Vaccarin suppresses diabetic nephropathy through inhibiting the EGFR signaling pathway

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Research Article

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Abstract

Background

Diabetic nephropathy (DN) is recognized as one of the primary causes of chronic kidney disease and end-stage renal disease. Vaccarin is a major component in traditional Chinese medicine Vaccaria with favorable effects on cardiovascular and metabolic diseases, including type 2 diabetes mellitus (T2DM). Nonetheless, the potential role and mechanism of vaccarin in the etiologies of DN have yet to be completely elucidated.

Methods

A classical T2DM was experimentally induced in mice via a high-fat diet (HFD)/streptozocin (STZ) regimen. The renal histological changes were assessed. Masson staining and immunohistochemistry (IHC) were employed to assess renal fibrosis. Quantitative real time-PCR (RT-PCR) was utilized to quantify the mRNA levels of renal fibrosis and inflammation markers. The levels of malondialdehyde (MDA) and reactive oxygen species (ROS), as well as the contents of glutathione peroxidase (GSH-Px) were measured. The protein expression of collagen, TGF-β1, α-SMA, E-cadherin, P-ERK, P-EGFR(Y845), P-EGFR(Y1173), T-ERK and T-EGFR was detected by western blot.

Results

Our study showed that vaccarin had a beneficial impact on DN mice by improving renal function and mitigating histological damage. This was achieved through its inhibition of renal fibrosis, the reduction of inflammation cytokine overproduction, and ROS levels. Moreover, vaccarin treatment effectively suppressed epithelial-to-mesenchymal transition (EMT), a crucial process in renal fibrosis, in high glucose (HG)-induced HK-2 cells. The underlying mechanism was explored through network pharmacology analysis and molecular docking, which identified epidermal growth factor receptor (EGFR) as a potential target for vaccarin. In support, vaccarin reduced the phosphorylation levels of both EGFR and its downstream mediator, extracellular signal-regulated kinase 1/2 (ERK1/2), in diabetic kidneys and HG-treated HK-2 cells. Notably, blocking either EGFR or ERK1/2 yielded similar renal benefits as observed with vaccarin treatment.

Conclusion

This study revealed that vaccarin held the strong ability to attenuate renal damage via inactivation of EGFR signaling in T2DM.

Introduction

For several decades, diabetic nephropathy (DN) is taken as a leading cause of chronic kidney disease and end-stage renal disease[1,2]. According to the latest research, it is estimated that 550 million people will suffer from diabetes all over the world by 2030, and approximately 50% of patients with type 2 diabetes
will develop into DN \cite{3, 4}. DN is characterized by proteinuria, excessive mesangial matrix formation and renal fibrosis \cite{5, 6}. It has been shown that renal fibrosis is the main pathological event in the development of DN \cite{7}. Renal fibrosis is usually manifested by overproduction of extracellular matrix (ECM), including collagen I, in the renal tubulointerstitium \cite{8}. Renal tubular epithelial cells have been damaged in the early stage of DN \cite{9}. Long-term exposure to hyperglycemia leads to their tubular structure to renal interstitial structure, also known as epithelial-to-mesenchymal transition (EMT). Once the process of EMT happened, ECM will be dispersed around tubular structures, which evolved into the accumulation of ECM in the tubulointerstitial, causing irreversible damage to the renal structure \cite{10, 11}. Accumulating evidence implicates that transforming growth factor-β1 (TGF-β1) plays a pivotal role in diabetic nephropathy (DN) progression by triggering the Smad signaling pathway, a significant fibrogenic pathway \cite{12, 13}.

Epidermal growth factor receptor (EGFR) is generally expressed in renal epithelial cells \cite{14, 15}. EGFR binds to ligand leads to activation of the intrinsic kinase domain at Y1173 \cite{14, 16}. In addition, EGFR can be phosphorylated at Y845 by non-ligand pathway mediated by oxidative stress \cite{17}. Mounting evidence indicates that EGFR is a pivotal mediator during the process of renal fibrosis \cite{18, 19}. HG-mediated EGFR phosphorylation and ERK activation, consequently promotes TGF-β expression and induces renal fibrosis \cite{17}. Despite the growing understanding of diabetic renal pathology, DN remains a leading cause of people's mortality in diabetic populations. Therefore, it is imperative to develop for novel drugs to treat DN.

