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REVIEW ARTICLE

Transient receptor potential vanilloid 4 channels as therapeutic targets in diabetes and diabetes-related complications

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ABSTRACT

With an estimated 425 million diabetes patients worldwide in 2019, type 2 diabetes has reached a pandemic proportion and represents a major unmet medical need. A key determinant of the development and progression of type 2 diabetes is pancreatic -cell dysfunction, including the loss of cell mass, the impairment of insulin biosynthesis and inadequate exocytosis. Recent studies have shown that transient receptor potential vanilloid 4 (TRPV4), a Ca^{2+}-permeable non-selective cation channel, is involved in -cell replication, insulin production and secretion. TRPV4 agonists have insulinotropic activity in pancreatic -cell lines, but the prolonged activation of TRPV4 leads to -cell dysfunction and death. In addition, TRPV4 is involved in a wide variety of pathophysiological activities, and has been reported to play an important role in diabetes-related complications, such as obesity, cardiovascular diseases, diabetic retinopathy, nephropathy and neuropathy. In a rodent type 2 diabetes model, Trpv4 agonists promote vasodilation and improve cardiovascular function, whereas Trpv4 antagonists reduce high-fat diet-induced obesity, insulin resistance, diabetic nephropathy, retinopathy and neuropathy. These findings raise interest in using TRPV4 as a therapeutic target for type 2 diabetes. In this review, we intend to summarize the latest findings regarding the role of TRPV4 in diabetes as well as diabetes-related conditions, and to evaluate its potential as a therapeutic target for diabetes and diabetes-related diseases.

INTRODUCTION

According to the International Diabetes Federation, the prevalence of diabetes is increasing rapidly, and the number of patients will reach approximately 629 million by 2045[1]. Approximately 90% of all cases of diabetes are type 2 diabetes[2], which is generally characterized by insulin resistance, during which the body does not fully respond to insulin for the proper control of blood glucose levels[3]. Insulin resistance triggers the exaggerated secretion of insulin to compensate for the insufficient metabolic actions of this hormone, and the persistence of this condition might lead to the "exhaustion" of pancreatic -cells. Such exhaustion ultimately results in the development of hyperglycemia. Various organs and tissues are damaged by prolonged exposure to high blood sugar levels, which leads to diabetes-related complications[4,5], such as cardiovascular disease (CVD), kidney disease, neuropathy, blindness and lower extremity amputation. These complications impact the quality of life of patients with diabetes[6], and have become economic and healthcare burdens in many countries[7].

Transient receptor potential vanilloid 4 (TRPV4) is a Ca^{2+}-permeable non-selective cation channel[8]. There is increasing evidence for the involvement of TRPV4 in a variety of pathophysiological conditions. For example, TRPV4 is involved in pancreatic -cell replication and insulin production, and the activation of TRPV4 induces insulin secretion[9]. Furthermore, inhibiting or attenuating TRPV4 activity might reduce high-fat diet (HFD)-induced obesity and inflammation[10]. Table 1 summarizes recent findings on the involvement of TRPV4 in the pathogenesis of type 2 diabetes and diabetes-related diseases[9-49]. Even though TRPV4 might play a significant role in the development of diabetes and related conditions, the underlying mechanism remains unclear. In this review, we intend to summarize the latest findings from the literature to explore the underlying mechanism of the role of TRPV4 in diabetes and

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evaluate the feasibility of using TRPV4 as a therapeutic target for diabetes and diabetes-related diseases.

BRIEF INTRODUCTION OF TRPV4 CHANNEL

TRPV4 belongs to the transient receptor potential vanilloid (TRPV) subfamily of transient receptor potential (TRP) cation channels, and is a vertebrate homologue of the *Caenorhabditis elegans Osm-9* gene[8,50]. To date, 28 TRP channels have been identified in mammals. Based on sequence homology, these proteins can be grouped into six subfamilies: TRPV, TRPC (transient receptor potential canonical), TRPM (transient receptor potential melastatin), TRPP (transient receptor potential polycystic), TRPML (transient receptor potential mucolipin) and TRPA (transient receptor potential ankyrin)[51,52]. TRPV4 is a widely expressed, polymodally gated, non-selective cation channel for ions, such as calcium, sodium, potassium and magnesium[8]. Various stimulating factors, such as moderate heat, osmotic pressure, cell swelling, and endogenous and exogenous chemical compounds, can affect the activity of TRPV4[53]. TRPV4 has the capability of being activated without stimulation[54], and is involved in various physiological functions, including osmoregulation[55], Ca^{2+} homeostasis[56], apoptosis and autophagy[57]. Furthermore, diseases, such as hyperalgesic, hypertensive, hypertrophic, degenerative, ischemic and metabolic disorders, can be attributed to the aberrant activity of TRPV4[58].

A number of endogenous small molecules have been shown to affect TRPV4 activity. These compounds include the arachidonic acid metabolite 5,6-epoxyeicosatrienoic acid (5,6-EET), acetylcholine, dimethylallyl pyrophosphate, and the endocannabinoid anandamide[59-62]. In addition, chemicals, such as gadolinium, lanthanum and ruthenium red, botanical extracts, such as bisandrographolide and apigenin[63,64], and synthetic compounds, including phorbol ester 4α-phorbol 12,13-didecanoate (4αPDD), can also affect TRPV4 activity[48,65-69]. TRPV4 antagonists have been successfully used *in vivo* for pulmonary edema induced by heart failure, and one of them, GSK2798745, is currently being evaluated in a clinical trial for heart failure[58,70].

