이학석사 학위논문

Exploring communication factors specific to hippocampal neural stem cell survival through exosome analysis

엑소좀 분석을 통한 해마 신경 줄기세포
생존에 특이적인 소통 인자 탐색

울산대학교 대학원
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이 논문을 이학석사 학위 논문으로 제출함

2024 년 8 월

울산대학교대학원
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문태권의 이학석사 학위 논문을 인준함

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Abstract

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Background

Hippocampal neural stem cells are highly sensitive to insulin and die in its absence. However, the impact of insulin deficiency on the interactions between hippocampal-derived neural stem cells and neighboring glial cells remains poorly understood. This contributes to the progression of diseases such as neurodegenerative cognitive disorders. Exosomes, secreted by various cells, contain substances like nucleic acids (DNA, RNA), proteins, and lipids involved in cell-to-cell signaling. miRNAs, in particular, are key communication vehicles for these interactions.

Aim

The aim of this study is to investigate exosome-derived miRNAs involved in the survival of hippocampus-derived neural stem cells and to determine their role in interactions with other nervous system cells. Furthermore, the potential of miRNAs as biomarker will be explored.

Results

I successfully isolated exosomes secreted by hippocampus-derived neural stem cells under insulin-deficient conditions using ExoQuick-TC precipitation solution. The typical EV markers such as CD9 were confirmed by western blotting. Candidate miRNAs whose expression changes were detected by microarray were screened, and the miRNAs with increased expression were studied. I performed RT-qPCR on the selected miRNAs to reconfirm their increased expression. Over-expression experiments were conducted with the

selected miRNAs, revealing that miR-4*1-5p affected the expression of the mTOR complex in astrocytes and microglia, while miR-2*3-3p had little effect in these cell types.

Conclusions

My experiments indicate that exosome-derived miRNAs, particularly miR-4*1-5p, can significantly influence intracellular signaling pathways in neighboring glial cells by affecting the mTOR complex in astrocytes and microglia. In contrast, miR-2*3-3p showed little effect on these pathways. This suggests that specific miRNAs play distinct roles in the regulation of cellular processes under insulin-deficient conditions.

Keywords

Hippocampal neural stem cells, Exosomes, MicroRNA, Cell signaling, Biomarkers

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Introduction

Recent research into cell therapy using neural stem cells for various neurodegenerative diseases has emerged as a promising alternative [1-3]. And a significant challenge in stem cell therapy research remains the low survival rate of cells post-transplantation [4]. Although the HCN cells utilized in our study were discovered relatively recently, it is established that basic fibroblast growth factor (B-FGF) and insulin play direct roles in their growth and differentiation [5]. Furthermore, HCN cells cultured in vitro have been transplanted into the brains of animal models, where they have demonstrated the ability to integrate into and function within the neural network at the transplant site [4].

A recent study reported that culturing HCN cells without insulin significantly impairs stem cell growth and induces cell death through the autophagy mechanism [6]. Autophagy is a process that regulates cellular homeostasis by removing abnormal cellular materials via autophagosome formation [7, 8]. Additionally, autophagy is involved in tissue differentiation and development and has been implicated in various diseases [9-11].

The nervous system is composed of neurons and glial cells, including astrocytes and microglia. These cells play a role in maintaining brain functional homeostasis and are responsive to various stimuli through interactions that can impact cell activity, function, and survival [12]. Phenomenological reports indicate that certain glial cells influence neurogenesis and the cell fate of neural stem cells; however, the underlying mechanisms of these actions remain unknown [13].

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs approximately 22 nucleotides in length that regulate genes and the genome at the post-transcriptional level [14-17]. These miRNAs are abundant in neural stem cells and are believed to function as signaling factors mediating cell-to-cell communication [18]. Additionally, they hold potential as biomarkers for various diseases [19].

Exosomes are small vesicles with a lipid bilayer membrane structure, approximately 30–150 nm in diameter [20], which are released into the extracellular matrix through the fusion of intracellular multivesicular bodies with the cell membrane [21]. Exosomes naturally exist in various body fluids, including blood, urine, saliva, ascites, and cerebrospinal fluid [22, 23]. They contain cell-specific proteins, lipids, and nucleic acids [24-26], which can be transported to target cells to regulate their transcription and phenotype [26, 27]. Therefore, exosomal miRNAs, which mediate cell-to-cell communication and are stably transferred, can be reliably stored under various conditions, highlighting their potential as biomarkers or therapeutic tools.

In this study, I successfully isolated exosomes secreted by hippocampus-derived neural stem cells under insulin-deficient conditions. Candidate miRNAs whose expression changes were detected by microarray were screened, and the miRNAs with increased expression were studied. RT-qPCR was performed on the selected miRNAs to reconfirm their increased expression. Over-expression experiments revealed that miR-4*1-5p affected the expression of the mTOR complex in astrocytes and microglia, while miR-2*3-3p had little effect. These findings suggest that specific miRNAs play distinct roles in the regulation of cellular processes under insulin-deficient conditions.

Materials and Methods

Adult hippocampal neural stem cell

Cells were cultured in Dulbecco's modified Eagle's medium nutrient mixture F-12 (Invitrogen) supplemented with 1.27 g/L of sodium bicarbonate (Invitrogen), 100 mg/L of transferrin (Sigma), 16mg/L of putrescine dihydrochloride (Sigma), 20-nM progesterone, 30-nM sodium selenite (Sigma), 100 μg/ml of streptomycin, and 100 U/ml penicillin (Invitrogen). Also, all plate for HCN cell culture were coated with 10 mg/L of poly-L-ornithine and 1 mg/L of Laminin.

miRNAs transfection

MiRNA mimics were obtained from Genolution. HCN cells were transfected with miRNAs using lipofectamine2000, Oligofectamine (Invitrogen) following the manufacturer's instruction.

Exosome isolation

HCNs were cultured at high density in 10 cm dishes in the absence and presence of insulin for 48 hr, then the cultures were centrifuged to concentrate and suspended in 200 μl of 1x PBS using ExoQuick-TC exosome precipitate (System Biosciences, Mountain View, CA, USA) according to the manufacturer's protocol, centrifuged at 13,000 rpm for 10 min, precipitated, and suspended in 200 ul of 1x PBS.