Vaccarin, a natural flavonoid glycoside \cite{20}, exhibits a broad of pharmacological effects, such as anti-oxidation, anti-inflammation and anti-hyperglycemic actions \cite{21-23}. Our group has shown that vaccarin alleviates HG- and hydrogen peroxide (H₂O₂)-induced endothelial cell injury via inhibiting the Notch signaling \cite{24, 25}. Besides, vaccarin treatment ameliorates nephropathy and cardiovascular remodeling in hypertensive rats \cite{26}. Recently, it has been demonstrated that vaccarin improves the disorder of glucose and lipid metabolism in type 2 diabetes mellitus (T2DM) mice \cite{27, 28}. Moreover, vaccarin prevents ox-LDL-induced injury in endothelial cells via suppressing reactive oxygen species (ROS)/mitogen-activated protein kinase (MAPK) signaling pathway \cite{20}. Overall, vaccarin is likely beneficial for attenuating cardiovascular and metabolic disorders. However, little is known regarding the role and underlying mechanism of vaccarin in DN. Moreover, EGFR was identified as a potential target of vaccarin. Whether and how EGFR mediated the renal benefits of vaccarin was largely unknown. The present study thus explored the potential effects of vaccarin on DN, and investigated whether vaccarin ameliorated renal injury by acting on the EGFR signaling pathway in T2DM.

## Materials and methods

### Reagents
Vaccarin was purchased from Shifeng Technology (Shanghai). D-glucose was bought from Sigma Chemical Co. (St Louis, MO, USA). Kits for serum creatinine (Scr), blood urea nitrogen (BUN) and urine protein were procured from Jiancheng Bioengineering Institute (Nanjing). Antibodies against β-actin, α-SMA, E-cadherin and P-ERK were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibodies against collagen, TGF-β1, T-EGFR, P-EGFR(Y845), P-EGFR(Y1173) and T-ERK from Abcam (Cambridge, MA, USA).

**Experimental animals**

Male C57BL/6J mice, aged 6–8 weeks, were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China). All experiments were approved by Institutional Animals Care and Use Committee at Jiangnan University (document number for animal use approval: JN.No20200710c0600131[174]). The animals were housed in a controlled environment with a 12-hour light-dark cycle, regulated temperature, and humidity. They were provided unrestricted access to both standard chow and tap water.

**Mice model establishment**

T2DM was experimentally induced in mice via a high-fat diet (HFD)/ streptozocin (STZ) regimen as we previously demonstrated [20, 27]. The normal control group (Ctrl) were given standard diet. Group received vaccarin daily (1 mg/kg, i.p.) for 8 weeks was served as vaccarin control group (VAC). While the other mice were given HFD (21.8 kJ/g, 60% fat, D12492). After feeding for 4 weeks, the HFD mice were received a single dose of STZ intraperitoneally (120 mg/kg, pH 4.0 dissolved in 10 mM citrate buffer). Mice with fasting plasma glucose higher than 11.1 mmol/L were diabetic [29]. Thereafter, T2DM mice were randomly allocated to two groups, model group (DN) and vaccarin-treated DN group (DN + VAC). DN + VAC group was given vaccarin (1mg/kg, i.p.) every day for weeks. The mice in DN group and (DN + VAC) group were maintained on a HFD feeding until sacrificed.

**Assessment of blood glucose level, albuminuria, blood urea nitrogen and serum creatinine**

Fast blood glucose (FBG) was measured using an AccuChek glucose meter weekly. At the end of the experiments, metabolic cages were employed to gather 24-hour urine samples for albuminuria analysis. Blood samples were obtained to extract serum, and the serum concentrations of blood urea nitrogen (BUN) and creatinine (Scr) were assessed following the guidelines provided by the manufacturer [30].

