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PEDF increases GLUT4-mediated glucose uptake in rat ischemic myocardium via PI3K/AKT pathway in a PEDFR-dependent manner

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ABSTRACT

Background: Targeted increase in glucose uptake of ischemic myocardium is a potential therapeutic strategy for myocardial ischemia. PEDF presents a profound moderating effect on glucose metabolism of cells, but its role is still controversial. Here, we try to demonstrate the direct effect of PEDF on glucose uptake in ischemic myocyte and to elucidate its underlying mechanism.

Methods and results: Lentivirus vectors carrying PEDF gene were delivered into the myocardium to locally overexpress PEDF in a myocardial ischemia/reperfusion rat model. PET imaging showed that PEDF local overexpression increased [^{18}F]-FDG uptake of ischemic myocardium. In vitro, PEDF directly increased the glucose uptake in hypoxic cardiomyocytes. The expression of glucose transporter 4 (GLUT4) on plasma membrane of hypoxic cardiomyocytes was significantly upregulated by PEDF, but its total amount was not changed. The increased glucose uptake and cardioprotective effects induced by PEDF were blocked by the GLUT4 inhibitor indinavir. PEDF-mediated GLUT4 translocation and glucose uptake increase in hypoxic cardiomyocytes were prevented by phosphatidyl-inositol-3 kinase (PI3K) inhibitor or AKT inhibitor. The PEDF-mediated glucose uptake was also diminished when PEDF receptor (PEDFR) was downregulated or potent phospholipase A2 enzymatic activity was inhibited.

Conclusions: PEDF can increase glucose uptake in ischemic myocardium through a PEDFR-dependent mechanism, involving PI3K/AKT signaling and GLUT4 translocation.

1. Introduction

Anaerobic glycolysis is the main source of ATP production in cardiomyocytes after myocardial ischemia (MI) [1]. Researchers have found that the increased glucose uptake by ischemic myocardium is critical for myocyte survival and contractile recovery [2]. Therefore, Targeted increase in glucose uptake in the ischemic myocardium, especially during the acute phase of cardiac ischemia, is a potential therapeutic strategy for MI [3]. The glucose uptake into cardiomyocytes is mediated by special glucose transporters (GLUTs) in the plasma membrane [4]. GLUT4 is the most abundant glucose transporter in the heart [5]. The translocation of GLUT4 from intracellular compartments to the plasma membrane is an important mechanism in the regulation of glucose uptake [6]. PI3K/AKT and AMPK are two main signal pathways that affect glucose uptake in cells [7,8].

Pigment epithelium-derived factor (PEDF), an endogenous protein of the SERPIN superfamily, is widely expressed in multiple tissues with many biological functions [9]. PEDF is thought to exert its biological activity by interacting with its receptor on cell surface. The PEDF receptor (PEDF-R) and laminin receptor (LR) are two crucial receptors among PEDF binding sites [10]. PEDF-R is a member of patatin-like phospholipase domain-containing 2 (PNPLA2). Upon PEDF binding, PEDFR exhibits potent phospholipase A2 (PLA2) enzymatic activity and lipase activity, and exerts corresponding cellular activities [11].

Clinical studies have demonstrated that serum levels of PEDF are elevated in patients suffering from obesity, metabolic syndrome and type 2 diabetes [12]. However, the role of PEDF on glucose metabolism is still controversial [13]. Famulla et al. demonstrated that PEDF reduced the insulin sensitivity by inducing ectopic lipid deposition and pro-inflammatory reactions in skeletal muscle [14]. Conversely, Yamagishi et al. found that PEDF increased the insulin sensitivity by blocking the AGE-induced insulin resistance in liver [15]. We and others have previously reported that PEDF protects cardiomyocytes from ischemic injury and significantly reduces the infarct size after MI [16,17]. However, the direct effect of PEDF on glucose uptake in ischemic cardiomyocyte is still unclear.

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In the present study, we used myocardial ischemia/reperfusion (MI/R) rat models and adult rat ventricular cardiomyocytes to demonstrate the direct effect of PEDF on glucose uptake in ischemic myocyte and to try to elucidate their underlying mechanism.

2. Materials and methods

Materials and expanded methods are presented in the Online Data Supplement.

3. Results

3.1. PEDF increases glucose uptake in the ischemic myocardium in rats

Positron emission tomography (PET) imaging has been considered as a viable technique for estimating glucose transport and metabolism [18]. 2-[^{18}F] fluoro-2-deoxy-D-glucose (FDG) is a glucose analog that reflects transmembranous transport of glucose and ^{13}N-NH_3 is commonly used as a perfusion tracer in heart [19,20]. In this study, Micro PET scans were performed to identify the ischemic region in the heart and to assess the glucose uptake in the ischemic region (Fig. 1A). Representative vertical long axis images are shown in Fig. 1B. There was no significant difference in relative volume of perfusion defect among all MI groups (Fig. 1C). The myocardial uptake of the [^{18}F]-FDG (SUV-$_{main}$) in the perfusion defective region was increased in the PEDF-LVs group compared to control group (Fig. 1D). These findings suggest that PEDF may increase ischemic cardiac glucose uptake. Since plasma insulin and glucose level can affect myocardial glucose uptake and PET metabolic imaging [21], we also examined these two targets before PET imaging. The results revealed no significant difference among all groups (Fig. 1E and F). Thus, the interference from systemic plasma insulin and glucose on glucose uptake was excluded.