Exosome RNA sequencing

Exosome preparation and RNA-seq analysis were performed by SBI's Exosome RNA NGS Service (https://www.systembio.com/services/exosome-services/exo-ngs). Nano particle Tracking Analysis (NTA) is an analytical technique that tracks nanoparticles in the 10 to

2000 nm diameter range in suspension, enabling the size and concentration of exosomes to be determined and visualized in real time. Illumina NGS libraries were prepared and sequenced using an Illumina HiSeq2000 sequence analyzer (Illumina Inc., San Diego, CA, USA). Data analysis occurred using the Banana Slug analytics platform (Banana Slug Genomics Center, Santa Cruz, CA).

RT-qPCR

TRIzol reagent (Invitrogen; 15596026) was used to isolate the total RNA of each tissue sample. For quantitation of miRNAs expression, reverse transcription was performed with the Mir-X miRNA first-strand synthesis kit (Clontech Laboratories, Mountain View, CA, USA; 638313), followed by PCR amplification using the iQ SYBR Green I mix (Bio-Rad, Hercules, CA, USA; 170-8885) on the CFX96 real-time system (Bio-Rad). miR-1*2-5p; 5'-AGGUUCUGUGAUACACUCCGACU-3' was used as the reference for relative quantification. The primer used for miR-3*1- 5p; 5' -TCCCTGAGGAGCCCTTTGAGCCTGA-3', let-*i-5p; 5' -UGAGGUAGUAGUUUGUGCUGUU-3', miR-2*a-5p; 5'-UUCAAGUAAUCCAGGAUAGGCU-3', miR-3*a-5p; 5'-TGG CAG TGT CTT AGC TGG TTG T- 3', miR-92b-5p; 5'-AGG GAC GGG ACG CGG TGC AGT GTT-3', miR-1*5a-5p; 5' -TCC CTG AGA CCC TTT AAC CTG TGA- 3', miR-1*9-3p; 5' -TGG AGA CGC GGC CCT GTT GGA G- 3', miR-1*8-5p; 5'-CAT CCC TTG CAT GGT GGA GGG- 3',miR-2*3-3p; 5' -TGTCAGTTTGTCAAATACCCC - 3', miR-4*1-5p; 5'-AAACCGTTACCATTACTGAGTT- 3', miR-2*2-3p; 5'-AGCUACAUCUGGCUACUGGGU -3', miR-1*2-5p; 5' -CAUAAAGUAGAAAGCACUACU -3', miR-7*1-3p; 5' -AAAGAUGCCACGCUAUGUAGAU-3', miR-2*b-5p; 5' -AGAGCUUAGCUGAUUGGUGAACAG - 3', miR-1*9a-3p; 5' -

ACAGUAGUCUGCACAUUGGUUA -3', miR-2*3a-3p; 5' -GUGAAAUGUUUAGGACCACUAG -3', miR-1*3-3p; AGCAGCAUUGUACAGGGCUAUGA-3', miR-*a-5p; 5' -UGGAAGACUAGUGAUUUUGUUGU-3', miR-4*4-3p; 5' -TGA AAC ACA CAC GGG AAA CCT C -3'

LDH assay

Cytotoxicity was measured using the LDH-Glo Cytotoxicity Assay Kit (#J2380, Promega, Madison, WI, USA) following the manufacturer's instructions. Cells were treated, and supernatants were collected. 50 μL of supernatant and 50 μL of LDH Detection Reagent were mixed in 96-well plates and incubated for 60 minutes at room temperature. Luminescence was measured using a plate-reading luminometer, with background. LDH release controls included. All experiments were performed in triplicate to ensure reproducibility and accuracy.

Western blotting

Proteins were resolved on sodium dodecyl sulfate (SDS)-polyacrylamide gels and then transferred to polyvinylidene fluoride (PVDF) membranes. After blocking with Tris-buffered saline (TBS) containing 0.1 % Tween-20 and 5 % (w/v) bovine serum albumin, membranes were incubated with various primary antibodies and appropriate secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Bands were detected using an enhanced chemiluminescence (ECL) system. . CD9 (60232-1, Proteintech) was used as the marker of exosome. Caspase-3 (9665S,Cell Signaling), Bcl-2 (2870S,Cell Signaling), Bax (556467,BD), Bak (12105S,Cell Signaling), mTOR (2983S,Cell Signaling), phospho-mTOR(2448) (5536S,Cell Signaling), phospho-mTOR(2481) (2974S,Cell Signaling), S6K (AF8962,R&D) phospho-S6K (AF8963,R&D), S6 (2217S,Cell Signaling), phospho-S6

(4856S,Cell Signaling), pkc alpha (2056S,Cell Signaling), phospho- pkc alpha (9375S,Cell Signaling), AKT (4691,Cell Signaling), phospho-AKT (4691,Cell Signaling), Beclin (3738,Cell Signaling), p62 (5114,Cell Signaling), LC3 (L8918,Sigma), AMPK (5831S,Cell Signaling), phospho-AMPK (2535S,Cell Signaling), ACC1 (3676S,Cell Signaling), phospho-ACC1(S221) (ab109540,abcam), phospho-ACC1(S79) (3661S,Cell Signaling) The β-Actin (A5441,Sigma) was used as the loading controls.

Statistical Analysis

Data were presented as mean ± standard deviation, and the experiments were performed at least in triplicate. Significant differences were determined at $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$. Graphs were used to visualize the data, including significance and mean ± SD values.