**Sample collection and morphological observations**

The kidneys were removed and weighed for renal/body weight index calculation. Kidney was fixed with 4% paraformaldehyde and kidney sections were cut at 5 μm. The renal histological changes were assessed by both hematoxylin and eosin (H&E) staining and periodic acid-schiff (PAS). Masson staining was used to evaluate the renal fibrosis. Images were captured by the Pannoramic SCAN (3DHISTECH Ltd., Budapest, Hungary).
**Immunohistochemistry assay (IHC)**

Renal sections were de-paraffinized, hydrated and underwent antigen retrieval. Following that, sections underwent hydrogen peroxide treatment (3%) to eliminate endogenous peroxidase activity, after which they were blocked with 5% BSA for 60 minutes. Primary antibodies against collagen, α-SMA, and E-cadherin were then applied and left to incubate overnight at 4°C. Subsequently, the sections were exposed to secondary antibodies coupled with horseradish peroxidase (HRP) for 60 minutes at room temperature. Finally, staining was accomplished using 3, 3’-diaminobenzidine (DAB). The resulting images were examined using the Pannoramic SCAN system.

**Quantitative real time-PCR (RT-PCR)**

Total RNA was extracted from tissues and cells using Trizol reagent (Cwbio, Beijing, China) in accordance with the provided guidelines. Subsequently, an equal amount of RNA was subjected to reverse transcription using HiScriptQ RT SuperMix (Vazyme, Nanjing, China), followed by quantitative real-time PCR utilizing ChamQTM SYBR®qPCR Master Mix (Vazyme, Nanjing, China). Relative gene expression levels were determined using the 2-ΔΔCT method\[^{31}\]. The primer sequences were provided in Table S1.

**Western blotting**

Total protein was extracted using RIPA lysis buffer (CWBIO, Taizhou China), and the protein concentration was determined using a BCA kit (Beyotime Biotechnology, Shanghai, China). Subsequently, 20 µg of proteins were loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and then transferred to polyvinylidene fluoride (PVDF) membranes. Afterward, these membranes were blocked with defatted milk in tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST) for 1 hour and incubated overnight at 4°C with primary antibodies. Following TBST washing, the membranes were exposed to a 1:2000 dilution of HRP-conjugated anti-rabbit IgG antibody (CWBIO, Taizhou China). The blots were visualized utilizing a chemiluminescence detection system (Millipore Darmstadt, Germany) and semi-quantified using Image J (National Institutes of Health, Bethesda, MD, USA).

**Cell culture**

HK-2 cells were cultured in low glucose DMEM medium (5.6 mM glucose, Gibco, Carlsbad, CA, USA) supplemented with 10% FBS and 1% penicillin/streptomycin (Gibco, Carlsbad, CA, USA) in a 5% CO\(_2\) incubator. The cells were treated with 5 µM of vaccarin for 12 h before HG (35 mM) for 48 h in the following experiments.

**Databases analysis**

Genes related to DN and diabetic kidney disease were screened from the DrugBank database (https://go.drugbank.com/) and Genecards database (https://www.genecards.org). The predicting targets of vaccarin were searched from PharmMapper database (http://www.lilab-ecust.cn/pharmmapper/). The DAVID database (https://david.ncifcrf.gov/) was applied to predict the biological processes (BP). High-
confidence proteins of the protein-protein interaction (PPI) network was constructed by the STRING database (https://string-db.org/) and visualized by Cytoscape.

**Molecular docking of EGFR and vaccarin**

The 3D structure of EGFR was downloaded from RCSB Protein Data Bank (PDB) (http://www.rcsb.org/). The molecular structure of vaccarin was provided from PubChem Database (https://pubchem.ncbi.nlm.nih.gov/). AutoDock (http://autodock.scripps.edu/) was used to dock EGFR and vaccarin based on network pharmacology. Binding energy was used as docking score to predict binding strength between the key target and the drug.

