The ARRIVE Guidelines Checklist

Cell Research: Reporting In Vitro Experiments

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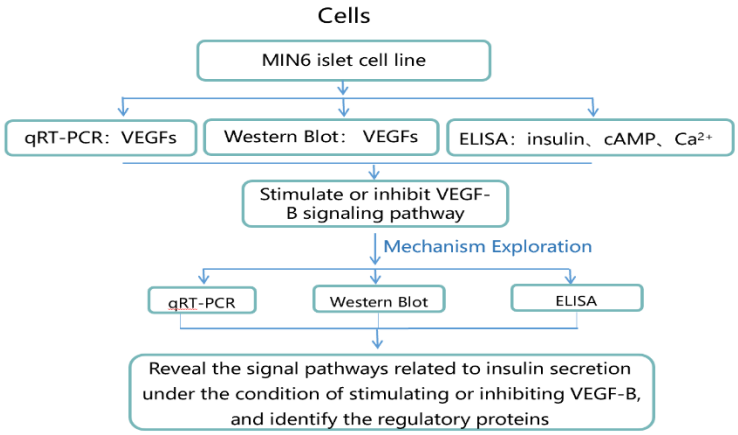
	ITEM	RECOMMENDATION	Reported on page #
Title	1	VEGF-B inhibits insulin secretion in MIN6 cells and reduces Ca2+ and cAMP levels through the PI3K/AKT pathway	1
Abstract	2	Type 2 diabetes (T2D) and its complications are serious threats to human health. However, the insulin secretion mechanism is currently unclear. The MIN6 cell line of mouse islets used in our study can simulate the function of normal pancreatic β-cells in vitro to analyze the mechanism of insulin secretion or biological effects. The purpose of this study is to explore the effect of vascular endothelial growth factor B (VEGF-B) on insulin secretion by stimulating and inhibiting the expression of VEGF-B in MIN6 cells. Studies have found that VEGF-B can inhibit the expression of VEGF-B in MIN6 cells, thereby providing mechanistic insights for insulin secretion and the prevention and treatment of T2D.	3
INTRODUCTION			4
Background	3	a. Diabetes and diabetic complications are serious threats to human health. Due to the high fatality rate, high disability rate and high medical cost, diabetes has become a common health problem faced by countries worldwide. The International Diabetes Federation (IDF) predicts that by 2040, there will be 642 million adults with diabetes worldwide, of which type II diabetes, which is caused by insulin secretion defects or decreased insulin sensitivity, accounts for more than 90% of the total number of diabetic patients [1]. VEGF-B is a member of the VEGF family. Seven VEGF family members have been identified in mammals, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth	

		factor (PIGF) [2, 3]. Members of the VEGF family and 3 tyrosine kinase receptors, namely, VEGFR1 (Fms-like tyrosine kinase, Flt-1), VEGFR2 (kinase insert domain containing receptor, KDR-Flk-1) and VEGFR3 (Flt-3), exert biological effects after binding. In addition, neuropilins, such as NRP1 and NRP2, can cooperate with VEGFR [4-6]. In 2012, Hagberg et al. proposed for the first time that abnormal lipid deposits in peripheral tissues can impair insulin sensitivity, which in turn affects glucose uptake and is one of the factors that triggers T2D. The researchers found that reducing VEGF-B levels in db/db mice could restore insulin sensitivity by reducing the accumulation of lipids in the heart and skeletal muscle; blocking VEGF-B signaling in high-fat diet (HFD)-fed rats could also restore insulin sensitivity and increase glucose uptake in skeletal muscle and the heart, thereby reducing blood glucose levels. Hagberg et al. proposed that VEGF-B may be involved in the lipid uptake process, opening the possibility of new strategies for regulating pathological lipid accumulation in diabetes, obesity and cardiovascular diseases [7]. Therefore, in this study, we investigated the effect of VEGF-B on insulin secretion in MIN6 cells by interfering with the expression level of VEGF-B. b. The MIN6 cell line was established from mouse insulinoma and is very similar to normal pancreatic islets in terms of glucose metabolism and glucose-stimulated insulin secretion. This cells line can be used as a model to simulate β-cell functions and study insulin secretion in vitro.	
Objectives	4	The main goal of this study is to study the effect of VEGF-B on insulin secretion in MIN6 cells. The secondary goal is to explore the mechanism of the effect of VEGF-B on insulin secretion.	
METHODS			6
Ethical statement	5	This study complies with cell use and biosafety instructions.	
Study design	6	a. Both the experimental group and the control group have at least 6 parallel experiments. b. In each group of experiments, the experimenter, environment, variables, etc. are kept consistent to minimize the influence of subjective factors.	

		c. 
Experimental procedures	7	a. MIN6 cells are stimulated by exogenous VEGF-B protein (PeproTech): adding VEGF-B protein to the culture medium to culture the cells. The method of adding SiRNA is the same as VEGF-B protein. b. MIN6 cells (Wuhan Feien Biotechnology, Hubei, China) were cultured in RPMI1640 (Hyclone, Sh30809) supplemented with 10% fetal bovine serum (FBS; gibco 10099-141), penicillin and streptomycin in a water-saturated atmosphere with 5% CO_2 at 37°C.
Experimental cells	8	a. The MIN6 cell line was established from mouse insulinoma and is very similar to normal pancreatic islets in terms of glucose metabolism and glucose-stimulated insulin secretion. b. The experimental cells were within 20 generations.
housing and husbandry	9	The MIN6 cells are cultured in a water-saturated atmosphere with 5% CO2 at 37°C.
Simple size	10	a. Cell counting is done by using a cell counter. b. Each experiment was replicated at least 6 times.
Allocating cells to experimental groups	11	The same batch of cells are processed uniformly and grouped randomly.
Experimental outcomes	12	a. Cell Counting Kit-8 was used to detect the effects of exogenous VEGF-B and knockdown VEGF-B on the proliferation of MIN6 cells. b. Flow cytometry was used to detect the effect of exogenous VEGFB on MIN6 cell apoptosis.
Statistical methods	13	SPSS 22 was used for statistical analysis of all experimental data. Measurement data were expressed as($\bar{x}\pm s$). One-way analysis of variance was used for comparison between multiple groups, $P<0.05$ is considered statistically different.
RESULTS		8

Baseline data	14	Observe the number, shape, size and other status of the cells before the experimental treatment, and evaluate the cell condition.	
Numbers analysed	15	Groups of all cells.	
Outcomes and estimation	16	Each result data are statistically analyzed by SPSS.	
Adverse events	17	a. Each group of adverse events is the group with greater difference. b. It can be processed by discarding or redoing the data.	
DISCUSSION			11
Interpretation/ scientific implications	18	a. Exogenous VEGF-B inhibited the secretion of insulin and simultaneously reduced the levels of Ca2+ and cAMP in MIN6 cells. Exogenous VEGF-B also reduced the expression of phospholipase C gamma 1 (PLCγ1), phosphatidylinositol 3-kinase (PI3K), serine/threonine kinase (AKT) and other proteins in the insulin secretion pathway. After knocking down VEGF-B in MIN6 cells, insulin secretion and Ca2+ and cAMP levels were increased, and the expression of PLCγ1, PI3K, AKT and other proteins was upregulated. b. Limitations: This study is conducted at the cellular level, and further exploration is required at the animal level.	
Generalisability/ tracslation	19	Our research at the cellular level will be further verified in animals. These results will provide mechanistic insights into the prevention and treatment of T2D.	
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