Validation of mouse phosphoprotein enriched in astrocyte 15 (mPEA15) expressing transgenic pig as a potential model in diabetes translational research

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Abstract

The present study aimed to investigate the characteristics of mPEA15 expressing transgenic pig (TG pig) as a potential model for diabetes. Expression analysis confirmed the ubiquitous expression of mPEA15 in TG pigs at F4. Oral glucose tolerance test results showed that restoration of normal glucose levels was significantly delayed in the TG pigs when compared with that in the wild-type pigs (WT pigs). Primary skeletal muscle cells isolated from TG pigs demonstrated reduced glucose uptake and reduced GLUT4 translocation to the plasma membrane in response to insulin treatment. Combined, these results suggest that mPEA15 expressing pigs has a glucose intolerance and insulin resistance which are known to mediate the pathophysiology of type 2 diabetes mellitus. Thus, mPEA15 transgenic pigs would serve as a promising model for diabetes translational research.

Keywords Diabetes · Glucose tolerance · GLUT4 · Insulin resistance · PEA15 · PED · Transgenic pig

Introduction

Diabetes mellitus (DM) is a worldwide problem in modern society and according to the World health organization, it will be the seventh leading cause of death by 2030 (World Health Organization 2015). DM is characterized by a common characteristic of high blood glucose levels and various complications such as diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy (Pearson 2019; Bingley et al. 2018). Type 1 DM (T1DM) considered as an autoimmune disease and is associated with insulin deficiency, weight loss, polyuria, and polydipsia. Type 2 diabetes

(T2DM) is the most common type of diabetes and associated with overweight and obesity (Association 2019). T2DM patients often suffered from reduction or failure of glucose uptake in peripheral tissues even in the presence of insulin and various degrees of β -cells dysfunction.

Phosphoprotein Enriched in Astrocytes (PEA15), also known as Phosphoprotein Enriched in diabetes (PED), was first identified in astrocytes, is a ubiquitously expressed phosphoprotein (Araujo et al. 1993; Danziger et al. 1995). Over-expression of PEA15 is commonly found in human diabetic patients and has a prominent role in glucose metabolism and DM (Fiory et al. 2009; Condorelli et al. 1998). PEA15 overexpression also contributes to insulin resistance in T2DM (Valentino et al. 2006; Vigliotta et al. 2004). PEA15 was shown to be overexpressed in the skeletal muscle and adipose tissues of T2DM patients, regardless of drug therapy and obesity (Condorelli et al. 1998). The overexpression of PEA15 in high fat-fed transgenic mice leads to diabetes (Vigliotta et al. 2004). Phospholipase D (PLD) mediated deregulation of protein kinase C signaling is known to cause insulin resistance in the muscle and adipose tissues. PEA15 binds to phospholipase D (PLD) and increases its stability thereby also indirectly contributes to diabetic symptoms (Viparelli et al. 2008; Zhang et al. 2000).

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To understand the pathogenesis of diabetes or to develop and test new therapeutics, appropriate animal models are required. With the extensive use of murine models copious data generated, which yielded significant insight into the biological mechanisms of DM at the cellular and molecular level (Al-Awar et al. 2016); however, murine models have several drawbacks, for example, rodents are physiologically and genetically not very close to humans compared to other mammals. Among mammals, pigs turned out to be an excellent diabetes model (Bellinger et al. 2006) because of their similarities to humans including cardiovascular anatomy, pancreatic development and morphology, metabolism, lipoprotein profile, the tendency to obesity, and the possibility of precise genetic modifications. Therefore, the generation and testing of transgenic pig models expressing diabetes-related genes are worthwhile. Previously, our laboratory generated mPEA15 transgenic pigs (Lee et al. 2011). The objective of the present study was to evaluate the mPEA15 transgenic pig as a potential diabetes model. We analyzed the efficacy of the integrated *mPea15* gene. We also attempted to confirm the occurrence of type 2 diabetes-related symptoms in five mPEA15 transgenic pigs.

Materials and methods

Animals

The standard operating procedures for the handling of the pigs and all other procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science, Rural Development Administration of Korea (Approval number: No.2010-053, C-grade). Domestic pigs (*Sus scrofa*, Landrace strain) were used for the development of transgenic pig. The transgenic pigs (TG pigs) were produced as described previously (Lee et al. 2009) and maintained at the National Institute of Animal Science (NIAS), Rural Development Administration (Suwon, Korea). All efforts were made to minimize animal suffering during the normal maintenance and the experimental periods.