TRPV4 AND TYPE 2 DIABETES

A critical determinant of type 2 diabetes is pancreatic -cell dysfunction, including -cell deficiency, impaired insulin biosynthesis and insufficient exocytosis[71]. It has been shown that the intracellular Ca^{2+} level ($[Ca^{2+}]i$) in pancreatic -cells affects insulin secretion[72,73]. Adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels and voltage-gated Ca^{2+} channels (VGCCs) are considered mediators of glucose-stimulated insulin secretion[74,75]. High blood glucose concentrations increase glucose metabolism in pancreatic -cells and lead to a higher cellular ATP/adenosine diphosphate ratio. A high ATP/adenosine diphosphate ratio induces the closure of K_{ATP} channels followed by the depolarization of the membrane and the opening of VGCCs to facilitate Ca^{2+} influx, thereby elevating the $[Ca^{2+}]i$ and stimulating insulin secretion.

A K_{ATP} channel-independent mechanism of glucose-induced insulin secretion has also been proposed[76,77]. Takii *et al.*[78] showed the secretion of insulin through hypotonic-induced -cell swelling, which activates Gd^{3+}-sensitive cation channels followed by membrane depolarization, the activation of VGCCs and an increased $[Ca^{2+}]i$. Trpv4 is expressed in the -cell lines, MIN6 and INS-1E, as well as in the rodent pancreas[9,17,41]. Immunohistochemistry has also shown a high abundance of TRPV4 protein in human islets[79]. TRPV4 can act as a mechano- and osmosensor; for example, TRPV4 activity can be induced by hypotonic stress or moderate heat, leading to the influx of extracellular Ca^{2+} and an increased $[Ca^{2+}]i$[9]. Therefore, TRPV4-mediated intracellular Ca^{2+} concentration changes might be involved in the regulation of glucose-induced -cell insulin secretion. Skrzypski *et al.*[9] found that 4αPDD-induced Trpv4 activation results in an increase in the intracellular Ca^{2+} concentration and insulin secretion in rat INS-1E cells. The induction of this effect by 4αPDD can be eliminated by TRPV4 inhibitors. However, Sawatani *et al.*[80] found that 4αPDD and GSK1016790A show no apparent effect on the $[Ca^{2+}]i$ in isolated mouse -cells or MIN6 cells. The authors speculated that the inconsistencies in the findings were caused by differences between mice and rats, as well as differences in experimental temperature; room temperature was used in the study by Skrzypski *et al.*, and 37°C was used by Sawatani *et al.* As TRPV4 can be activated by a warm temperature (>27°C), the lack of effects of 4α-PDD and GSK1016790A on the $[Ca^{2+}]i$ might have been caused by the higher temperature used by Sawatani *et al.*

The activation of TRPV4 is not only associated with insulin secretion, but also affects the level of insulin messenger ribonucleic acid (mRNA) in -cells. Billert *et al.*[41] found that the GSK1016790A-induced activation of TRPV4 promotes the expression of insulin mRNA after 1 and 3 h of treatment. In contrast, when cells are incubated with GSK1016790A for 24 h, the mRNA expression of insulin and -cell specific genes, such as Ins1, Ins2, Pdx1 and Gck, are suppressed, and this suppression is accompanied by an increase in cell death[41]. Extracellular signal-related kinase 1 and 2 (ERK1/2) are activated by glucose in a Ca^{2+}-dependent manner, and ERK1/2 regulates other transcription factors associated with insulin gene expression and -cell survival[81-83]. GSK1016790A can promote ERK1/2 phosphorylation in INS-1E cells, and the pharmacological blockade of ERK1/2 weakens insulin mRNA expression induced by GSK1016790A over time[41]. Therefore, Billert *et al.* proposed that TRPV4 stimulates insulin mRNA expression through ERK1/2 activation; however, the involvement of ERK1/2 in TRPV4 activation-induced insulin mRNA expression has yet to be confirmed. In contrast, TRPV4 activation can stimulate nitric oxide (NO) production and inducible NO synthase mRNA expression, resulting in NO-induced endoplasmic reticulum stress and the suppression of insulin mRNA expression in -cells[41]. Casas *et al.*[17] also found that the activation of TRPV4 by human islet amyloid polypeptide in mouse MIN6 -cells induces apoptosis (Figure 1).

Table 1 | Involvement of transient receptor potential vanilloid 4 in a wide range of pathophysiological conditions in diabetes and diabetic complications