Result

Exosome characterization of HCN in the presence and absence of insulin

Exoquick-TC precipitation solution was used for exosome isolation, and the expression of the typical EV marker CD9 was confirmed by western blotting (Figure 1C). CD9 was well expressed regardless of the I (+) or I (-) conditions. Additionally, there was no significant difference in surface markers between the exosomes secreted by control HCN and damaged HCN. Nanoparticle Tracking Analysis (NTA) demonstrated that the insulin (+) exosomes particles had a diameter of 129 ± 6.1 nm and a concentration of 3.01 ± 0.14 × 10^8 particles/ml, whereas insulin (-) exosomes had a diameter of 113 ± 4.3 nm and a concentration of 4.84 ± 0.3 × 10^8 particles/ml (Figure 1A). EVs from both groups were seen as round-shaped structures on nanosight (Figure 1B). Collectively, these results indicated no significant differences in the mass, number of particles, or structure of the exosomes released from insulin (+) and insulin (-) HCN cells.

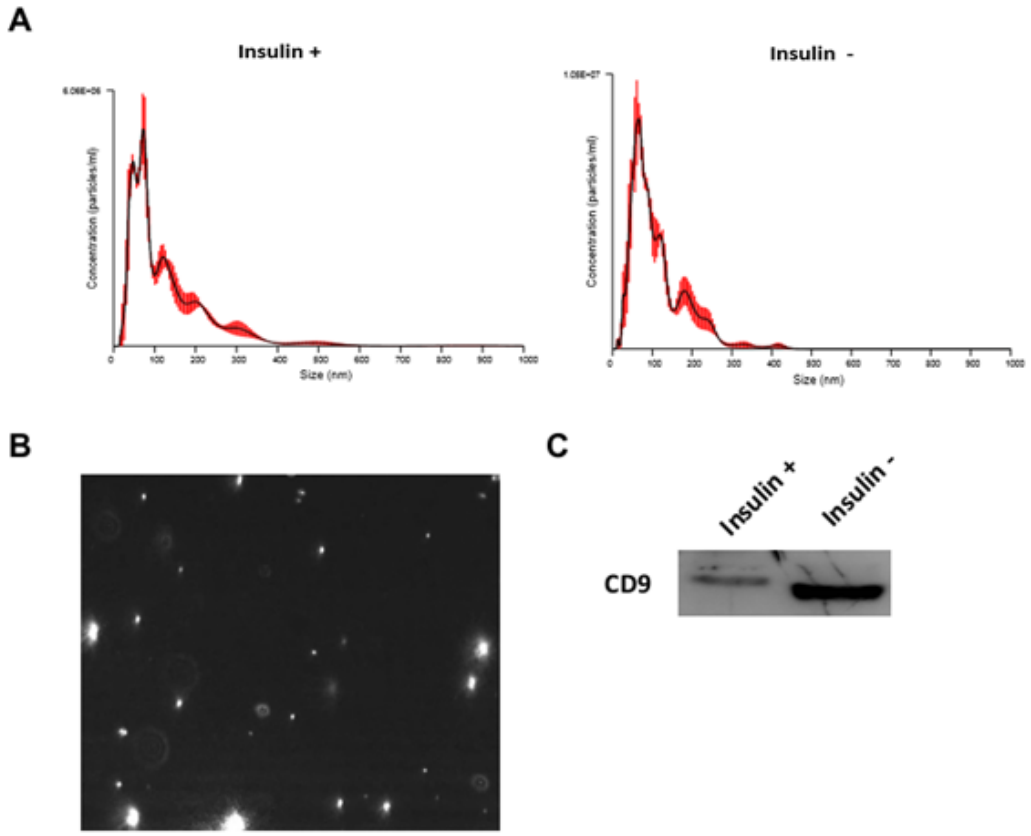

Fig. 1. Characterization of HCN-derived exosomes in the presence and absence of insulin. (A) Size distribution and concentration of exosomes measured by Nanoparticle Tracking Analysis (B) Nanosight image of exosomes (C) Western blotting for exosome marker protein (CD9)

Exosome RNA-seq analysis and candidate exosomal miRNAs

To identify DEGs expressed in exosomes, I collected conditioned media in the presence or absence of insulin and analyzed by RNA-seq. A total of 7,088 DEG types were analyzed, of which miRNAs were identified in approximately 3% of them (Figure 2). From the results obtained by NGS analysis, I listed up the top 8 mature form miRNAs with high fold-value ratio of expression when insulin (+) compared to insulin (-) (Table 1). Then, I used miRWalk database (http://mirwalk.uni-hd.de/) to obtain a list of targetable genes for these 8 miRNAs, and performed Gene Ontology pathway analysis through Enrichr analysis (http://amp.pharm.mssm.edu/Enrichr.), and found that genes related to mTOR signaling were the most abundant, indicating that these exosomal miRNAs are likely to be involved in cell proliferation (Figure 3A). Using volcano plots of candidate miRNAs predicted for exosomes using RNA-Seq data, I confirmed the expression levels of candidate miRNAs in exosomes (Figure 3B). Additionally, differentially expressed genes (DEG) analysis was used to identify genes based on their p-value in insulin (+) and (-) conditions (Figure 3C).

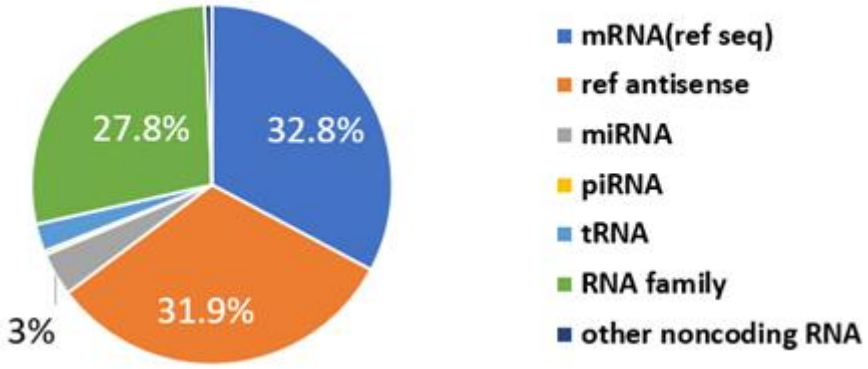

Fig 2. RNA-Seq analysis of the DEGs in exosomes released by HCN. (A) characterization of exosomal RNA contents.

Table 1. List of top 8 fold-value mature form miRNAs with high expression ratios in the absence of insulin in HCN.