Restriction fragment length polymorphisms (RFLPs)

Genomic DNA was isolated using a phenol–chloroform method from liver tissues. DNA from each sample was amplified using PCR with a specific primer set (Sense 5'-GGACTCCCCAGTGAGAAGA-3' and Antisense 5'-TACGGGTTAGCTTGGTGTCC-3'). The PCR was performed under following conditions: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a 5 min at 72 °C. PCR product was incubated with 10U of *EcoRV* (Toyobo, Osaka, Japan) in a final reaction volume of 20 µl

at 37 °C for 4 h. Restriction fragments were separated on 2% agarose gel electrophoresis.

Southern blotting

Genomic DNA was digested overnight with *SphI* (NEB, Massachusetts, USA) at 37 °C. DNA fragments were separated on 1.0% agarose gel and transferred onto a nylon membrane overnight. A 4006 bp *SphI* fragment of PEA15 cDNA was DIG-labeled and hybridized to blots. The DIG labeling was carried out using DIG DNA labeling and detection kit (Roche, Indianapolis, USA) according to the manufacturer's instructions. The amplicons with 318 bp were produced using PCR and used as a hybridization probe.

Western blotting analysis

Proteins from tissues or cultured cells were extracted using Mammalian Protein Extraction Reagent (M-PER) buffer (Thermo Fisher Scientific, Massachusetts, USA) containing a protease inhibitor cocktail (Santa Cruz Biotechnology, Texas, USA). Plasma membrane protein lysates were extracted using a Plasma Membrane Protein Extraction Kit (Abcam, Cambridge, UK). Samples were mixed with 4X Laemmli's sample buffer (Bio-Rad, California, USA) and boiled for 5 min. Protein samples were separated on 10% sodium dodecyl sulfate–polyacrylamide gel and transferred onto nitrocellulose membranes (Amersham Biosciences, Little Chalfont, UK). The membranes were incubated 90 min in blocking buffer containing 5% non-fat milk and 0.05% Tween-20 at room temperature and then immunoblotted with respective primary antibodies (PEA15, 1:1000, Cell Signaling Technology, Massachusetts, USA; FLAG, 1:3000, Sigma-Aldrich, Missouri, USA) at 4 °C for overnight. The nitrocellulose membranes were washed thrice, 10 min each, with TBST washing buffer. The membranes were then incubated with horseradish peroxidase (HRP)-conjugated Goat polyclonal anti-Rabbit IgG (1:3000, Abcam, Cambridge, UK) 1 h at room temperature and immunoreactivity bands were visualized using the enhanced chemiluminescence (ECL solution, Bio-Rad, CA, USA) method under an automatic X-ray film processor (JP-33, JPI health care, Seoul, Korea). The respective band densities were quantified by ImageJ software.

Immunohistochemistry

To immuno-localize PEA-15, 5 µm paraffin sections were mounted on 3-aminopropyl triethoxysilane-coated glass slides and dried overnight at 37 °C. Immunohistochemistry was performed using EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (Abcam, Cambridge, UK) according to the manufacturer's guidelines. Briefly, after

rehydration, the slides were incubated for 90 min in blocking buffer supplemented with 5% non-fat dry milk and 0.05% Tween-20 at room temperature and probed with a primary antibody against PEA15 (1:100, Cell Signaling Biotech. Inc.) or FLAG (1:200, Sigma-Aldrich, Missouri, USA) at 4 °C overnight. Goat polyclonal antibody anti-Rabbit IgG (1:400) was used as a secondary conjugation molecule and incubated for 1 h at room temperature. Proteins were localized using diaminobenzidine (DAB; Sigma-Aldrich, Missouri, USA) in 50 mM Tris-HCl (pH 7.6). Slides were observed using a fluorescence microscope (DMI6000 B, Leica, Wetzlar, Germany).

Oral glucose tolerance test (OGTT)

OGTT was performed on overnight (18 h) fasted animals (WT pigs, $n = 4$; TG pigs, $n = 3$). 50% Glucose solution (400 ml/pig, 200 g) was administrated to the about 11-month-old pigs using an oral injection sonde. Blood samples were drawn from the cervical vein before and 5, 10, 30, 60, 120, 180, 240 min after glucose loading. The blood glucose concentration of the samples was immediately analyzed using Accu-Check glucose test kit (Roche Diagnostics, Basel, Switzerland).