3.2. PEDF increases glucose uptake in hypoxic cardiomyocytes in vitro

To determine the direct effect of PEDF on ischemic cardiac glucose uptake, we treated rat adult ventricular cardiomyocytes with recombinant PEDF protein under low-glucose and hypoxic conditions. Fluorescent glucose analog 2-NBDG was used to investigate the glucose uptake of cultured cardiomyocytes (Fig. 1G). First, we tested the glucose uptake of cardiomyocytes after different durations hypoxia (0, 1, 2, 4, 8, 12 h) pre-treated or not with PEDF (10 nmol). We found that the glucose uptake in PEDF pre-treated cardiomyocytes was significantly higher when compared to control groups at the corresponding time points, and reached a peak after 4 h of hypoxia (Fig. 1H). Interestingly, PEDF did not increase glucose uptake in cardiomyocytes during normoxia. Next, to evaluate the effect of PEDF concentration on glucose uptake, different concentrations of PEDF were used under hypoxic conditions. Results showed that PEDF potently increased glucose uptake in cardiomyocytes after 4 h of hypoxia (Fig. 1J). Since 10 nmol of PEDF pre-treatment induced a significant increase in glucose uptake, we used this concentration for the subsequent experiments. These results demonstrated that PEDF had direct glucose uptake stimulatory effects on hypoxic cardiomyocytes. It was important to note that CCK-8 was not only used for normalization of the 2-NBDG concentration but also for determination of cell viability in these experiments. CCK-8 assay revealed that hypoxia insult reduced cardiomyocyte viability and PEDF pre-treatment significantly alleviated the decrease of cell viability (Fig. 1I and K).

3.3. PEDF increases glucose uptake through stimulating GLUT4 membrane translocation in hypoxic cardiomyocytes

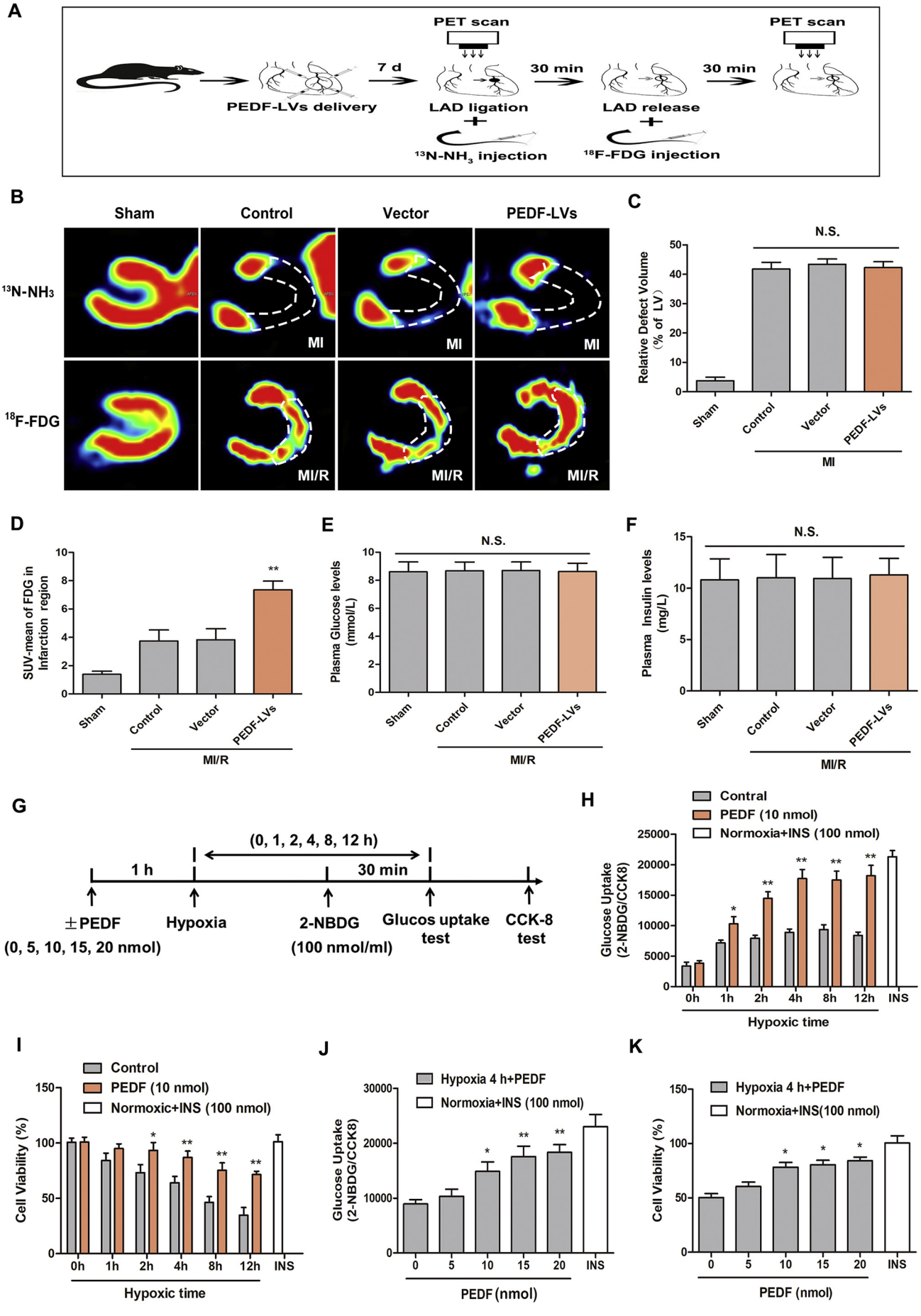
To clarify if the influence of PEDF on glucose uptake in hypoxic cardiomyocytes is associated with GLUT4 or GLUT1, the protein expressions on the plasma membrane and total amount of these two glucose transporters were analyzed by Western blot (Fig. 2A). We found that the presence of GLUT4 on the plasma membrane was significantly upregulated by PEDF under hypoxic condition, and reached a relatively stable high level after 4 h of hypoxia (Fig. 2B). Remarkably, PEDF did not change the total amount of GLUT4 expression (Fig. 2C). The plasma membrane protein and total protein of GLUT1 were not changed due to PEDF treatment (Fig. 2D and E). It is noteworthy that PEDF did not affect the expression or translocation of GLUT1 and GLUT4 in the absence of hypoxia. Therefore, we presume that PEDF may increase the glucose uptake of hypoxic cardiomyocytes through GLUT4 but not GLUT1, and only affects the transposition of GLUT4 rather than the protein expression. Considering this, cycloheximide (10 mg/ml), a global protein synthesis inhibitor, was used to treat cardiomyocytes at the same time as the PEDF treatment. We found that cycloheximide did not inhibit PEDF-induced glucose uptake increase after 4 h of hypoxia, which suggests that the increase of glucose uptake does not occur due to the increase of glucose transporters synthesis (Fig. 2F). Furthermore, immunofluorescence was used to mark the expression and location of GLUT4 in cardiomyocytes after 4 h of hypoxia. As shown in Fig. 2G, a specific GLUT4 staining concentrated on the plasma membrane of hypoxic cardiomyocytes with PEDF pre-treatment and the content of fluorescence in cytoplasm markedly decreased, which did not occur in non-treated cardiomyocytes.