**Measurement of oxidative stress markers**

Cellular superoxide anion production was assessed using dihydroethidium (DHE, 10 µM) or 2,7-dichlorofluorescein diacetate (DCFH-DA, 10 µM) staining, conducted in a dim environment for 30 minutes at 37°C. Fluorescence microscope (Zeiss, Germany) was employed to capture the resulting images. Additionally, malondialdehyde (MDA) levels and glutathione peroxidase (GSH-Px) activity in diabetic kidney tissue were evaluated following the manufacturer's instructions [32].

**Statistical analysis**

Data were presented as mean ± SEM and subjected to statistical analysis using one-way analysis of variance (one-way ANOVA) through GraphPad Prism 8 software (California, USA). A significance level of P < 0.05 was considered statistically significant.

**Results**

**Vaccarin alleviates renal dysfunction in diabetic mice**

Firstly, we assessed the therapeutic effects of vaccarin on renal damage induced by diabetes *in vivo*. Compared with control mice, the FBG level was significantly elevated in DN mice and reversed by vaccarin treatment (Fig. 1A). Moreover, the levels of urine albumin, BUN and Scr, as well as renal/body weight index were significantly higher in DN group. However, treatment of vaccarin alleviated these parameters in diabetic mice (Fig. 1B-E). Morphological analysis from H&E staining and PAS staining revealed that the glomeruli was hypertrophic in T2DM mice, which was obviously ameliorated by vaccarin (Fig. 1F-G). Of note, there were no significant changes on renal morphology and renal function tests in normal mice and DN mice administrated with vaccarin. These findings indicated that vaccarin protected kidney from damage in T2DM mice.

**Vaccarin attenuates renal fibrosis in diabetic mice**

Renal fibrosis is recognized as a fundamental priming stage in the pathogenesis of DN [33]. Accumulation of ECM is the primary feature of renal fibrosis [34]. Collagen I is one of the essential components in the
process of ECM \[35\]. Masson staining showed that vaccarin attenuated renal fibrosis in diabetic mice (Fig. 2A-B). Immunohistochemistry (IHC) results further confirmed that vaccarin reduced renal fibrosis in diabetic mice, as reflected by lower collagen I immuno-positive signals (Fig. 2A, C). In similarity, increased collagen I mRNA level were detected in diabetic mice compared with Ctrl group. However, treatment with vaccarin substantially reversed these abnormalities (Fig. 2D). Abundant evidence underscores the involvement of EMT in the accumulation of ECM \[36, 37\]. This process entails the elevation of fibroblast markers, such as α-smooth muscle actin (α-SMA), and the reduction of epithelial indicators, including E-cadherin \[38\]. IHC results indicated that vaccarin diminished EMT process in T2DM mice, as indicated by higher E-cadherin and lower α-SMA immuno-positive signals (Fig. 2A, C). Consistent with the IHC results, vaccarin was found to downregulate α-SMA mRNA level (Fig. 2F) and upregulate E-cadherin mRNA level (Fig. 2G) in diabetic mice. TGF-β1 may play a key role in the processes of EMT under diabetic conditions \[39, 40\], RT-PCR results demonstrated that upregulation of TGF-β1 in T2DM mice was reduced by vaccarin treatment (Fig. 2E).

**Vaccarin attenuates oxidative stress and inflammation in the kidneys**

Abnormal inflammatory response and oxidative damage are the driving factors of DN \[32\]. Hyperglycemia resulted in inflammatory responses in the renal system, as evidence by increased mRNA levels of IL-1β, VCAM-1, COX-2 and p65-NFκB, effects that were effectively reversed by vaccarin (Fig. 3A-D). Additionally, we observed elevated renal MDA content and reduced renal GSH-Px activities in diabetic mice. However, these anomalous alterations were mitigated by vaccarin treatment (Fig. 3E-F). Furthermore, DHE staining and DCFH-DA staining of kidney tissues demonstrated diminished renal oxidative stress in diabetic mice treated with vaccarin (Fig. 3G-I). Collectively, these outcomes suggest that vaccarin mitigated the inflammatory response and curtailed ROS production within diabetic kidneys.