#	ID	logFC	P.Value
1	rno-miR-4*1-5p	5.543389	0.102014
2	rno-miR-2*3-3p	4.580108	0.003686
3	rno-miR-1*4-3p	3.962223	0.023018
4	rno-miR-2*2-3p	2.573526	0.189296
5	rno-miR-1*2-5p	2.428042	0.21598
6	rno-miR-7*1-3p	2.113726	0.372223
7	rno-miR-2*b-5p	2.029436	0.399021
8	rno-miR-1*2-5p	2.025144	0.441125

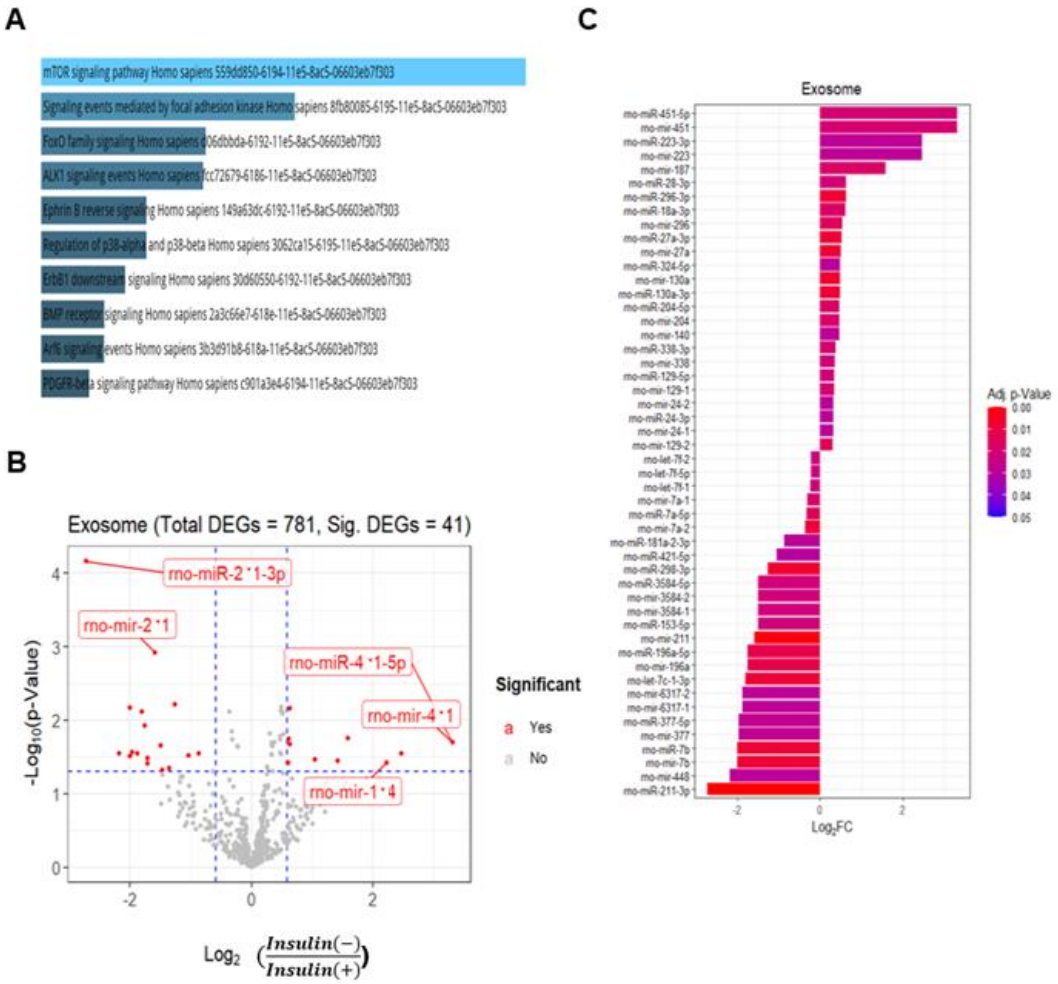

Fig 3. Analysis of Differentially Expressed Genes (DEGs) and Candidate miRNAs in Exosomes. (A) GO pathway analysis results of DEGs (B) Volcano plots of predicted candidate miRNAs for exosomes using RNA-Seq data, depicting the expression levels of candidate miRNAs in exosomes (C) DEG analysis identifying genes differentially expressed under insulin (+) and (-) conditions

Evaluation of exosomal miRNA expression levels

I focused on miR-4*1-5p and miR-2*3-3p, which showed a significant increase in expression in exosomes without insulin. The two selected miRNAs were analyzed by GO pathway to confirm the role of the candidate genes and found that they affect the process of vesicle release from the cell and the mitochondria outer membrane (Figure 4A). Following the examination of the expression of candidate miRNAs in insulin (+) and (-) cells, it was observed that miR-1*2-5p exhibited minimal variation in expression levels. Consequently, it was selected as a reference gene for normalization. As a result, I found that the expression of miR-2*3-3p was significantly upregulated, and most of the miRNAs were also upregulated (Figure 4B).