To further evaluate GLUT4 and GLUT1 participation in PEDF-stimulated glucose uptake, hypoxic cardiomyocytes were treated with indinavir and apigenin, respectively. Indinavir is a relatively selective inhibitor of glucose transport mediated by GLUT4, and apigenin can selectively inhibit glucose transport through GLUT1 [22,23]. We found that PEDF-stimulated glucose uptake was blocked by indinavir but was not affected by apigenin, which suggests that the induction of glucose uptake by PEDF occurs through GLUT4 (Fig. 2H). From all these results we confirm that PEDF increases glucose uptake in hypoxic cardiomyocytes by promoting GLUT4 membrane translocation.

3.4. PEDF promotes GLUT4 translocation to the plasma membrane and glucose uptake through PI3K/AKT pathway

To clarify the pathway participated in PEDF-mediated glucose uptake in hypoxia cardiomyocytes, we investigated the protein expression of AMPK, phospho-AMPKα (p-AMPKα, Thr172), AKT, phospho-AKT (p-AKT, Ser473) in cardiomyocytes (Fig. 3A). We found that PEDF did not change the expression of these 4 proteins during normoxia. In contrast, after 4 h of hypoxia, PEDF decreased the expression of P-AMPK and AMPK (Fig. 3B and C) and increased the phosphorylation of AKT while total AKT was not changed (Fig. 3D). Cardiomyocytes were then treated with MK-2206 dihydrochloride (an inhibitor of AKT) and Wortmannin (an inhibitor of PI3K), respectively (Fig. 3E). The findings revealed that both MK-2206 dihydrochloride and wortmannin diminished the increase of PEDF-mediated GLUT4 expression at the cell surface and glucose uptake, which suggest that PI3K/AKT pathway may be necessary for PEDF to