**Vaccarin attenuates HG-induced EMT in HK-2 cells**

We extended our investigation to evaluate vaccarin’s potential impact on HG-induced HK-2 cells. Western blotting and RT-PCR analyses demonstrated that prior exposure to vaccarin effectively counteracted elevated levels of collagen I, α-SMA, and TGF-β1, while raising the diminished E-cadherin level in HG-exposed HK-2 cells (Fig. 4A-E). Furthermore, as anticipated, vaccarin-treated control cells exhibited no alterations in ECM deposition or EMT progression. These findings strongly suggest the involvement of the EMT process in vaccarin’s renal protective effects.

**Vaccarin attenuates HG-induced inflammation response and oxidative injury in HK-2 cells**

Subsequently, we investigated whether vaccarin could alleviate inflammation and oxidative stress induced by HG in HK-2 cells. The mRNA levels of inflammatory markers, including IL-1β, VCAM-1, COX-2,
and \( \text{p65-NFkB} \), displayed significant increases in response to HG exposure, which were notably suppressed by vaccarin (Fig. 5A-D). Furthermore, vaccarin preincubation effectively mitigated the excessive intracellular ROS generation triggered by HG in HK-2 cells (Fig. 5E-G).

**Vaccarin ameliorates renal fibrosis by inhibiting EGFR signaling pathway**

To further explore the underlying mechanism of vaccarin in alleviating renal fibrotic lesions of diabetic mice, we conducted a network pharmacological analysis. 107 intersection gene of vaccarin and DN were identified (Fig. 6A). A protein-protein interaction (PPI) network of common targets was built \cite{41}. EGFR was obviously at the center of the network (Fig. 6B). Biological Process (BP) is a significant aspect of GO (Gene Ontology) enrichment analysis \cite{31}, we found that ERBB2 signaling pathway, epidermal growth factor receptor signaling pathway and negative regulation of apoptotic process significantly enriched (Fig. 6C), which were verified to contribute to renal fibrosis \cite{34,42,43}. Molecular docking demonstrated a direct interaction between vaccarin and EGFR with binding energy of \( \sim 8.8 \text{ Kcal/mol} \) (Fig. 6D).

It is highly probably that EGFR is the key target of renal injury based on bioinformatics and molecular docking. More importantly, EGFR activation is responsible for the development of DN through the activation of ERK1/2 signaling pathway \cite{15}. Thereafter, we sought to determine whether vaccarin ameliorated renal injury by acting on the EGFR/ERK signaling pathway in T2DM. Our results showed that the phosphorylated levels of EGFR (Y845, Y1173) and ERK1/2 in the kidney were augmented, whereas this was abrogated by treatment with vaccarin (Fig. 7A, C-E). Moreover, the HG-induced upregulation of EGFR/ERK phosphorylation in HK-2 cells were also attenuated by vaccarin treatment, as demonstrated by western blotting (Fig. 7B, F-H). Additional experiments were performed to test whether the EGFR/ERK signaling contributed to hyperglycemia-induced renal damage, therefore, HK-2 cells were treated with or without EGFR inhibitor AG1478 or ERK inhibitor U0126 in the context of HG. We found AG1478 or U0126 significantly suppressed HG-induced fibrosis (Fig. 8A-D), inflammation (Fig. 8E-H) and oxidative stress (Fig. 8I).

**Discussion**

In the present study, we found that administration of vaccarin efficiently attenuated diabetic renal injury via preserving renal function, improving biochemical parameters, ameliorating morphological abnormalities and antagonizing renal fibrosis in mice. We also observed that vaccarin inhibited HG-induced HK-2 cells fibrosis, inflammation response and oxidative stress via inactivation of EGFR/ERK signaling. In summary, our study suggested that vaccarin may serve as a new potential approach for the treatment of DN.

