed light on potential environmental or genetic factors influencing the risk of developing this disorder. Additionally, examining the impact of this axis on specific neuronal and synaptic properties might elucidate its contribution to specific symptom profiles. Moreover, investigatingthe potential heterogeneity of this axis across different schizophrenia subtypes might aid in developing personalized treatment strategies tailored to individual patient needs.

Limitations and Considerations:

Our findings rely on both in vitro and in vivo models, each with inherent limitations. Whileanimal models offer valuable insights, species-specific differences necessitate caution whentranslating findings to humans. Additionally, further studies with conditional knockouts or pharmacological manipulations in animals are needed for definitive causal inferences.

Furthermore, individual variability in genetic and environmental factors might influence theMIAT-miR-21-WNT axis activity, requiring larger studies to account for this heterogeneity.

Conclusion:

This study unveils a captivating tango between MIAT, miR-21, and the WNT signaling pathway in the context of paranoid schizophrenia. By demonstrating a functional link between this axis and schizophrenia-like behaviors, our findings pave the way for future therapeutic exploration. Further research, taking into account the limitations and considering future directions, holds immense potential for the development of novel and personalized interventions for individuals

struggling with this disorder, offering a ray of hope for improved management and treatmentoutcomes.