Fig. 1. PEDF increases glucose uptake in ischemic myocardium and hypoxic cultured cardiomyocytes. (A) The protocol of MI/R rat modal and PET experiments. (B) Representative vertical long axis images of ^{13}N-NH_3 (perfusion) after LAD occlusion and ^{18}F-FDG uptake at the beginning of reperfusion generated by PET scan. The ranges selected by the dashed indicate ischemic regions after MI or ischemic/reperfusion regions after MI/R. (C) Relative ^{13}N-NH_3 (perfusion) defect volume (% of LV) of the heart. (D) Main Standard uptake value (SUV-$_{main}$) which indicates cardiac [^{18}F]-FDG (glucose) uptake in ischemic region. (E and F) Plasma glucose levels and insulin levels measured before PET scan. Data was expressed as mean ± SD. (n = 4). $^{**}p < 0.01$ vs. control group. N·S., no statistical difference. (G) Experimental protocol used in cultured adult rat cardiomyocytes. (H) Hypoxic time course of glucose uptake in cardiomyocytes pre-treated or not with PEDF (10 nmol). (I) Cell viability (%) assessed by Cell counting kit-8 (CCK-8) in cardiomyocytes at different hypoxic time. Data was expressed as mean ± SD. (n = 6.) $^{*}p < 0.05$, $^{**}p < 0.01$ vs. relative control group. (J) Glucose uptake in cardiomyocytes subjected to hypoxia 4 h with different content of PEDF pre-treatment. (K) Cell viability (%) assessed by CCK-8 in cardiomyocytes with different content of PEDF pre-treatment. 0 h or 0 nmol represents normal control. Data was expressed as mean ± SD. (n = 6.) $^{*}p < 0.05$, $^{**}p < 0.01$ vs. normal control. Insulin (100 nmol) treatment for 30 min under normoxic condition was used as a positive control during above tests.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PEDF-LVs
13N-NH3
18F-FDG
MI
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Relative Defect Volume (% of LV)
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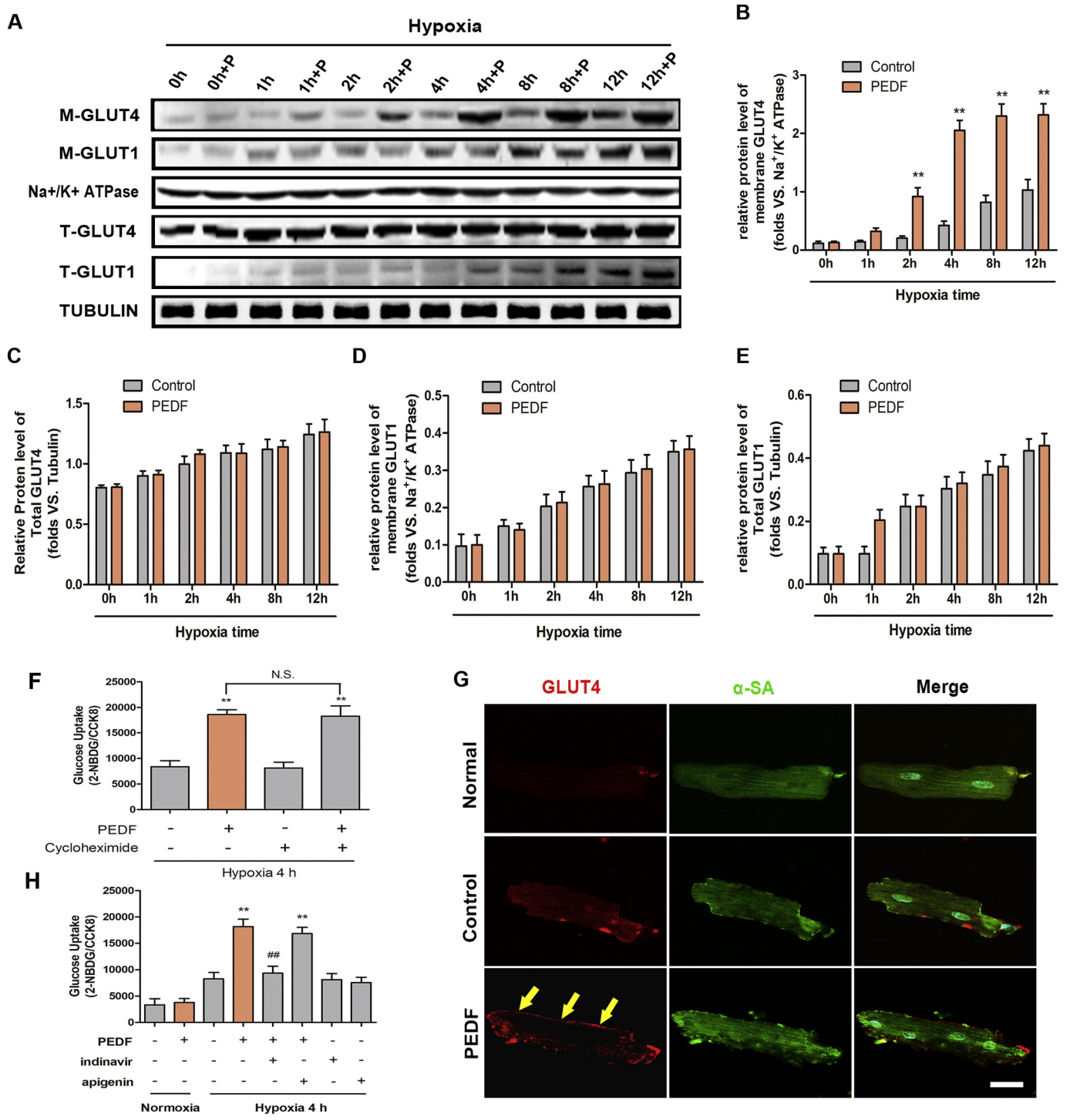

