Exosomal microRNA-let-7b-5p Derived From Pancreatic Cancer Cells Possibly Promotes Insulin Resistance in C2C12 Myotube Cells by Targeting SLC6A15

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Research

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Abstract

Background Cancers trigger systemic metabolic disorder usually associated glucose intolerance, which as initial apparent phenomenon. One of the features of pancreatic cancer (PC) metabolic reprogramming is the crosstalk between PC and peripheral tissues (skeletal muscle and adipose tissues), emphasized by insulin resistance (IR). In our previous study (Sci Rep. 2017;7(1):5384), we reported that mice pancreatic cancer derived exosomes could induce skeletal muscle cells(C2C12) IR and exosomal microRNAs (miRNAs) may exert an important effect. This work was carried out to investigate whether there exist a direct functional relationship between PC exosomal miRNAs and C2C12 cell genes, in the pathological process of IR.

Methods The expression profiles of exosomal miRNAs were evaluated using the Agilent Mouse miRNA V21.0 chip (GSE95741). The differentially expressed genes were screened through Agilent SurePrint G3 Mouse GE V2.0 chip (GSE174058). TargetScan and miRbase databases were used for target genes prediction and the prediction results were verified by dual-luciferase reporter gene assay.

Results The biochips (GSE95741 and GSE174058) revealed that exosomes derived from mouse pancreatic cancer cells had higher levels of miRNA-let-7b-5p (Log FC 8.6); SLC6A15 gene expression was down-regulated in C2C12 cells (Log FC -4.7). Related mice and human studies has showed that SLC6A15 is associated with IR of metabolic disorder. In this work, luciferase assays confirmed that a direct interaction between miRNA-let-7b-5p and the SLC6A15 3´-untranslated region (3´-UTR) was established, as predicted by the TargetScan and miRbase.

Conclusions Our data suggest that exosomal miRNA-let-7b-5p may promote IR in C2C12 myotube cells targeting SLC6A15.

Background

Pancreatic cancer(PC) is a morbid disease and there are currently no reliable forms of early detection, which leads to a 5-year survival rate for PC patients that is under 9%[1]. Therefore, the main goals of PC research lie in looking for markers of the early stage, understanding the biological behaviors of the tumor and developing novel therapeutic methods and targets.

Multiple clinical studies have shown that pancreatic cancer-associated new-onset diabetes mellitus (PC-DM) may be one of the early indicators of PC[2–5]. The fundamental cause is “metabolic reprogramming” and “metabolic crosstalk”[6–8], characterized by PC. In other words, PC affects peripheral tissue (skeletal muscle and adipose tissues) through certain mechanisms, and results in insulin resistance (IR) in these tissues, which is the main pathological component of PC-DM and type II diabetes mellitus (T2DM)[9].

How does PC exert its influence on peripheral tissues from afar? In our previous study (Sci Rep. 2017;7(1):5384), we reported that mice pancreatic cancer derived exosomes could induce skeletal muscle
cells(C2C12) IR and exosomal microRNAs (miRNAs) may exert an important effect. But the mechanisms between specific exosomal miRNAs and target genes are still waiting to be elucidated.

This work aims to find the candidate exosomal miRNAs and target genes, through microarray technology and bioinformatics analysis, and then check the functional relationship by dual-luciferase reporter gene assay. This may help clarify PC biological behaviors and develop potential therapeutic targets for PC.

**Material And Methods**

**Microarray assay**

The miRNA microarray analysis was performed by OE Biotech.Co.,Ltd. (Shanghai, China; [http://www.oebiotech.com/](http://www.oebiotech.com/)), using the Agilent Mouse miRNA V21.0 chip (GSE95741). The differentially expressed genes were screened through Agilent SurePrint G3 Mouse GE V2.0 chip (GSE174058). TargetScan, and miRbase databases were used for target genes prediction.

**Cell Culture and Transfection**

Human embryonic kidney 293T (HEK-293T) cell lines were purchased from National Collection of Authenticated Cell Cultures (shanghai, China). All cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 μg/ml streptomycin sulfate at 37°C in a humidified incubator containing 5% CO2. The miRNA-let-7b-5p mimics were synthesized by Sangon Biotech Co.,Ltd. (Shanghai,China) and transfected into 293T cells using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's recommendations.

**Dual Luciferase Reporter Assay**

The 3'-UTR of SLC6A15(NM_001358821) containing the miRNA-let-7b-5p binding sites and its corresponding mutated sequence were cloned into pmir-GLO dual luciferase reporter vector (Promega), named Wt-SLC6A15-3'UTR-pmir-GLO and Mut-SLC6A15-3'UTR-pmir-GLO, respectively. Using Lipofectamine 2000 (Invitrogen), HEK-293T cells were cotransfected with the reporter constructs, miRNA-let-7b-5p mimics and negative control. Luciferase activity was determined after 48h using the Dual-Glo substrate system (Promega) and a Beckman Coulter LD400 luminometer. Data are presented as the ratio of experimental (Renilla) luciferase to control (Firefly) luciferase.