Disease	Tissue/cell type	Species	Experiment	Effect	References
Type 2 diabetes	Pancreatic -cells	Murine	*In vitro/in vivo*	Modulation of insulin secretion; mediated -cell apoptosis	Casas 2008[17], Skrzypski 2013[9], Billert 2017[41]
Obesity	Adipocytes	Rat, murine, human	*In vitro/in vivo*	Adipogenesis and energy metabolism; adipose Ca^{2+} homeostasis and inflammation	Ye 2012[10], Che 2014[29], Chen 2015[31], Janoschek 2016[37], Sanchez 2016[38], Sun 2017[44]
	Skeletal muscle	Murine	*In vitro/in vivo*	Heightened metabolic capacity	Pritschow 2011[22], Kusudo 2012[25]
	MSC, ASC	Murine	*In vitro/in vivo*	Increased obesity susceptibility	O'Conor 2013[28]
	Sebocyte	Human	*In vivo*	Influences glucose and lipid metabolism	Olah 2014[30]
	Blood	Human	Cross-sectional studies	Increased genetic susceptibility to obesity	Duan 2015[32], Tabur 2015[34]
Diabetes-related CVD	MAECs, Smooth muscle cells	Rat, murine	*In vitro/in vivo*	Vasodilator response	Earley 2009[19], Mendoza 2010[20], Ma 2013[27], Ye 2018[47]
	Mesenteric arteries	Rat	*In vitro/in vivo*	Endothelium-dependent relaxation	Zou 2015[36], Matsumoto 2017[43], Bihzad 2017[40]
	CAECs	Rat	*In vivo*	Shear stress-induced vasodilation	Kohler 2006[15]
Diabetic nephropathy	HCD cells	Human	*In vivo*	Role in RVD	Hills 2006[14], Hills 2012[12]
	Collecting ducts, tubules	Murine	*In vitro/in vivo*	Control of mechanosensitivity	Berrout 2012[24], Wu 2007[16]
Diabetic retinopathy	RMECs, RPE	Rat, murine, human, bovine	*In vitro/vivo*	Water diffusion and BRB breakdown in the retina; endothelial dysfunction; role in RVD	Monaghan 2015[13], Zhao 2015[35], Arredondo 2017[39], Orduna 2019[49]
	HCECs	Human	*In vivo*	Role in RVD	Pan 2008[18], Mergler 2011[21]
	RGCs, Müller cells	Murine	*In vitro/in vivo*	Polymodal sensory transduction; modulation of calcium flux and apoptosis	Ryskamp 2011[23], Lakk 2017[42], Lakk 2018[46]
Painful diabetic neuropathy	DRGs, TGs	Rat, murine	*In vitro/in vivo*	Modulates mechanosensation; mechanical hyperalgesia	Alessandri 2008[11], Alexander 2013[26], Hinata 2018[45]
	DRGs, sciatic nerve, hind paw plantar skin	Murine	*In vitro/in vivo*	Mechanical allodynia	Dias 2019[48]
	SGCs	Murine	*In vitro/in vivo*	Nociceptors for inflammatory pain	Rajasekhar 2015[33]

ASC, subcutaneous adipose-derived stem cells; BRB, blood–retina barrier; CAECs, carotid artery endothelial cells; CVD, cardiovascular disease; DRGs, dorsal root ganglia; HCD, human collecting duct; HCECs, human corneal endothelial cells; MAECs, mesenteric artery endothelial cells; MSC, bone marrow derived stem cells; RGCs, Retinal ganglion cells; RMECs, retinal microvascular endothelial cells; RPE, retinal pigment epithelium; RVD, regulatory volume decrease; SGCs, satellite glial cells; TGs, trigeminal ganglia.

TRPV4 AND DIABETES-RELATED COMPLICATIONS

Diabetes patients have an increased risk of developing heart, blood vessel, eye, tooth, kidney and nerve conditions as a result of prolonged high blood glucose levels[1]. Hyperglycemia-associated conditions are often accompanied by dysregulated metabolic processes of carbohydrates, fats, proteins and electrolytes, all of which have significant impacts on the normal function of the cardiovascular system[84]. Furthermore, endothelial capillary cells, including those in the retina and renal glomerulus, are damaged by an excessive accumulation of glucose in cells[85]. The consequent complications are termed "microvascular diseases" and "macrovascular diseases" owing to damage to small blood vessels and the arteries, respectively[4]. One of the common comorbidities of type 2 diabetes is obesity caused by calorie overload, a lack of physical activity and insulin resistance, which all contribute to the vascular complications associated with diabetes[4,86].

Several studies have shown an association between TRPV4 activity and obesity[87] and the progression of diabetic macrovascular and microvascular complications, such as diabetes-related cardiovascular diseases, diabetic retinopathy, nephropathy and neuropathy (Figure 2)[8,88-90]. In type 2 diabetes rodent models, TRPV4 agonists promote vasodilation and improve cardiovascular function[43]. In addition, TRPV4 antagonists reduce HFD-induced obesity and insulin resistance, and alleviate the progression of diabetic nephropathy, retinopathy and neuropathy[10,12,39,48].

TRPV4 AND OBESITY

Obesity is caused by an imbalance between energy intake and consumption, and is a common comorbidity in individuals with type 2 diabetes. Compared with individuals in the healthy weight range, obese men have a sevenfold higher risk of developing type 2 diabetes, whereas obese women have a 12-fold higher risk[91]. Adipose tissue (AT) was previously thought to be a passive tissue that preserves excessive energy in the form of lipids, and modulates the distribution of lipids in the body[92]. It is now becoming clear that AT can also serve as an endocrine organ, releasing many biologically active factors, such as adipokines, to communicate with different organs and regulate metabolic pathways[93]. In addition, brown and beige AT have been found to sustain euthermia by diffusing energy in the form of heat[94].