Establishment of primary skeletal muscle cells

Skeletal muscle cells were isolated and established as described previously (Ueyama et al. 1999) with minor modifications. After pig sacrifice, Quadriceps (QUAD) muscles were collected, minced, and digested with 1.25 mg/ml pronase (Sigma-Aldrich, Missouri, USA) for 1 h at 37 °C. Cells were dissolved in PBS and centrifuged at 3000 rpm for 4 min. Pellet was washed with PBS and centrifuged at 1000 rpm for 8 min. To remove mononuclear cells from the muscle fibers and myofibrils fragments, cells were centrifuged at 3000 rpm for 5 min. Cells were plated on poly-L-lysine- and fibronectin-coated dishes to remove fibroblasts. Cells were plated on 8-well culture plates and cultured with Dulbeccos' modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 50 Unit/ml penicillin, 50 µg/ml streptomycin and 50 µg/ml gentamicin at 37 °C, 5% CO₂.

Skeletal muscle cell culture and glucose uptake measurement

Skeletal muscle cells were grown DMEM culture medium containing 0.5% FBS and 2.5 mM glucose for 24 h to induce differentiation and fusion of myoblasts into myotubes, and to prevent further division. The cells were then incubated with or without insulin for 15 min or 24 h. The myotube monolayers were washed with glucose-free KRB buffer at 22°C, then incubated with 1 ml of KRB buffer containing 0.1 mM

2-deoxy-D-glucose (2DG) and 2-deoxy-D-[³H]glucose (0.2 µCi) for 15 min or 24 h at 22 °C. Buffer was removed, and the cells were washed twice with ice-cold KRB buffer, lysed with 0.3 ml of 1 M NaOH solution, and the radioactivity of ³H was counted with a liquid scintillation counter (1450 Micro Beta, TriLux, PerkinElmer, Massachusetts, United States).

Glucose transporter (GLUT4) detection

Detection of glucose transporters at the cell surface was accomplished as previously reported (Caruso et al. 1997). Briefly, cells were pre-incubated in serum-free DMEM (low glucose) supplemented with 2% FBS for 24 h and then further incubated with or without 100 nM insulin for 2, 5, 10, 30, and 60 min. After incubation for pre-determined time periods, cells were removed from culture plates. Subsequently, plasma membrane protein was extracted from cells using a Plasma Membrane Protein Extraction Kit. Total proteins from the cells were extracted with Mammalian Protein Extraction Reagent containing a protease inhibitor cocktail (Santa Cruz Biotechnology, Texas, USA). Western blot experiment to detect GLUT-4 was performed with polyclonal anti-GLUT4 antibody (1:2000, Cell Signaling Technology, Massachusetts, USA).

Statistical analysis

Statistical analysis was performed using sigma plot software. The data presented as mean ± SEM from at least three separate experiments.

Results

mPEA15 ubiquitously expressed in transgenic pigs

The PEA15 peptides of different species share a high degree of homology (Fig. S1). Previously, we developed a transgenic pig expressing mouse-originated PEA15 (Lee et al. 2011). The construct used for the mPEA15 expression shown in Fig. 1a. Briefly, *mPea15* was cloned under the control of CAG promoter (CMV and chicken β -actin promoter fused) and the EGFP gene was fused at 5' ends. To check the integrity of introduced *mPea15* at F4, we performed both PCR amplification followed by RFLP and Southern blot analysis. PCR-RFLP analysis confirmed that all the five transgenic pigs (TG pigs) used in the study carry *mPea15* gene (Fig. 1b). As shown in Fig. 1c, in the southern blot experiment, we detected an expected 4 kb band in TG pig but not in wild-type pig (WT pig) (Fig. 1c).

To identify whether integrated *mPea15* resulted in the mPEA15 expression, first, we performed western blot

Fig. 1 **a** Diagram of expression cassette used to express mPEA15 in pigs. CMV, cytomegalovirus promoter; IRES, internal ribosome entry site; EGFP, enhanced green fluorescent protein; PA polyadenylation. **b** Agarose gel showing representative bands after PCR and RFLP. Genomic DNA isolated from liver tissue was amplified using transgene specific primers, the PCR product was digested with *EcoRV* and separated on 2% agarose gel. M 100 bp size marker; P.P. PCR

product (318 bp); P.C. positive control (179 bp and 139 bp); N.C. negative control; W1–W3 wild-type pigs; T1–T5 transgenic pigs. **c** Southern blot showing *mPea15* integration in the transgenic pig. Genomic DNA of liver tissue was digested with *SphI* and detected using DIG-labeled *mPea15* cDNA fragment. WT wild type; TG transgenic pig