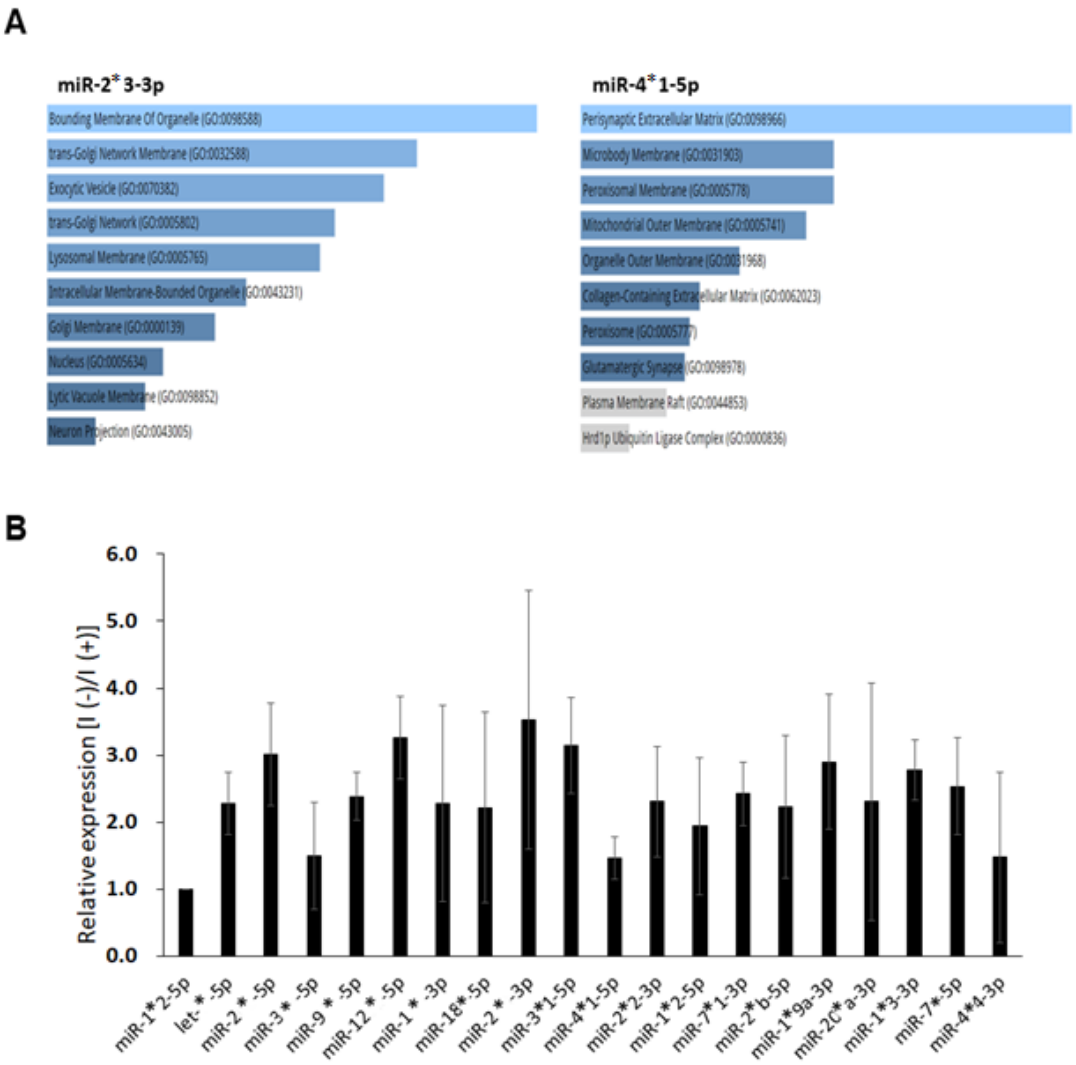

Fig 4. The selection and evaluation of exosomal miRNA candidates. (A) GO pathway analysis results of miR-2*3-3p (B) GO pathway analysis results of miR-4*1-5p (C) The expression of miRNAs was evaluated by RT-qPCR.

Non-lethal expression of miR-2*3-3p and miR-4*1-5p

The influence of miR-2*3-3p and miR-4*1-5p on LDH activity was evaluated, and their effects on LDH activity in astrocytes and microglia cells, which are known to communicate with HCN cells, were assessed. No significant effect on LDH activity was observed in samples overexpressing either miRNA (Figure 5A). Subsequently, to analyze the signaling involved in proliferation and apoptosis, Western blot analysis was performed to evaluate the expression of caspase-3, Bcl-2, Bax, and Bak. The results demonstrated that there was no significant change in the expression of caspase-3, Bcl-2, Bax, and Bak, apoptosis-related proteins (Figure 5B).