TRPV4 is highly expressed in human preadipocytes, and is involved in lipogenesis through the phosphorylation of protein kinase B. In addition, TRPV4 activation affects ERK1/2 activity,

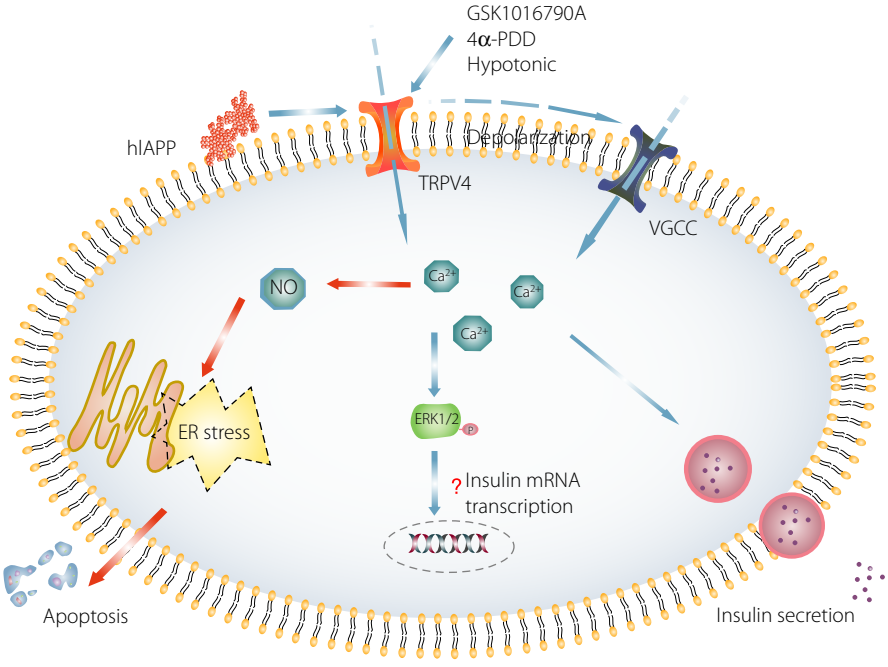

Figure 1 | Transient receptor potential vanilloid 4 (TRPV4) activation affects insulin release and apoptosis from pancreatic -cells. In pancreatic -cells, the activation of TRPV4 can be involved in the regulation of insulin release and apoptosis through different mechanisms. In contrast, the activation of TRPV4 induced by hypotonia and agonists (GSK1016790A and phorbol ester 4α-phorbol 12,13-didecanoate [4α-PDD]) can promote Ca^{2+} inflow, leading to plasma membrane depolarization, which elevates intracellular Ca^{2+} levels by activating voltage-gated Ca^{2+} channels (VGCCs) in -cells, ultimately leading to insulin secretion. In addition, elevated intracellular Ca^{2+} levels promote Extracellular signal-related kinase 1 and 2 (ERK1/2) phosphorylation, which is involved in the regulation of insulin messenger ribonucleic acid (mRNA) transcription. However, few studies have reported that TRPV4 activation is involved in the regulation of insulin mRNA expression through the ERK1/2 pathway, and the mechanism has not yet been elucidated. In contrast, protracted or human islet amyloid polypeptide-induced TRPV4 activation can stimulate nitric oxide (NO) production in -cells, resulting in endoplasmic reticulum (ER) stress and the promotion of cell apoptosis. hIAPP, human islet amyloid polypeptide; P, phosphorylation.