Fig. 2 mPEA15 expressed ubiquitously in transgenic pigs. **a** Representative western blot image showing mPEA15 expression in muscle tissues of the wild type and transgenic pigs. The tissue lysate was immunoblotted with anti-mPEA15 and anti-FLAG antibodies as mentioned in materials and methods. WT: Wild-type pigs ($n=3$) TG: Transgenic pigs ($n=5$). **b** Graph showing the mPEA15 fold differ-

ence between wild type and transgenic pigs. Results represent means SEM from three separate experiments. * $P<0.001$ compared to WT. **c** Representative blots showing PEA15 expression. Tissue sections obtained from muscle, liver, and pancreas were stained with anti-PEA15 and anti-FLAG antibodies. Scale bar is 200 μ m

analysis of muscle tissue. Anti-PEA15 antibody detected a protein band about 15kD in all the pig samples but detected an additional high molecular weight band specifically in the TG pig samples (Fig. 2a, b). Moreover, the high molecular weight bands seen in the TG pigs were detected by anti-FLAG antibody. So, we concluded that the expression of N-terminal fused FLAG tag along with mouse PEA15 contributed to the band size upshift. We next determined the spatial expression of PEA15 by performing immunohistochemistry of tissue sections obtained from muscle, liver, and pancreas of WT and TG pigs. As shown in Fig. 2c, the mPEA15 expression was much stronger in the TG pigs when compared WT pigs. Additionally, we have also quantified the mRNA expression of *mPea15* in several other tissues (heart, fat, muscle, spleen, lung, bladder, stomach, spermary, liver, kidney small intestine, large intestine, and pancreas) using qRT-PCR and the results showed that mPEA15 expression was significantly stronger in TG pigs when compared to WT pigs (data not shown). Collectively, these results indicate that TG pigs express mPEA15 ubiquitously.

mPEA15 transgenic pigs display glucose intolerance in vivo

To investigate whole-body glucose tolerance, we performed an oral glucose tolerance test (OGTT) in overnight fasted about 11-month-old TG and WT pigs (Fig. 3). After fast, the pigs were orally administrated with glucose solution (400 ml/pig, 200 g) and the blood glucose levels of the pigs were measured at various time points (0, 5, 10, 30, 60, 120 and 240 min). After glucose loading, the blood glucose levels of the WT pigs was increased (96 ± 17 mg/dl) within 5 min and started declining to basal levels. In contrast, the TG pigs manifested biphasic glucose curves; blood glucose levels constantly raised and reached to 141 ± 11 mg/dl in 30 min and reduced greatly (94 ± 8 mg/dl) by 60 min. Again,

the blood glucose levels were peaked to 109 ± 21 mg/dl at 120 min before reaching to basal level (74 ± 1) at 240 min. Moreover, the blood glucose levels of TG pigs at 10, 30, and 120 min after glucose loading were significantly higher than that of WT pigs, indicating that the cellular uptake of glucose is weaker in mPEA15 expressing TG pigs.

mPEA15 TG pig derived skeletal muscle cells showed the symptoms of insulin resistance

Insulin enhances glucose disposal from the blood and defects in insulin expression are associated with impaired glucose tolerance (Abdul-Ghani and DeFronzo 2010). To investigate whether the addition of insulin enhances the glucose uptake, primary cultures of skeletal muscle cells were established both from TG and WT pigs. Skeletal muscle cell cultures at the myoblast stage (basal glucose uptake is more at the myoblast stage compared to myotube stage) were incubated in the presence or absence of 100 nM insulin. After 15 min and 24 h of insulin treatment, the glucose uptake of the cell was assessed by estimating 2-deoxy-D-[3 H] glucose in the cells. As evident in OGTT in vivo (Fig. 3), the glucose uptake of TG pig cells in vitro was significantly lower than that of the WT pig cells (Fig. 4). Moreover, even in the presence of insulin the glucose uptake of the TG cells was not further enhanced, suggesting that TG pig cells are insulin resistance.