Fig. 2. PEDF increases glucose uptake through stimulation of GLUT4 translocation in hypoxic cardiomyocytes. (A) Western blot for M-GLUT4, M-GLUT1, T-GLUT4 and T-GLUT1 protein in cardiomyocytes pretreated by PEDF (10 nmol) or not and subjected to hypoxic insult at 0–12 h. 0 h represents normal control. (B and C) Densitometric analysis of M-GLUT4 and T-GLUT4 protein. (D and E) Densitometric analysis of M-GLUT1 and T-GLUT1 protein. Data was expressed as mean ± SD. (n = 3.) $^{**}p < 0.01$ vs. relative control group. (F) Cycloheximide (10 mg/ml) was used to inhibit protein synthesis. Data was expressed as mean ± SD. (n = 6). $^{**}p < 0.01$ vs. versus basal. N.S, no statistical difference. (G) Representative images of GLUT4 exposure in cardiomyocytes analyzed by confocal immunofluorescence after 4 h of hypoxia. The red fluorescent marked GLUT4 and green fluorescent marked α-SA. The yellow arrows indicate the GLUT4 on plasma membrane. (Bar = 60 μm, n = 3.) (H) Glucose uptake of cardiomyocytes with or without PEDF (10 nmol), indinavir (100 μmol), apigenin (100 μmol) pre-treatment. Data was expressed as mean ± SD. (n = 6). $^{**}p < 0.01$ vs. purely hypoxic group. $^{\#\#}p < 0.01$ vs. hypoxia + PEDF group. M-GLUT, membrane protein of glucose transporter. T-GLUT, total protein of glucose transporter.

increase glucose uptake through GLUT4 translocation in hypoxic cardiomyocytes (Fig. 3F and G).

We try to verify the feasibility that glucose uptake could be increased via PI3K/AKT pathway in hypoxic cardiomyocytes in the case of P-AMPK reduction was conducted. IGF-1 (a PI3K/AKT activator) and compound C (an AMPK inhibitor) were used together to mimic the effect of PEDF in hypoxic cardiomyocytes (Fig. 3H). We found that IGF-1 and compound C simultaneous treatment could significantly increase the phosphorylation level of AKT and decrease P-AMPK expression in cardiomyocytes at 4 h of hypoxia (Fig. 3I and J). Furthermore, IGF-1