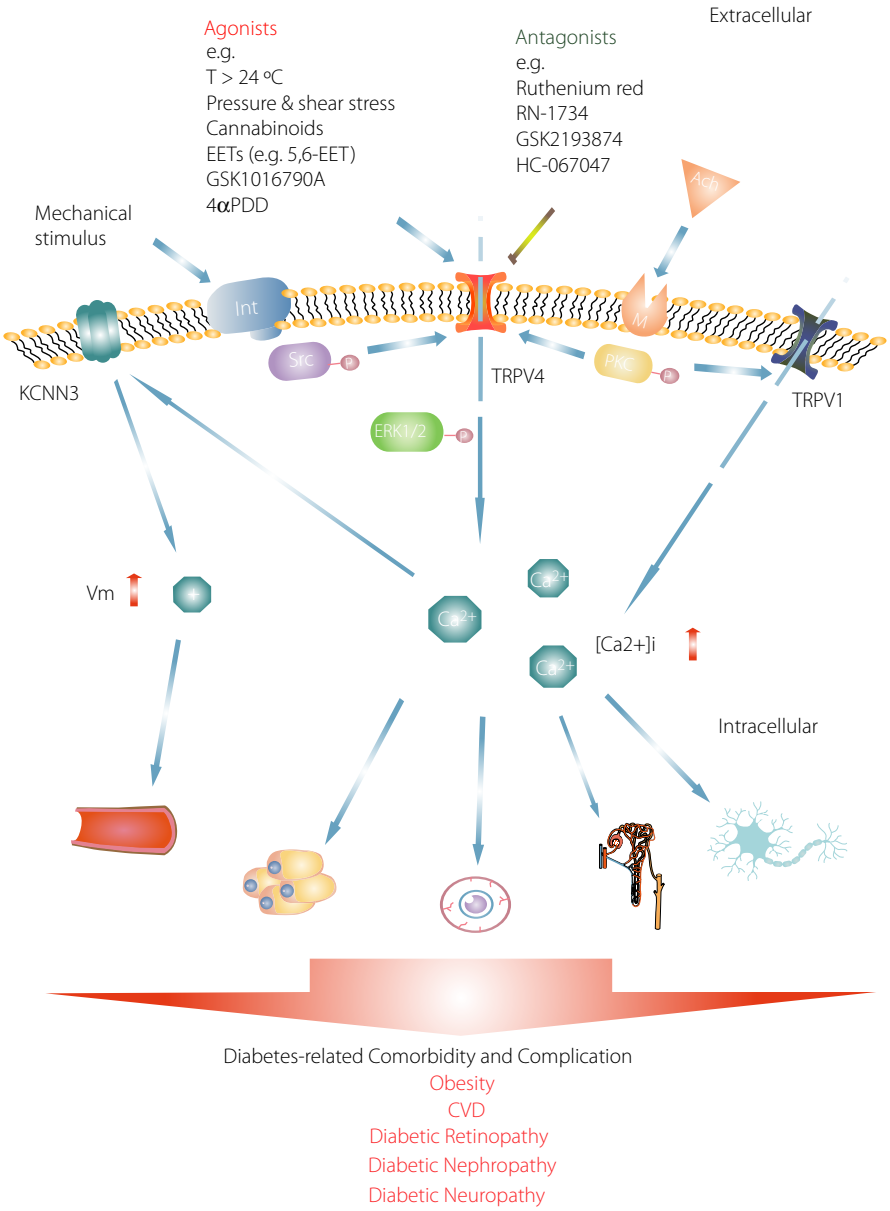

Figure 2 | Diabetes leads to cellular damage mediated by transient receptor potential vanilloid 4 (TRPV4) activity. Pressure and shear stress activate TRPV4, which is a mechanosensitive channel. Integrin (Int) signaling is stimulated by the mechanical activation of TRPV4 through Src tyrosine kinase (Src). The stimulation of muscarinic (M) receptors by acetylcholine (ACh) also activates the colocalization of TRPV4 and TRPV1 through protein kinase C (PKC). TRPV4 and TRPV1 activation increase the intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$), which can result in damage to various cell types, such as artery cells, retinal microvascular endothelial cells, collecting duct cells, Müller cells and satellite glial cells. In addition, the activation of TRPV4 promotes extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation, and an elevated $[Ca^{2+}]i$ in adipocytes can lead to inflammation and insulin resistance. However, an increase in the $[Ca^{2+}]i$ can activate the KCNN3 channel, which can result in an increase in membrane potential and promote vasodilation. In diabetes, the dysfunction of TRPV4 can result in diabetes-related diseases such as obesity, cardiovascular disease (CVD), diabetic retinopathy, nephropathy and neuropathy. KCNN3, potassium calcium-activated channel subfamily N member 3; P, phosphorylation; Vm, membrane potential.

which disrupts normal glucose and lipid metabolism[29,30]. Ye *et al.*[10] showed that Trpv4 deficiency positively regulates white adipocyte "browning" and beige adipocyte differentiation, and TRPV4 knockdown has been found to cause the elevated expression of thermogenic genes, including *Ppargc1a* and *Ucp1*, in 3T3-F442A adipocytes. Furthermore, the activation of TRPV4 has been found to cause the rapid phosphorylation of ERK1/2 and c-Jun N terminal kinase 1/2, which inhibits thermogenic gene expression. As found in a study carried out in cells[10], *Trpv4*$^{-/-}$ mice have elevated levels of thermogenic genes

and lower bodyweight when fed a HFD[25]. Kusudo *et al.* reported that skeletal muscle metabolic capacity is increased in *Trpv4*$^{-/-}$ mice, resulting in resistance to HFD-induced obesity[25]. The activation of TRPV4 controls resting Ca^{2+} influx in skeletal muscle, which regulates skeletal muscle contraction[22]. However, O'Conor *et al.*[28] found that HFD-fed *Trpv4*$^{-/-}$ mice are prone to obesity, which is contradictory to the findings of Kusudo *et al.*[25]. This inconsistency was probably caused by differences in diet and animal age. In the report by Kusudo *et al.*, mice were fed a HFD with 41.9% kcal for 16 weeks beginning at 4 months-of-age (approximately 12 weeks)[25], whereas a HFD with 60% kcal was administered for 22 weeks beginning at 10 weeks-of-age in the study by O'Conor *et al.*[28]. Nevertheless, the pathophysiological role of TRPV4 in obesity is complicated and further study is warranted. In humans, *TRPV4* mRNA levels in peripheral blood leukocytes are markedly reduced in patients with metabolic syndrome[34].

Studies have shown that the Trpv4 level is significantly increased in the white AT and brown AT of HFD-induced obese mice and diabetes (*db*/*db*) mice[31,37,44]. In addition, maternal obesity causes a high level of Trpv4 in the white AT of offspring on postnatal day 21[37]. Increased *Trpv4* mRNA levels might be the result of macrophage infiltration in AT, as macrophages also express *Trpv4*, promoting the expression of pro-inflammatory genes in adipocytes[10,95]. Chronic low-grade inflammation of AT is a trigger for systemic inflammation and insulin resistance[96]. Weight loss procedures, such as exercise and dietary intervention, are able to revert the Trpv4 level in AT back to the normal level[31,37].