**Statistical analysis**

Results are expressed as the means ± standard error. Analyses between different groups were performed using the two-tailed Student t-test or one-way analysis of variance. Statistical significance was set as follows: a P value of 0.05 was significant, and a value of 0.01 was highly significant.

**Results**
MiRNA-let-7b-5p Directly Targeting SLC6A15

To investigate whether SLC6A15 is a direct target of miR-let-7b-5p, we cloned the SLC6A15 3’-untranslated region fragment containing the miR-let-7b-5p binding site and mutated targeting sequence into pmir-GLO dual luciferase reporter vectors, named Wt-SLC6A15-3’UTR-pmir-GLO and Mut-SLC6A15-3’UTR-pmir-GLO, respectively. The result showed that ectopic expression of miR-210 significantly inhibited the luciferase activity in HEK-293T cells transfected with Wt-SLC6A15-3’UTR reporter vector. The luciferase activity levels in HEK-293T transfected with Mut-SLC6A15-3’UTR reporter vector were restored (Fig. 1). Taken together, our results demonstrate that SLC6A15 is a target of miR-let-7b-5p.

Discussion

PC is one of the most invasive carcinomas worldwide, which causes distinct symptoms of malnutrition and altered glucose homeostasis. The only curative therapeutic treatments rely on surgical resection, yet the efficiency of this approach is limited by the highly aggressive behaviors and lack of inceptive diagnosis. About 90% of PC patients who lost the chance of surgery eventually show cachexia—a severe complication involving pathological weight loss due to the wasting of skeletal muscle and adipose tissue [10,11]—The clinical syndrome also indicates high metastasis rate, dangerous condition, poor prognosis and low survival rate.[12].

During the disease progression, the severity of IR is closely related to the development of cancer cachexia [13,14]. In the state of IR, the normal signal pathway is inhibited, and the proteolysis system is activated, leading to the degradation of muscle protein [15], which is the primary characteristic of cancer cachexia.

How does PC exert its influence on peripheral tissues IR from afar? As previously reported (Sci Rep. 2017;7(1):5384), mice pancreatic cancer derived exosomes could induce skeletal muscle cells (C2C12) IR and exosomal microRNAs (miRNAs) may exert an important effect. But the mechanisms in this filed are still waiting to be clarified.

In this work, the biochips (GSE95741 and GSE174058) revealed that exosomes derived from mouse pancreatic cancer cells had higher levels of miRNA-let-7b-5p (Log FC 8.6); SLC6A15 gene expression was down-regulated in C2C12 cells (Log FC -4.7). Related mice and human studies has showed that SLC6A15 is associated with IR of metabolic disorder [16,17], and luciferase assays confirmed that a direct interaction between miRNA-let-7b-5p and the SLC6A15 3’-untranslated region (3’-UTR) was established, as predicted by the TargetScan, and miRbase.

Conclusion

This work suggest that exosomal miRNA-let-7b-5p may promote IR in C2C12 myotube cells by targeting SLC6A15. Our data paves the way towards discovering potential targets for correcting metabolic disorders and improving the diagnosis/treatment of PC.
Abbreviations

PC: pancreatic cancer; IR: insulin resistance; miRNAs: microRNAs; PC-DM: pancreatic cancer-associated new-onset diabetes mellitus.

Declarations

Acknowledgments

Not applicable

Authors' contributions

Lantian Wang conceived and designed the study, performed the experiments and was the contributor to the writing of the manuscript.

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Availability of data and materials

The author confirm that all regarding data are available.

Ethics approval and consent to participate

Not applicable

Consent for publications

Not applicable

Patient consent for publication

Not applicable.

Conflict of interest

The author declare that there is no conflict of interest.

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Figures
miRNA-let-7b-5p directly regulates SLC6A15 expression in HEK-293T cells. (A). The complementary sequences of miR-let-7b-5p were discovered in 3'UTR of SLC6A15 mRNA using TargetScan and miRBase. The mutagenesis was performed in the complementary sites for the seed region of miR-let-7b-5p (wt, wild type; mut, mutant type.) (B). miR-let-7b-5p inversely modulated the luciferase activity of plasmids that carried wt 3'UTR of SLC6A15 rather than mut 3'UTR of SLC6A15. n=four independent experiments. Values are the MEAN ± SD. *P < 0.05 vs Control. **P < 0.01 vs Control.