DN is a severe renal microvascular complication, arising as a consequence of persistent hyperglycemia \cite{44}. Uncontrolled hyperglycemia causes renal structural damage, including glomerular lesions,
microalbuminuria, mesangial expansion and interstitial fibrosis [4,44]. In this study, vaccarin decreased the level of blood glucose of diabetic mice. We also found that vaccarin improved diabetic renal dysfunction by reducing the level of creatinine, BUN, and albuminuria. Histological examination further suggested the therapeutic effect of vaccarin in DN. Renal fibrosis plays an important role in the pathophysiology of DN [45]. The development of renal fibrosis is related to the increase of fibroblasts and myofibroblasts, which produce ECM in the renal tubular interstitial space [46]. Moreover, hyperglycemia can lead to EMT of renal tubular epithelial cells in DN, and EMT is one of the reasons for myofibroblast activation and synthesis of ECM [47]. Additionally, TGF-β1 is an essential mediator of renal fibrosis in diabetes [48]. A previous study showed that HG-mediated EGFR phosphorylation and ERK activation, consequently promoting renal fibrosis [17]. Our results showed that vaccarin retarded renal fibrotic processes, as manifested by upregulated E-cadherin, downregulated collagen, α-SMA and TGF-β1. These results indicated that vaccarin ameliorated the progression of renal fibrosis in diabetic mice via antagonizing the process of EMT.

Inflammation and oxidative stress are considered the drivers of DN [49]. In this study, our results found that vaccarin exhibited an anti-inflammatory effect by inhibiting expressions of inflammatory factors. Also, vaccarin alleviated oxidative stress in hyperglycemia-induced kidneys and HK-2 cells. These results demonstrated that vaccarin may be promising agent for the treatment of DN by inhibiting inflammation response and clearing overproduction of reactive oxygen species.

Using the network pharmacology and molecular docking, we speculated that vaccarin might be able to ameliorate renal fibrosis and injury through targeting EGFR. Compelling evidence indicates that EGFR plays a necessary role in the process of DN through the activation of ERK1/2 signaling pathway. Our data showed that vaccarin inhibited EGFR phosphorylation and ERK1/2 activation. Meanwhile, we found that blockade of either EGFR or ERK1/2 ameliorated DN. Thus, vaccarin may ameliorate renal fibrosis via inhibiting the EGFR-ERK signaling pathway. Further confirming whether the overexpressed EGFR/ERK signaling pathway counteracted renal protection of vaccarin.

In conclusion, the available results of our study showed that administration of vaccarin effectively ameliorated renal damage via lowering blood glucose, restoring histological alterations in an experimental model of HFD/STZ-induced T2DM. Moreover, we found that vaccarin preserved diabetes-induced renal injury by inhibiting EMT of renal tubular epithelial cells, inflammation response and oxidative damage via inactivating the EGFR/ERK signaling pathway.

**Declarations**

**Ethics approval and consent to participate**

All experiments were approved by Institutional Animals Care and Use Committee at Jiangnan University (document number for animal use approval: JN.No20200710c0600131[174])
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

Xuexue Zhu, Xinyao Du, Xinyu Ma performed experiments and wrote the manuscript. Zhijun Han, Xinyu Meng, Chenyang Zhao, Yuanyuan Wen analyzed and processed data. Bao Hou, Weiwei Cai provided technical assistance. Liying Qiu, Haijian Sun and Fei Xu coordinated and conceived the experiments. All authors corrected and approved the manuscript.

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Availability of data and material

All data produced or examined during this study are incorporated within the published article and its supplementary online materials. For additional inquiries, please contact the corresponding author.