The relationship between TRPV4 and obesity-associated pathologies is still unclear, as very few studies have reported the role of TRPV4 in human adipocytes. Sanchez *et al.*[38] first carried out an electrophysiological study of TRPV4 in human adipocytes, and confirmed that the cation current in cultured human adipocytes can be activated under hypotonic conditions. This current is mediated by TRPV4 channels and can depolarize cells, thereby increasing Ca^{2+} levels in adipocytes. In addition, Duan *et al.*[32] also reported the impact of TRPV4 gene polymorphisms on BMI and body fat mass.

TRPV4 AND DIABETES-RELATED CARDIOVASCULAR DISEASES

CVD is one of the major morbidities associated with diabetes. Compared with individuals without diabetes, diabetes patients are two- to fourfold more likely to be hospitalized for CVD or CVD-related clinical events[6]. In addition, CVD is one of the leading causes of death in diabetes patients[97], and the incidence of cardiovascular mortality in diabetes patients is three- to fivefold higher than that in individuals without diabetes[98]. It is now known that endothelial dysfunction in resistance vessels is a hallmark of conditions, such as hypertension and vascular complications, associated with diabetes[99].

TRPV4 is expressed in the endothelium of small mesenteric arteries and is involved in the regulation of endothelial Ca^{2+} levels[8,20]. *In vitro* experiments have shown that the activation of TRPV4 by mild hypothermia leads to the production of acetylcholine by endothelial cells, which activates muscarinic receptors in adjacent cells to promote endothelium-dependent relaxation[36]. In addition, in *Trpv4*$^{-/-}$ mice, acetylcholine-induced hyperpolarization and vasodilation are reduced by approximately 75% in mesenteric resistance arteries[19]. Vascular smooth muscle tone is dynamically regulated by vasoactive substances secreted by the endothelium[10]. Luminal flow in normal animals results in endothelial-dependent dilation through the release of NO and endothelium-derived hyperpolarizing factor; however, in *Trpv4*$^{-/-}$ mice, these responses are significantly reduced[15,101]. Although the basal blood pressure of wild-type and *Trpv4*$^{-/-}$ mice is comparable, transient hypertension induced t the suppression of NO synthase is higher in *Trpv4*$^{-/-}$ mice, suggesting that the activation of Trpv4 counteracts hypertensive stimuli *in vivo*[19]. In normal mice, 11,12-EET (11,12-epoxyeicosatrienoic acid) induces the hyperpolarization of vascular smooth muscle cell membranes and the vasodilation of mesenteric arteries, but after the activity of Trpv4 in the endothelium is disrupted, hyperpolarization and vasodilation induced by 11,12-EET are decreased by half[19]. Likewise, the 11,12-EET-induced vasodilation response is also markedly reduced in the tissues of diabetic animals or in tissues incubated with the TRPV4 inhibitor ruthenium red[40].

Ma *et al.*[27] showed that the colocalization of Trpv4 with Ca^{2+}-sensitive K+ channels 2.3 (potassium calcium-activated channel subfamily N member 3 [Kcnn3]), which induces membrane hyperpolarization and vascular relaxation in smooth muscle, occurred in mesenteric artery endothelial cells in rats. However, the endothelial expression of *Trpv4* and the downstream *Kcnn3* is decreased in the mesenteric arteries from streptozotocin-induced diabetic rats, and Trpv4 expression is also decreased in microvascular endothelium cultured in a hyperglycemic environment[27]. Furthermore, Matsumoto *et al.*[43] found that vasodilation responses induced by various TRPV4 activating factors, such as acetylcholine, GSK1016790A and NS309, are impaired in the superior mesenteric arteries of female Otsuka Long-Evans Tokushima Fatty rats, a diabetic rat strain. TRPV4 interacts with the protein fibronectin type III domain-containing 5[47], an exercise-induced myokine with vasoprotective effects on endothelial function; however, its endothelium-dependent vasodilation can be abolished by TRPV4 antagonist[12,103]. These findings suggest that the dysregulation of TRPV4 activity contributes to vasodilation dysfunction in cardiovascular diseases.

TRPV4 AND DIABETIC NEPHROPATHY

Approximately 20–40% of diabetes patients develop microalbuminuria, a precursor to diabetic nephropathy[14], and approximately 30–40% of them further develop diabetic nephropathy[15], which is the main cause of end-stage renal disease[16]. In diabetes patients, the elevated blood glucose tends to be excreted into the urine, which induces osmotic drag to increase tubular flow,

and increases the formation of hyperosmotic urine. These physical alterations induce renal epithelial cell contractions and eventually trigger compensatory mechanisms to restore cell volume[12]. TRPV4 is widely distributed in the renal vasculature, and is considered to be a sensor or transducer that senses the mechanical stress caused by the flow of liquid into the collecting duct of the kidney[16,24,43]. The rise of the cytosolic Ca^{2+} concentration through TRPV4 activation after hypo-osmotic fluid-induced cell swelling has been shown in various volume-regulating cells, such as bladder urothelial cells, chondrocytes and bronchial epithelial cells[17-109]. Therefore, TRPV4 is a potential mediator of osmoreceptors, which play a role in cell volume recovery[24].