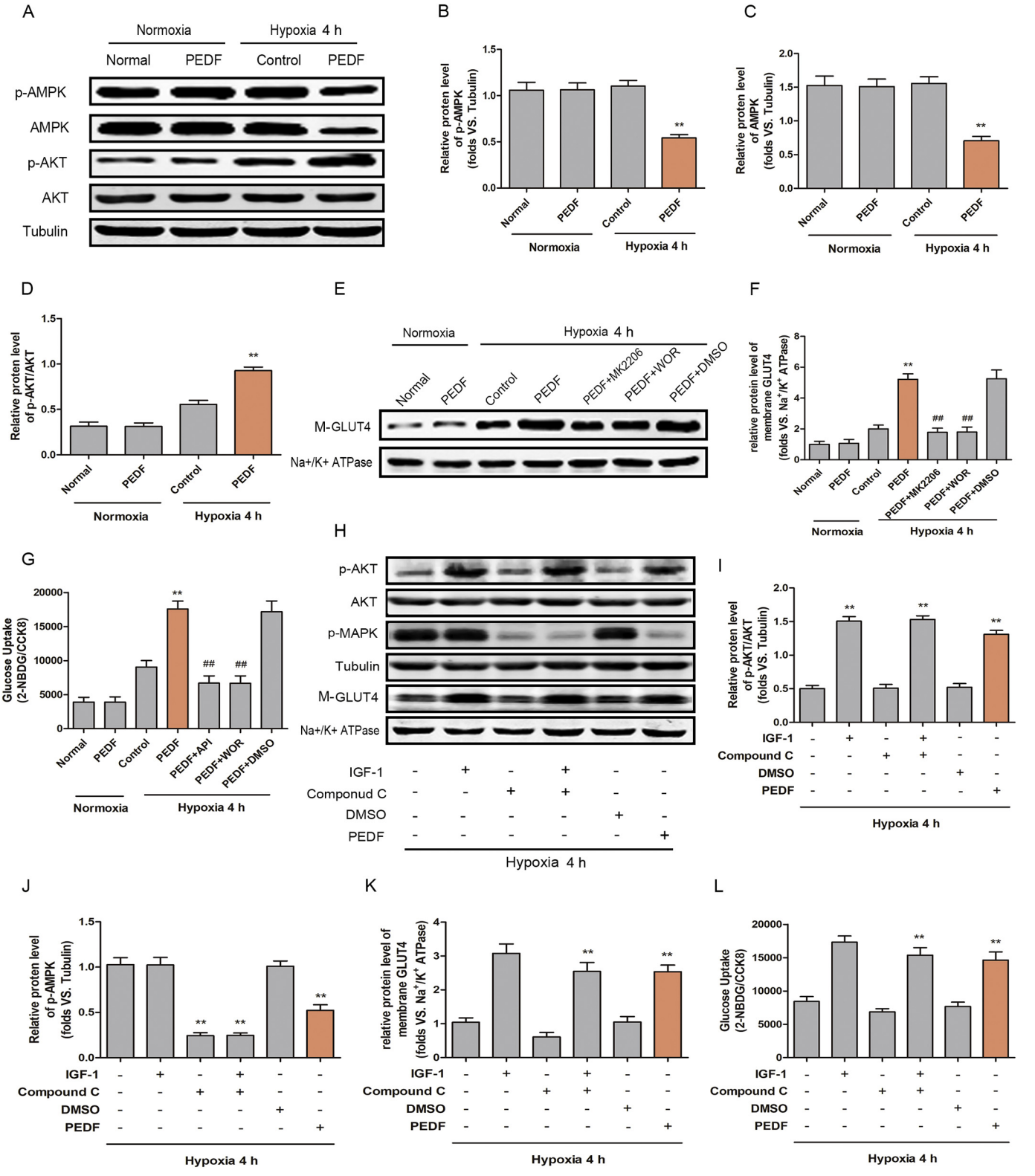

Fig. 3. PEDF promotes GLUT4 translocation to the plasma membrane and glucose uptake through PI3K/AKT pathway. (A) Western blot for p-AMPK, AMPK, p-AKT and AKT in cardiomyocytes with or without PEDF (10 nmol) pre-treatment. (B and C) Densitometric analysis of p-AMPK and AMPK protein. (D) Densitometric analysis of phosphorylation level of AKT protein. Data was expressed as mean ± SD. (n = 3.) **p < 0.01 vs. control group. (E) Western blot for M-GLUT4 in cardiomyocytes with or without PEDF (10 nmol), MK-2206 dihydrochloride (5 μmol) and Wortmannin (5 ng/ml) pre-treatment. (F) Densitometric analysis of M-GLUT4 protein (G) Glucose uptake of cardiomyocytes. Data was expressed as mean ± SD. (n = 3). **p < 0.01 vs. control group. ##p < 0.01 vs. hypoxia + PEDF group. (H) Western blot for p-AKT, AKT, p-AMPK and M-GLUT4 protein in cardiomyocytes with or without PEDF (10 nmol), IGF-1 (100 ng/ml), compound C (10 μmol/L)) pre-treatment. (I and J) Densitometric analysis of phosphorylation level of AKT and p-AMPK protein. (K) Densitometric analysis of M-GLUT4 protein. (L) Glucose uptake of cardiomyocytes. Data was expressed as mean ± SD. (n = 3.) **p < 0.01 vs. purely hypoxic group. WOR, Wortmannin. M-GLUT, membrane protein of glucose transporter. DMSO, Dimethyl sulfoxide. IGF-1, insulin-like growth factors −1.