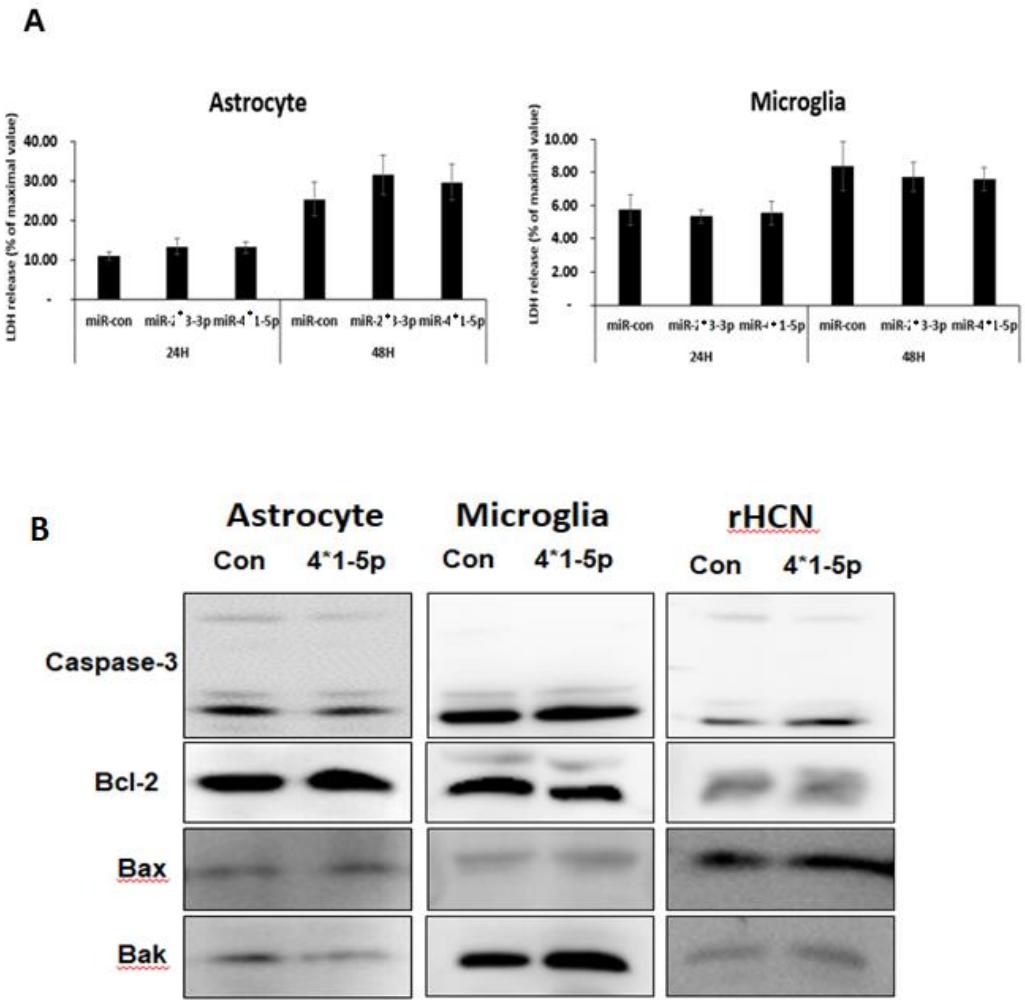

Fig 5. Cell death after overexpression of exosomal miRNAs in astrocytes and microglia cells. (A) Overexpression of miRNAs and subsequent LDH activity measurement 24 hours post-transfection (B) Expression patterns of proteins associated with cell death, including caspase-3, Bcl-2, Bax, and Bak

Regulation of mTOR signaling pathway and autophagy-related pathways in astrocytes, microglia cells were identified after miRNA treatment.

To examine cell signaling regulation by exosomal miRNAs on astrocytes and microglia, miRNAs were overexpressed to assess the specific changes that occurred. When I analyzed the effects of miR-2*3-3p and miR-4*1-5p treatment on mTOR signaling, I found that miR-4*1-5p increased the protein levels of S6K, S6, and PKC alpha in astrocytes, confirming the activation of mTORC1 and 2 (Figure 6C). Interestingly, there were no significant changes in other signaling pathways. Whereas, to investigate changes in cellular metabolism, I looked at ACC1, a gene involved in fatty acid synthesis, and found that the level of repressive phosphorylation of ACC1 was reduced in microglia (Figure 6A). For miR-2*3-3p, no activation of mTOR signaling or other signaling was observed (Figure 6B). In contrast, miR-4*1 and miR-2*3 appear to have different effects on the regulation of metabolism, suggesting distinct roles for these miRNAs.

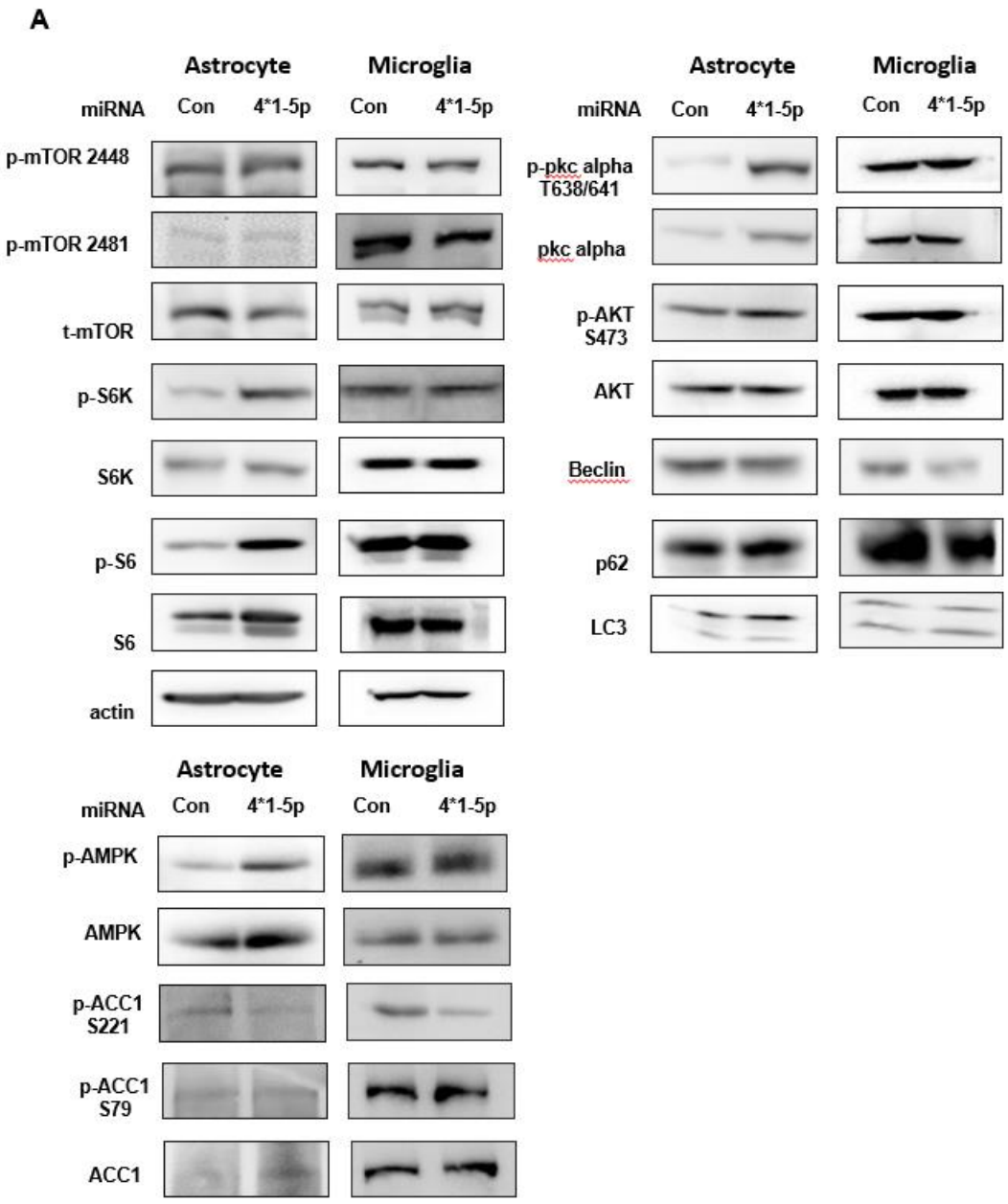

Fig 6. Expression level of signaling after overexpression of exosomal miRNAs in astrocyte and microglia cells. (A) Signaling in miR-4*1-5p-treated astrocytes and microglia cells (B) Signaling in miR-2*3-3p-treated astrocytes and microglia cells (C) Changes in protein levels of p-S6K, p-S6, and p-PKC alpha, components of the mTOR complex, following the overexpression of miR-4*1-5p in astrocytes