GLUT4 translocation is altered in mPEA15 TG pig derived skeletal muscle cells

It was well established that GLUT4 and its translocation to the plasma membrane play a major role in insulin-stimulated muscle glucose uptake (Wasserman et al. 2011). Thus, we assumed that the TG pigs may have defects in GLUT4 expression and/or translocation. To find out this, the skeletal muscle cells were treated in vitro with insulin

Fig. 3 Glucose uptake is impaired in mPEA15 transgenic pigs. Oral glucose tolerance test was performed as described in materials and methods. Blood glucose was determined at indicated times points after loading of glucose solution. WT wild-type pigs ($n=4$), TG transgenic pigs ($n=3$). Results represent means SEM from three separate experiments. * $P < 0.05$ compared to WT

Fig. 4 Basal and insulin-stimulated cellular glucose uptake is weaker in skeletal muscle cells obtained from mPEA15 transgenic pigs. Skeletal muscle cells were incubated in the absence or presence of 100 mM insulin as indicated and then cells were assayed for 2-deoxy-

D-glucose (2-DG) uptake as described in Materials and methods. Cells were incubated with insulin for either **a** 24 h or **b** 15 min. Results represent means SEM from three separate experiments. * $P < 0.001$ compared to WT

Fig. 5 Insulin-stimulated GLUT4 translocation to the plasma membrane is reduced in skeletal muscle cells obtained from mPEA15 transgenic pigs. Cultured skeletal muscle cells were incubated with 100 nM insulin for the indicated time points and GLUT4 protein levels of the plasma membrane fraction were analyzed by western blot

analysis. **a** Representative western blot images showing GLUT4 at indicated time points. **b** The graph showing the relative expression levels of GLUT4. WT wild type pigs ($n = 3$); TG transgenic pigs ($n = 5$). Results represent means SEM from three separate experiments. * $P < 0.05$ compared to WT; ** $P < 0.001$ compared to WT

for predetermined time periods (0, 2, 5, 10, 30, and 60 min) and subsequently analyzed for total and plasma membrane-associated GLUT4 using western blot experiment. There was

no difference in the total GLUT4 (Fig. 5). Upon insulin stimulation, in WT pig cells, the GLUT4 protein in the plasma membrane fraction was increased in a time-dependent

fashion; however, there was no difference in the GLUT4 protein in the plasma membrane fraction of TG pig cells, indicating a defective insulin-stimulated GLUT4 translocation in mPEA15 TG pig skeletal muscle.

Discussion

PEA15 is a multifunctional protein that controls a number of cellular functions and diseases (Greig and Nixon 2014; Formstecher et al. 2001; Trencia et al. 2003; Kitsberg et al. 1999), and increased expression of PEA15 was found in the T2DM patients (Condorelli et al. 1998). Moreover, PEA15 overexpressing mice showed diabetes-related symptoms (Vigliotta et al. 2004). Considering the significance of PEA15 in diabetes, earlier, we had successfully developed mPEA15 overexpressing transgenic pigs (Lee et al. 2011). However, the implications of mPEA15 overexpression in the pigs remain to be elucidated. In the present work, we describe the consequences of mPEA15 expression in pigs, particularly focusing on glucose uptake in the skeletal muscle, a major insulin-sensitive organ within the body.

The TG pigs overexpressed PEA15 (Fig. 2) comparable to the levels appear in human T2DM (Condorelli et al. 1998). The TG pigs displayed a decreased glucose clearance ability from the circulation post glucose administration when compared to WT pigs (Fig. 3). The blood glucose level of TG pigs is significantly higher than that of WT pigs at even after 30 min glucose loading and greatly delayed to reach normal values (TG pigs, 240 min vs WT pigs 10–30 min), indicating that overexpression of mPEA15 alone sufficient to impair glucose tolerance in pigs. These results are consistent with the earlier report that overexpression of PEA15 in mice resulted in the impairment of glucose tolerance (Vigliotta et al. 2004). Impairment in glucose uptake represents an early step in the development of T2DM (Pearson 2019) and in diabetes patients, high blood glucose (two-fold higher than the normal blood glucose) at 120 min is considered as an indicator of impaired glucose tolerance (Eyth et al. 2019).

Our in vitro analysis of differentiated skeletal muscle cells obtained from TG pigs revealed that basal cellular glucose uptake is lower in TG pigs. Moreover, the addition of insulin also failed to enhance the cellular glucose uptake in TG pig obtained skeletal muscle cells, which imply that the mPEA15 overexpression leads to insulin resistance in pigs. Indeed, the PEA15 overexpressing transgenic mice showed the symptoms of insulin resistance (Vigliotta et al. 2004). These findings are further supported by Condorelli et al. previous works in rat myoblast cells overexpressing PEA15 (Condorelli et al. 1998, 2001). Insulin resistance leads to a reduction in decreased glucose uptake by peripheral tissues and is considered as an initial event leading to the development of T2DM (Pearson 2019). Thus, it is

plausible that insulin resistance in peripheral tissues could make a significant contribution to the impaired glucose tolerance of mPEA15 TG pigs.