References


Figures
Figure 1

**Vaccarin alleviated renal function in T2DM mice.** (A) Fasting blood glucose. (B) 24-hour urine albumin analysis. (C) Serum BUN levels. (D) Serum creatinine levels. (E) Kidney/body weight ratio. (F) Glomerular mesangial matrix expansion analysis. (G) Representative images of H&E and PAS staining. Scale bar = 50 μm. ***P < 0.001 vs Ctrl. #P < 0.05, ##P < 0.01, ###P < 0.001 vs DN. n = 4-6.
Figure 2

Vaccarin alleviated renal fibrosis in T2DM mice. (A) The image of kidney sections stained with Masson and immunohistochemistry of collagen1, α-SMA and E-cadherin in the mice kidney. (B) Quantification of fibrosis area of Masson staining results. (C) Quantification of collagen, α-SMA and E-cadherin expression. (D) Relative mRNA levels of collagen, TGF-β1, α-SMA and E-cadherin. Scale bar = 50 μm. *P < 0.05, **P < 0.01, ***P < 0.001 vs Ctrl. #P < 0.05, ##P < 0.01, vs DN. n = 4-6.
Figure 3

**Vaccarin alleviated renal inflammation response and oxidative stress in T2DM mice.** (A-D) Relative mRNA levels of IL-1β, VCAM-1, COX-2 and p65-NFκB. (E) MDA content of the diabetic kidney tissues. (F) GSH-Px activity of the diabetic kidney tissues. (G-I) DHE fluorescence staining or DCFH-DA fluorescence staining of the diabetic kidney tissues. Scale bar = 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001 vs Ctrl. #P < 0.05, ##P < 0.01, ###P < 0.001 vs DN. n = 4.
Figure 4

Effects of vaccarin on fibrosis in HK-2 cells. (A) Representative blot images and quantitative analysis of collagen1, TGF-β1, α-SMA, and E-cadherin in HK-2 cells after 48 hours of culture, both with and without vaccarin. (B-E) Relative mRNA levels of collagen1, TGF-β1, α-SMA, and E-cadherin. Scale bar: 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001 vs NG. #P < 0.05, ##P < 0.01 vs HG. n = 3-6.
Vaccarin alleviated inflammation response and oxidative stress in diabetic kidneys. HK-2 cells were pre-incubated with 5 μM vaccarin for 12 hours, followed by exposure to 35 mM HG for 48 hours. (A-D) Relative mRNA levels of IL-1β, VCAM-1, COX-2, and p65-NFkB. (E-G) DHE fluorescence staining or DCFH-DA fluorescence staining of HK-2 cells. Scale bar = 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001 vs NG. #P < 0.05, ##P < 0.01, ###P < 0.001 vs HG. n = 4.

Figure 5
Figure 6

Network pharmacology analysis of core targets of vaccarin and molecular docking model of core target with vaccarin. (A) A Venn diagram depicted the overlap between candidates and interaction targets of vaccarin in the context of diabetic nephropathy. (B) The PPI network of core targets was generated using the STRING database. (C) A bar graph illustrated the results of biological processes (BP) enrichment analysis. (D) The molecular docking of vaccarin with EGFR was performed.
Vaccarin inhibited EGFR/ERK phosphorylation in T2DM mice and HG-induced HK-2 cells. (A) Representative blot images in the diabetic kidney tissues. (B) Representative blot images in HG-induced HK-2 cells. (C-E) Quantitative analysis of p-EGFR and p-ERK in the diabetic kidney tissues. (F-H) Quantitative analysis of p-EGFR and p-ERK in HG-induced HK-2 cells. **P < 0.01, ***P < 0.001 vs Ctrl or NG. #P < 0.05, ##P < 0.01 vs DN or HG. n = 3.
Figure 8

Vaccarin ameliorated fibrosis, inflammation response and oxidative stress in HG-induced HK-2 cells exposed to EGFR inhibitor AG1478 or ERK inhibitor U0126. (A-D) Relative mRNA levels of collagen1, TGF-β1, α-SMA, and E-cadherin in HK-2 cells. (E-D) Relative mRNA levels of IL-1β, VCAM-1, COX-2, and p65-NFκB in HK-2 cells. (D) DHE staining performed on HG-induced HK-2 cells. Scale bar = 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001 vs NG. #P < 0.05, ##P < 0.01, ###P < 0.001 vs HG. n = 4.

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