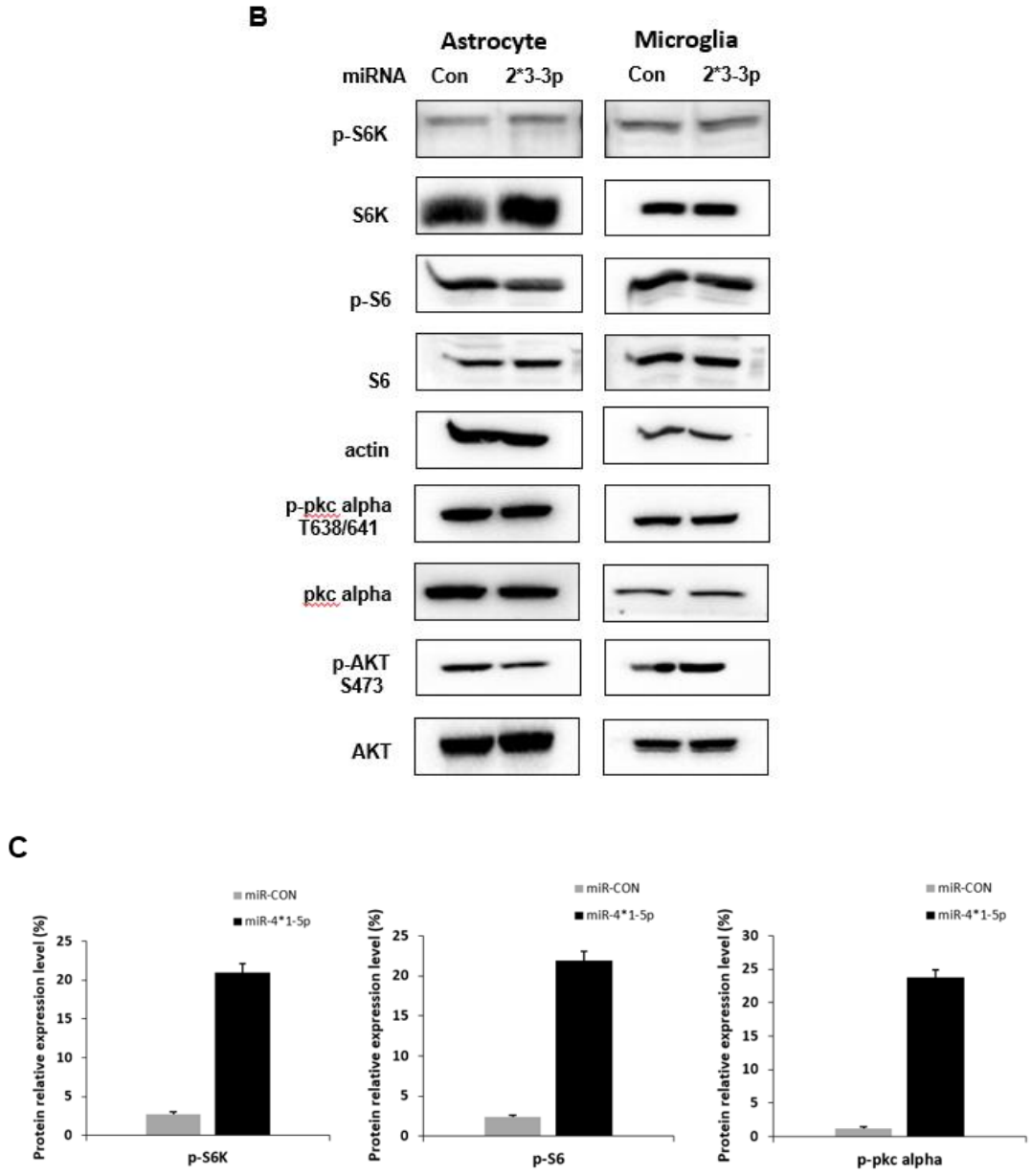

Fig 6. Expression level of signaling molecules after overexpression of exosomal miRNAs in astrocyte and microglia cells.

Discussion

The present study provides a comprehensive analysis of the role of exosome-derived miRNAs in the survival and interaction of hippocampal neural stem cells (HCNs) with neighboring glial cells under insulin-deficient conditions[6]. The findings elucidate significant insights into the molecular mechanisms by which exosomal miRNAs influence cellular communication and survival, particularly in the context of insulin availability[18].

I began by characterizing the exosomes isolated from HCN cells cultured under both insulin-sufficient and insulin-deficient conditions. Despite the differences in culture conditions, the physical properties of the exosomes, including their size and concentration, remained consistent. This consistency suggests that the observed functional effects are due to alterations in the exosomal cargo, rather than changes in the exosome structure itself[26, 27]. The Western blot analysis of CD9 and the nanoparticle tracking analysis (NTA) reinforced that the exosomes from both conditions retained their typical vesicular characteristics, emphasizing the role of their molecular contents in mediating cellular effects.

The RNA-Seq analysis identified a substantial number of differentially expressed genes (DEGs) between the insulin-sufficient and insulin-deficient conditions. Among these, a specific subset of miRNAs exhibited significant changes in expression. Notably, miR-4*1-5p and miR-2*3-3p were significantly upregulated in the exosomes derived from insulin-deficient conditions. This differential expression points to a potential regulatory mechanism by which insulin availability influences miRNA profiles within exosomes.