Our study also focused on the possible alterations of GLUT4 expression and translocation in the TG pigs. Both these phenomena are critical determinants of glucose uptake in the skeletal muscle cells and adipose tissue (Shepherd and Kahn 1999). Insulin stimulates glucose uptake in the muscle by enhancing the recruitment of GLUT4 from intracellular storage vesicles to the cell membrane (Leto and Saltiel 2012; Bryant et al. 2002). Our results showed that the total GLUT4 levels are similar in WT and TG pigs; however, insulin-stimulated GLUT4 translocation was weaker in TG pig muscle cells. These results are in agreement with the previous report that PEA15-overexpressing muscle cells are defective in cell surface recruitment of GLUT4 (Condorelli et al. 1998). The impaired glucose tolerance of TG pigs was therefore likely due to the defective GLUT4 translocation to the plasma membrane. It remains to be determined the pathways and molecular mechanisms through which mPEA15 controls the translocation of GLUT4 to the plasma membrane in the TG pigs.

It is well known that T2DM is a complex disease involving many genes such as regulatory, hormonal, and signaling. Therefore, it is unlikely that overexpressing a single gene can create a perfect model animal to study all disease parameters (Wolf et al. 2014). The mPEA15 pig is just one step toward the creation of a perfect model animal. Follow-up studies are in progress to further enhance and evaluate the T2DM phenotype of mPEA15 TG pigs. So far, there is no approved ideal pig model that is fully mimic the pathophysiology of diabetes (Maqbool et al. 2019). TG pigs that express human dominant-negative GIP receptor mutant (*GIPR^{dm}*) displayed reduced insulin secretion (Renner et al. 2010). Another study on TG pigs expressing recombinant human erythropoietin (rhEPO) suggested a potential anti-diabetic effect of EPO (Baek et al. 2019). Recently, Zou et al. developed TG pigs expressing human islet amyloid polypeptide (hIAPP), a protein required for glucose and lipid metabolism (Zou et al. 2019). Considering one of these protein co expressions in mPEA15 TG pigs might induce much stronger diabetes phenotypes.

A study conducted on first-degree relatives (FDR) of T2DM subjects found that FDR of T2DM expresses two-fold higher PEA15 than FDR of non-diabetes subjects and these increased PEA15 levels are strongly correlated with insulin action (Valentino et al. 2006). Consistent with these observations in humans, our mPEA15 overexpressing TG pig model showed insulin resistance. Moreover, Vigliotta et al. reported that the impaired glucose tolerance PEA15 overexpressing transgenic mice was more aggravated and became diabetic under the influence of a high-fat diet, and this phenotype was

much stronger than high-fat-fed nontransgenic littermates (Vigliotta et al. 2004). It remains to be elucidated this phenomenon in the mPEA15 expressing TG pigs.

Advancements in genetic engineering, facilitate the tailoring of transgenic pigs for specific problems in diabetes research and therapy. Genetically engineered pigs could serve as translational diabetes research models to bridge between mouse and human. Our mPEA15 pig are manifested weaker glucose uptake and insulin sensitivity. At this moment we believe that the reason behind these phenotypes is defective GLUT4 translocation. So, these pigs would be effectively applied for the screening of drugs that can modulate GLUT 4 translocation. Indeed, drugs that stimulate GLUT4 translocation, for example, metformin (Lee et al. 2012), are proven to be the best anti-diabetic medication.

In conclusion, based on the above results the present study demonstrates that overexpression of mPEA15 in the pigs appears to develop impaired glucose uptake and insulin resistance. We believe that the mPEA15 TG pigs would serve as an appropriate animal model for testing the drugs and understanding pathophysiology of T2DM, at least, those related to glucose tolerance, insulin sensitivity, and GLUT4 translocation.

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Compliance with ethical standards

Conflicts of interest None.

Ethical approval I testify on behalf of all co-authors that: this article has not been submitted in whole or in part elsewhere. This article is not under consideration for publication in any other journal. All the authors read and approve the final content of the manuscript.

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