Mechanosensitive TRPV4 channels regulate cell volume by Ca^{2+}-dependent mechanisms[110]. Human collecting duct (HCD) cells express TRPV4[14], and mechanical stimulation activates TRPV4, which leads to the elevation of the Ca^{2+} level in HCD cells to induce a regulatory volume decrease[111]. Recent studies have shown a decrease in TRPV4 levels in HCD cells when they are cultivated in a high-glucose environment, undermining TRPV4-mediated regulatory volume decrease[12]. Furthermore, *Trpv4* knockdown eliminates changes in cellular Ca^{2+} levels caused by mechanical stimulation and inhibits the ability of HCD cells to respond to membrane deformation caused by osmotic or mechanical stress[12]. Cell volume regulation plays an important role in maintaining the overall integrity and function of nephrons. Failure to properly respond to osmotic stimuli might have adverse outcomes for fluid and electrolyte balance in the kidney, which eventually leads to various kidney conditions, such as diabetic nephropathy and end-stage renal disease.

TRPV4 AND DIABETIC RETINOPATHY

Approximately one-third of diabetes patients might develop diabetic retinopathy, which is the primary reason for blindness associated with diabetes[112]. Non-proliferative diabetic retinopathy and proliferative diabetic retinopathy are the two main categories of diabetes-associated retinopathy[113], and they have incidences of approximately 27.9% and 7.5%, respectively, among diabetes patients[114]. Non-proliferative diabetic retinopathy is a disorder without neovascularization, and might progress to proliferative diabetic retinopathy with neovascularization. Macular edema with swelling or thickening of the macula was attributed to sub- and intraretinal fluid accumulation in the macula triggered by the breakdown of the blood–retinal barrier (BRB), which can occur in non-proliferative diabetic retinopathy[115], is the most common cause of vision loss[116].

The BRB is formed by vascular endothelial (inner layer) and retinal pigment epithelial (outer layer) cells[117]. Under normal circumstances, the inner layer of the BRB is formed by close-fitting intraretinal vascular endothelial cells and thereby restricts the entry of liquid. High-glucose conditions damage the integrity of vascular endothelial and retinal pigment epithelial cells, which increases the permeability of the retinal barrier and leads to protein leakage into the interstitial retinal tissue[118-121]. Recent studies have found that TRPV4 participates in regulating BRB permeability in retinal microvascular endothelial cells and retinal pigment epithelial cells[13,35,39]. For example, Arredondo *et al.*[39] observed that BRB breakdown can be attenuated by *Trpv4*-selective antagonists, including RN-1734 and GSK2193874, in diabetic rats. Under diabetic conditions, BRB breakdown is aggravated by increased water diffusion into the retina, which can be stopped by inhibiting TRPV4 activity[49]. These results suggest that TRPV4 is involved in maintaining the homeostasis and structural integrity of the retina[49]. In hyperglycemia and diabetes, TRPV4 activity is decreased in retinal microvascular endothelial cells[13]. The activation of TRPV4 induces inflammatory responses that result in edema[122-125], which can be resolved by using TRPV4 inhibitors[126,127]. TRPV4 is expressed in human corneal epithelial cells[21], and the activation of TRPV4 results in Ca^{2+} influx, which might induce regulatory volume decrease[18]. Therefore, a decrease in TRPV4 levels during hyperglycemia and diabetes might be a compensatory mechanism aimed at maintaining retinal homeostasis.

The main pathogenesis of diabetic retinopathy involves not only structural alterations in retinal blood vessels, but also the dysfunction of perivascular neurons or glial tissue[128]. Using continuous fixed-focus electroretinography, retinal ganglion cells (RGCs) and bipolar cells were shown to exhibit abnormal activity in early diabetic retinopathy. In addition, RGCs and Müller cells, a type of retinal glial cell, show increased apoptosis[129]. Trpv4 is expressed in RGCs, Müller cells and the optic nerve head in mice[42,46]. Cholesterol molecules act as sentinels for metabolic, osmotic, mechanical and inflammatory signals within the retina, and are involved in regulating a range of pathophysiological functions, such as the loss of RGCs and photoreceptors, the hypertrophy and pathological swelling of Müller cells, and the maintenance of the BRB. Lakk *et al.*[42] reported that Trpv4 can mediate cholesterol-dependent multimodal transduction in Müller cells. Furthermore, hypercholesterolemic retinas show similar pathologies, including reactive gliosis, elevated retinal microvascular endothelial barrier permeability, RGC degeneration and pathological glial swelling, with excessive TRPV4 activation[42]. A study by Ryskamp *et al.*[23] showed that TRPV4 activation in RGCs mediates responses to membrane stretching, resulting in elevated Ca^{2+} levels in the cells and augmented excitability. Sustained exposure to a TRPV4 agonist leads to excessive Ca^{2+} influx, which might activate Ca^{2+}-dependent pro-apoptotic signaling pathways and induce time- and dose-dependent apoptosis in RGCs[23].

TRPV4 AND DIABETIC NEUROPATHIC PAIN

Diabetic sensorimotor polyneuropathy is the most common diabetes-associated microvascular complication, and develops in 10–54% of diabetes patients[130], approximately one-third of which will develop diabetic neuropathy[131]. Patients with neuropathic pain can experience spontaneous and stimulus-induced pain, including hyperalgesia and allodynia[132]. Mechanical

allodynia often manifests as an adverse reaction to innocuous stimuli, including the touch of bedsheets, clothing and shoes, or can be activated by slight motion, such as walking and position adjustment[133]. Therefore, diabetic neuropathic pain has a markedly negative impact on quality of life[134].