The overexpression studies of miR-4*1-5p and miR-2*3-3p provided insights into their functional roles. Despite the significant upregulation of these miRNAs in insulin-deficient conditions, their overexpression did not induce cytotoxicity, as indicated by unchanged lactate dehydrogenase (LDH) activity levels in astrocytes and microglia. This finding suggests that these miRNAs are involved in non-lethal regulatory processes rather than

directly triggering cell death. Further, the protein expression analysis of apoptosis-related markers (caspase-3, Bcl-2, Bax, Bak) did not show significant changes upon miRNA overexpression. This reinforces the notion that miR-4*1-5p and miR-2*3-3p are not primarily involved in apoptotic pathways under the experimental conditions used.

One of the notable findings was the differential impact of miR-4*1-5p on cell signaling pathways. Overexpression of miR-4*1-5p in astrocytes led to the activation of key components of the mTORC1 and mTORC2 pathways, including S6K, S6, and PKC alpha. These pathways are crucial for cell growth, proliferation, and metabolic regulation, indicating that miR-4*1-5p plays a significant role in modulating these processes. The reduction in repressive phosphorylation of ACC1 in microglia further supports the involvement of miR-4*1-5p in metabolic regulation, particularly in fatty acid synthesis. The lack of significant changes in signaling pathways following miR-2*3-3p overexpression suggests a more specific or context-dependent role for this miRNA. These differential effects underscore the complexity of miRNA-mediated regulation and the necessity of context-specific studies to fully elucidate their roles.

The ability of exosomal miRNAs to mediate intercellular communication and modulate key signaling pathways positions them as crucial players in neural stem cell biology and potential therapeutic targets[26, 27]. The stability of miRNAs within exosomes and their ability to be transferred between cells without degradation highlight their potential as biomarkers for early diagnosis and as therapeutic tools in neurodegenerative diseases. Given the impact of miR-4*1-5p on mTOR signaling, which is known to influence neurogenesis and neural stem cell proliferation, targeting this miRNA could provide novel therapeutic strategies to enhance neural stem cell survival and function in neurodegenerative conditions. The findings from this study pave the way for further in vivo investigations to explore the therapeutic potential of exosomal miRNAs.

Future studies should focus on the detailed mechanisms by which these miRNAs regulate

cell signaling pathways and their effects in animal models of neurodegenerative diseases. The Otsuka Long-Evans Tokushima fatty (OLETF) rat model, a human type 2 diabetes model, shows promise as a biomarker of neurodegenerative brain disease if miRNAs are altered in the early stages of diabetes[28-30]. In addition, exploring the therapeutic delivery of miRNAs via exosomes may provide new therapeutic avenues. The potential use of exosomal miRNAs as biomarkers for early detection and monitoring of disease progression also requires extensive study.

In conclusion, our findings highlight the critical role of exosomal miRNAs in regulating the survival and function of neural stem cells under insulin-deficient conditions. These results highlight the potential of miRNAs as therapeutic targets and biomarkers, and provide a foundation for the development of miRNA-based strategies for the treatment of neurodegenerative diseases. The significance of this study is that it provides new insights into the molecular interactions between exosomal miRNAs and neural stem cells and highlights the importance of insulin availability in these processes. However, it is important to note the limitations of the study, including the need for further validation in in vivo models and the need to explore additional miRNAs and signaling pathways involved in these regulatory mechanisms.

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국문 요약

배경

해마 유래 신경줄기세포는 인슐린에 매우 민감하여 인슐린 결핍 시 사멸하는 특성을 보이고 있다. 하지만 당뇨 병증과 같은 상태에서 나타나는 해마 유래 신경줄기세포의 손상이 주변 교세포들과의 상호작용에 어떠한 영향을 미치는지. 이를 통해 궁극적으로 신경 퇴행성 인지장애 등의 질병 발생과 진행에 어떠한 영향을 주는지는 밝혀진 바가 거의 없다. 다양한 세포에서 분비되는 것으로 알려진 엑소좀은 세포 간 신호전달에 관여하는 핵산(DNA, RNA), 단백질 지방 등 다양한 물질을 포함하고 있는데, 그 중에서도 miRNA 는 세포 간 상호작용의 주요 소통 수단으로 간주되고 있다.

목적

본 연구에서 해마에서 유래한 신경줄기세포의 생존에 관련된 엑소좀 유래 miRNA 를 조사하고, 이들이 다른 신경계 세포와의 상호작용에서 어떤 역할을 하는지 규명하고자 한다. 또한, 바이오 마커로서 miRNA 의 가능성을 탐색하고자 한다.

결과

인슐린 결핍 조건에서 해마 유래 신경줄기세포가 분비하는 엑소좀을 ExoQuick-TC 침전 용액을 사용하여 성공적으로 분리하였다. CD9 와 같은 대표적인 EV 마커를 웨스턴 블롯팅으로 확인하였다. 마이크로어레이를 통해 발현 변화가 감지된 후보 miRNA 를 선별하고, 발현이 증가된 miRNA 를 연구하였다. 선별된 miRNA 를 대상으로 RT-qPCR 을 수행하여 발현 증가를 재확인하였다. 선별된 miRNA 를 대상으로 과발현 실험을 수행한 결과, miR-4*1-5p 는 성상세포와 미세아교세포에서 mTOR 복합체의 발현에 영향을 미치는 반면, miR-2*3-3p 는 이러한 세포 유형에 거의 영향을 미치지 않는 것으로 나타났다.

결론

본 연구결과에 따르면 엑소좀 유래 miRNA, 특히 miR-4*1-5p 는 성상교세포와 미세아교세포의 mTOR 복합체에 영향을 미침으로써 인접한 아교세포의 세포 내 신호 경로에 상당한 영향을 미칠 수 있다. 반면, miR-2*3-3p 는 이러한 경로에 거의 영향을 미치지 않는 것으로 나타났다. 이는 인슐린 결핍 조건에서 세포 과정의 조절에 특정 miRNA 가 뚜렷한 역할을 한다는 것을 시사한다.

중심 단어

해마 유래 신경줄기세포, 엑소좀, MicroRNA, 세포 신호, 바이오 마커