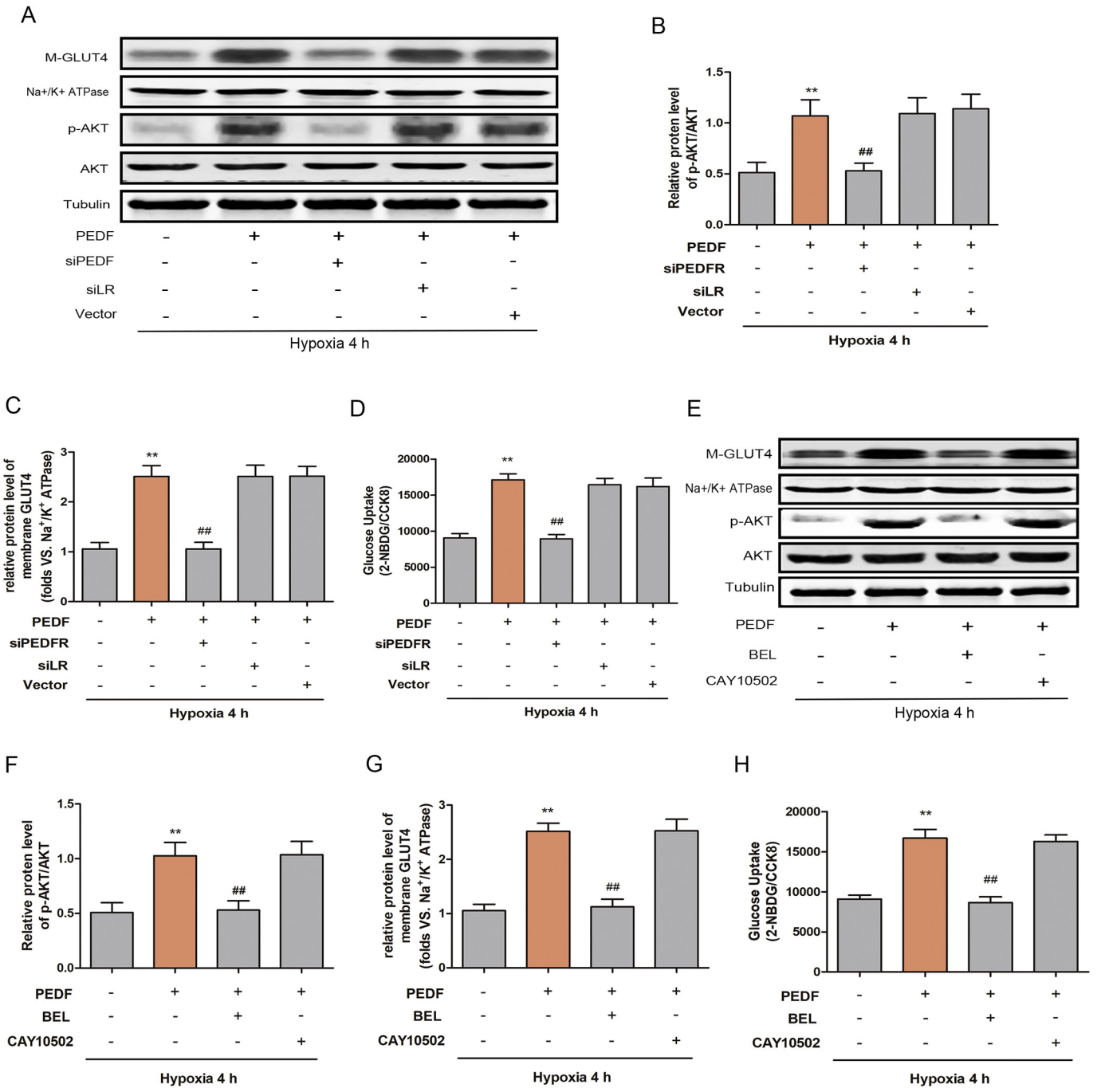

Fig. 4. PEDF increase glucose uptake in hypoxic cardiomyocytes in a PEDFR-dependent manner. (A) Western blot for M-GLUT4, p-AKT, AKT protein in hypoxic cardiomyocytes with or without PEDF (10 nmol) pre-treatment and lentivirus vector (vector), short interference RNA against PEDFR (siPEDFR), short interference RNA against LR (siLR) transfection. (B) Densitometric analysis of phosphorylation level of AKT protein. (C) Densitometric analysis of M-GLUT4 protein. (D) Glucose uptake in hypoxic cardiomyocytes with or without PEDF (10 nmol) pre-treatment and lentivirus vector (vector), siPEDFR, siLR transfection. (E) Western blot for M-GLUT4, p-AKT, AKT protein in hypoxic cardiomyocytes with or without PEDF (10 nmol), BEL (25 μmol) or CAY10502 (50 nmol) pre-treatment. (F) Densitometric analysis of phosphorylation level of AKT protein. (G) Densitometric analysis of M-GLUT4 protein. (H) Glucose uptake in hypoxic cardiomyocytes with or without PEDF (10 nmol), BEL (25 μmol) or CAY10502 (50 nmol) pre-treatment. Data was expressed as mean ± SD. (n = 3). $^{**}p < 0.01$ vs. purely hypoxic group. $^{\#\#}p < 0.01$ vs. hypoxia + PEDF group. M-GLUT, membrane protein of glucose transporter. P-AKT, phosphorylated AKT.

and compound C simultaneous treatment also increased GLUT4 exposure at the cell surface and glucose uptake in hypoxic cardiomyocytes, which were similar to the effect of PEDF (Fig. 3K and L). These results suggest that it is feasible that PEDF increases glucose uptake in hypoxic cardiomyocytes via PI3K/AKT pathway even in the case of P-AMPK reduction. Taken together, all these results indicate that PEDF-induced glucose uptake increase in hypoxic cardiomyocytes is mediated by PI3K/AKT pathway.

3.5. PEDF increases glucose uptake in hypoxic cardiomyocytes in a PEDFR-dependent manner

To investigate which receptor was involved in the effect of PEDF on increasing glucose uptake in hypoxic cardiomyocytes, short interference RNA against PEDFR (siPEDFR) and short interference RNA against LR (siLR) were administered (Fig. 4A). We found that it was siPEDFR rather than siLR that diminished the increase of PEDF-mediated

phosphorylation of AKT and GLUT4 expression on the cell surface and glucose uptake in hypoxic cardiomyocytes (Fig. 4B, C and D). These results indicate that the influence of PEDF on glucose uptake in hypoxic cardiomyocytes is PEDFR dependent. Next, BEL (PLA2 inhibitor) or CAY10502 (lipase activity inhibitor) was used to treat cardiomyocytes (Fig. 4E). We found that Bel instead of CAY10502 inhibited PEDF-mediated increase in phosphorylation of AKT and GLUT4 translocation and glucose uptake (Fig. 4F, G and H). Altogether, our findings suggest that the effect of PEDF on glucose uptake in hypoxic cardiomyocytes is mediated through binding to PEDFR and activating its PLA2 activity.