A cluster of primary somatosensory neurons, also called nociceptors, originating from the trigeminal ganglion and the dorsal root ganglion (DRG) are responsible for regulating the ability to perceive pain[133]. TRPV4 is expressed in the DRG and trigeminal ganglion, and serves as a detector and transducer in nociceptive neurons[135]. TRPV4 has been reported to maintain neuropathic pain induced by alcohol, vincristine, paclitaxel, 2'-3'-dideoxycytidine and the chronic compression of the DRG[11,136,137]. Dias *et al.*[48] showed that HC-067047, a TRPV4 inhibitor, can hinder the progression of mechanical allodynia in diabetic mice induced by streptozotocin. Similarly, streptozotocin-induced mechanical hyperalgesia is also weakened in *Trpv4*$^{-/-}$ mice[11]. These findings suggest that the reduced TRPV4 activity can modulate the hyperactivity of mechanosensitive afferent nerves and effectively prevent diabetes-induced mechanical allodynia.

Immunohistochemical analysis shows no difference in the level of Trpv4 in the hind paw skin, sciatic nerve, and DRG between diabetic and non-diabetic mice[48]. As TRPV4 is constitutively expressed and capable of spontaneous activation in the absence of agonist stimulation, Dias *et al.*[48] suggested that TRPV4 can transduce neuropathic pain independent of the TRPV4 level. In fact, TRPV4 has been shown to be constitutively active, and might be involved in controlling neuronal excitability[138]. Furthermore, sensory nerve damage, inflammation, swelling and mechanical stimulation can trigger an integrin signal cascade transmitted by SRC family tyrosine kinases that causes the membrane insertion and activation of TRPV4 without changing the TRPV4 level[11,45]. Furthermore, protein kinase C is activated in the DRG neurons of monoiodoacetate-induced arthritis rats, causing the sensitization of Trpv4 and Trpv1, which are coexpressed in the DRG, thereby aggravating pain signaling[139-142]. However, Alexander *et al.*[26] showed by immunohistochemical analysis that TRPV4 is not expressed in cultured DRG and trigeminal ganglion neurons, and that there is no difference in the Ca^{2+} concentration induced by hypo-osmotic solution or 4αPDD between neurons from wild-type and *Trpv4*$^{-/-}$ mice. Approximately 73% of Trpv4 expression in mouse dorsal root ganglia is derived from satellite glial cells, which form a sheath around the neurons of the sensory ganglia, and can regulate pain and inflammation-related neuronal excitability[33]. TRPV4 is also expressed in other nonneuronal cells, such as skin keratinocytes, and regulates nociception, itching and inflammation[33,143-145]. Collectively, these findings imply that TRPV4 plays an important role in diabetic neuropathic pain.

CONCLUSION AND PERSPECTIVE

TRPV4 has been shown to play important roles in various diseases. TRPV4 is constitutively expressed and capable of spontaneous activation without stimulation, which is in accordance with its participation in controlling homeostatic functions through the regulation of cellular Ca^{2+} levels, and intracellular and systemic water balance. TRPV4 is expressed in different cell types throughout the body, which indicates its diverse activities under normal physiological conditions. For example, TRPV4 is highly expressed in the cilia of the bronchial epithelium in the lung, and is involved in mucociliary transport and ciliary beat frequency[146]. In addition, it is also found in osteoclasts, osteoblasts and chondrocytes, and is probably involved in bone remodeling and development[147]. It has been shown that TRPV4 activity is increased in hippocampal astrocytes after ischemia and hypoxia, which suggests its involvement in post-stroke oxidative stress-associated cell activities[148,149].

Regarding its involvement in type 2 diabetes, TRPV4 is expressed in pancreatic -cells, and is involved in insulin secretion and -cell apoptosis[9,41]. In addition, it is abundant in the vascular endothelium and smooth muscle of arteries, which suggests its involvement in vascular tone[19,20]. TRPV4 is also expressed in epithelial cells of the nephron and urothelial cells of the bladder, where it plays a role in osmosensation[17,150]. In sensory neurons, TRPV4 is expressed in the DRG and is involved in pain perception[135]. Accordingly, TRPV4 seems to be an important protein involved in type 2 diabetes and type 2 diabetes-associated complications.

It seems difficult to develop TRPV4 as a therapeutic target for specific diseases, such as type 2 diabetes, because of its ubiquitous expression and involvement in various pathophysiological processes. However, there are several clinical trials investigating TRPV4 antagonists for different conditions, including a trial studying the use of GSK2798745 for congestive heart failure. The results of these trials will allow us to evaluate the feasibility of using TRPV4 as a therapeutic target for specific diseases and the implications of its off-target effects[70].

Based on the current findings, TRPV4 agonists have insulinotropic activities, but the continuous activation of TRPV4 leads to -cell dysfunction and death[9,41]. TRPV4 reduces obesity and insulin resistance induced by HFD, and alleviates the development of diabetic complications, including diabetic nephropathy, retinopathy and neuropathy[10-11,25,39]. Therefore, TRPV4 has apparent clinical potential for managing diabetes and diabetic complications. However, the development of TRPV4 as a therapeutic target for type 2 diabetes might face multiple challenges associated with its interesting biological properties, including its wide expression profile and some contradictory indications.

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DISCLOSURE

The authors declare no conflict of interest.

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