4. Discussion

In this study, we described a novel role for PEDF as a positive regulator of glucose uptake in ischemic myocardium. In addition, we tested and verified in vitro that this effect of PEDF on glucose uptake occurred via PEDFR-dependent PI3K/AKT pathway and GLUT4 translocation.

We observed that PEDF was downregulated in the infarct region after MI (Fig. S1A and B). To investigate the potential effect of PEDF on glucose uptake in ischemic myocardium, PEDF-LVs were delivered into the myocardium to locally overexpress PEDF. The PEDF local overexpression was investigated by Western blot and Immunohistochemically staining (Fig. S1C–E). Furthermore, the efficiency of viral transduction was further confirmed by GFP co-expression on the construct (Fig. S1F). From all the results we confirm that PEDF-LVs intra-myocardial transfection can maintain PEDF local overexpression in myocardium.

Researchers have found that ischemia results in increased glucose uptake in cardiomyocytes [24]. This may be mediated by AMPK pathway and may protect cardiomyocytes from hypoxia to some extent [25]. However, our previous and current studies have shown that PEDF can reduce the expression of AMPK and P-AMPK [26]. In view of this, PEDF-mediated glucose uptake in hypoxia cardiomyocytes should not be considered as a synergistic effect with hypoxia but a unique biological process. PI3K/AKT pathway may participate in the PEDF-induced regulation of glucose uptake in hypoxic cardiomyocytes, because MK-2206 dihydrochloride or wortmannin was sufficient to inhibit this effect of PEDF [27].

Previous studies investigating the PEDF effect on glucose metabolism mainly focused on its impact on insulin sensitivity [13]. However, there was little evidence for the link between robust measures of insulin sensitivity and circulating PEDF [28]. It is possible that its impact on glucose metabolism is tissue dependent [29]. In this study, we found that PEDF has no effect on cardiomyocytes under normoxic condition. This discrepancy may be attributed to the significantly decreased PEDFR expression in normoxic cardiomyocytes [30]. Reports conclude that PEDFR is necessary for the effects of PEDF on insulin resistance [31]. PEDF-induced glucose uptake increase in hypoxic cardiomyocytes was also PEDFR dependent. The difference was that phospholipase A2 activity rather than lipase activity was activated here. These contrasting findings of PEDF are difficult to reconcile. The interpretation may relate to differences in the source of PEDF, target tissue and receptor activity among studies.

Several studies have provided supportive evidence for the protective effect of glucose uptake in myocardial ischemia, especially for improving functional recovery [2]. In this study, we investigated the cardio-protective effects of PEDF (Fig. S2). Results demonstrate that PEDF protects myocardium against ischemic injury and may improve cardiac functional recovery after ischemia. We also tested the relationship between PEDF-mediated cardioprotective effect and increase of glucose uptake in vitro (Fig. S3). We found that inhibiting the increase of glucose uptake is sufficient to diminish the protective effects of PEDF on hypoxic cardiomyocytes. While other factors may be involved, PEDF-mediated increase in glucose uptake is closely associated with its cardioprotective roles. These findings are relevant to the therapeutic strategies of MI. As demonstrated in the current study, when PEDF overexpression after an experimentally induced ischemic insult, represent a potential therapeutic modality to preserve the myocardium and improve functional recovery. However, the PEDF administration by intra-myocardial transfection has obvious limitation. This window of opportunity for PEDF administration may be more appropriate for “planned” ischemic events, such as coronary artery bypass surgery (CABG). Therefore, further studies will be required to determine the key constituents of PEDF, which mediate its glucose uptake actions, and to explore a more convenient way for PEDF administration. In addition, stimulation of myocardial glucose uptake plays a key role in alleviating MI/R injury [2]. PEDF overexpression might also reduce the MI/R injury in rat heart. Although other mechanisms mediated by PEDF may be involved, such as antioxidative effect via inhibition of reactive oxygen species (ROS) generation [32], glucose uptake is likely to play an important role as well. These findings have potential translational relevance for management of acute coronary syndromes. PEDF may represent a potential treatment strategy to preserve the myocardium and improve functional recovery after MI.

5. Limitations

In this study, we lack a detailed research on how PEDF activates the PI3K pathway. We examined the interaction between PEDF and tyrosine phosphorylated sites of insulin receptor substrate-1 (IRS-1) which is a key step in PI3K stimulation induced by insulin, and found that PEDF had no effect on the phosphorylation of IRS-1 (Fig. S4D). Thus, PEDF may activate a different PI3K/AKT pathway from insulin. More studies are needed to fully understand the interaction between PEDF and the PI3K/AKT signaling pathway in cardiomyocytes.

6. Conclusion

In conclusion, our data provide evidence that PEDF can increase ischemic myocardium glucose uptake. Supporting in vitro data suggest that modulation of myocardium glucose uptake by PEDF is mediated through a membrane receptor-dependent mechanism involving PI3K/AKT signaling and GLUT4 translocation. These findings complement a novel role of PEDF in glucose metabolism and provide a rationale for the myocardial protective mechanism of PEDF.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.02.035.

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Disclosures

The authors disclose no conflict of interest.

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