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Invited Review

TRPM2: a multifunctional ion channel for oxidative stress sensing

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Abstract: Transient receptor potential (TRP) superfamily is a superfamily of cation channels that can be divided into seven subfamilies. TRPM2 is the second member of the TRPM subfamily, which includes eight members, namely TRPM1–8. TRPM2 is widely expressed in excitable and non-excitable cells, where it forms a Ca^{2+}-permeable cation channel and performs diverse cellular functions. TRPM2 channels are activated by ADP-ribose (ADPR), Ca^{2+}, H_2O_2 and other reactive oxygen species (ROS). It is established that TRPM2 serves as a cellular sensor for oxidative stress, mediating oxidative stress-induced $[Ca^{2+}]_i$ increase and contributing to pathological processes in many cell types. Accumulating evidence has indicated that TRPM2 is a potential therapeutic target for oxidative stress-related diseases. This review will highlight recent progress in this field.

Key words: TRPM2; oxidative stress; Ca^{2+} signaling; cardiovascular diseases; inflammation

TRPM2：氧化应激敏感的多功能离子通道

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摘 要：瞬时受体电位(transient receptor potential, TRP)超家族是一组非选择性阳离子通道，分为7个亚家族。TRPM亚家族包括8个不同的成员，TRPM1~8。TRPM2广泛表达于可兴奋细胞和非兴奋性细胞，形成Ca^{2+}通透性阳离子通道，并发挥不同的细胞功能。TRPM2通道可被ADP-核糖(ADPR)、Ca^{2+}、H_2O_2以及其他活性氧(ROS)所激活。现已证明，TRPM2作为氧化应激传感器，介导了氧化应激引起的细胞内Ca^{2+}浓度升高，并参与多种细胞的生理/病理过程。丰富的证据表明，TRPM2可作为氧化应激相关疾病的一个潜在的治疗靶点。本文对以上方面的研究进展做一综述。

关键词：TRPM2；氧化应激；Ca^{2+}信号；心血管疾病；炎症
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1 Introduction

Transient receptor potential (TRP) proteins represent a large superfamily of cation channels. Most TRP channels are non-selective cation channels permeable to Na^+, Ca^{2+} and Mg^{2+} [1]. The TRP protein was first discovered from a spontaneously mutant strain of *Drosophila melanogaster* that showed an abnormal response to prolonged illumination and consequently impaired vision [2]. Since then, at least 29 TRP proteins have been identified, which are classified into seven subfamilies based on the amino acid sequence homology: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin), TRPML (mucolipin) and TRPN (NOMPC-like). Except for TRPN, all subfamilies can be found in mammals. The structure of TRP proteins is characterized by six-transmembrane domains (namely segment 1–6), with a pore region loop located between segment 5 and segment 6, and with both the N- and C-termini oriented

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inward to the cytoplasm [1]. TRP proteins typically assemble into homo-tetramers or hetero-tetramers to form functional channels [3]. The topology of TRP channels is similar to voltage-gated channels. However, the positively charged amino acid residues for voltage-sensing are not preserved in most TRP members. Thus, TRP channels usually act as non-voltage-gated channels [4,5].

TRP channels are conserved through evolution and are ubiquitously expressed in numerous cell types from worms to humans, located in both the plasma membrane and organelle membrane [4,6]. Activation of TRP channels in the plasma membrane leads to the influx of Ca^{2+} and/or Na^{+}, increasing intracellular concentration of these ions and depolarizing the cell, whereas intracellular TRP channels might function as Ca^{2+} release channels [4,6–8]. TRP channels act as polymodal cellular sensors for a wide range of physical and chemical stimuli. They participate in diverse biological processes, including mechanosensation, perception of vision, taste, auditognosis, pain and temperature sensing, pheromone sensing, salivary fluid secretion, inflammation, Ca^{2+} and Mg^{2+} homeostasis, lysosomal function, smooth muscle tone modulation, cell survival/growth/differentiation/death, and cardiovascular function regulation [4,9,10].

The mammalian TRPM subfamily is named after the tumour suppressor melastatin (TRPM1) and consists of eight members: TRPM1–TRPM8, which are sub-divided into four homologue pairs on the basis of sequence homology: TRPM1/TRPM3, TRPM2/TRPM8, TRPM4/TRPM5 and TRPM6/TRPM7 [11,12]. The TRPM subfamily is characterized by very long N- and C-termini. The N-termini are conserved, containing four stretches of residues named TRPM subfamily homology domain (MHD) [13]. The C-termini have a TRP domain near the transmembrane segments [4]. TRPM1–6 and TRPM8 have splice variants, either a full-length protein or a short variant. These splice variants are believed to play a role in modulating the channel selectivity, localization and function [4]. This review focuses on the molecular and channel properties of TRPM2, and highlights its physiological/pathological significance as an oxidative stress sensor.

2 TRPM2 structure, expression and channel properties

2.1 Structure

TRPM2 is a multifunctional Ca^{2+} permeable, non-selective cation channel, previously known as LTRPC2 or TRPC7 [9,14]. The human TRPM2 gene is located in chromosome 21q22.3. It consists of 32 exons spanning about 90 kb [14]. The human TRPM2 protein contains 1 503 amino acids (1 507 in mouse and rat), with a molecular mass of ~170 kDa [5,14]. TRPM2 has six transmembrane segments (S1–S6) and intracellular N- and C-termini, with a predicted pore-forming loop located between S5 and S6 [13,14]. The N-terminus has four MHD of about 700 amino acids and a calmodulin binding IQ-like motif [11–13]. The C-terminus contains a TRP domain, a TRPM2-specific NUDT9 homology region (NUDT9-H), and a coiled-coil domain that has been reported to be engaged in the homotetrameric assembly of TRPM2 [4,5,15]. There is a unique ADP-ribose (ADPR) pyrophosphatase domain (namely Nudix box) located in the NUDT9-H region, responsible for TRPM2 channel activation by ADPR [11–13]. The structure of TRPM2 is closest to TRPM8, with 42% identical. TRPM8 is a sensor for cold temperature and cool taste. However, the biological functions of TRPM2 are quite different from those of TRPM8 [16,17].

2.2 Alternative splicing isoforms

Besides the full-length isoform of TRPM2, several alterative splicing isoforms have been reported. The first two identified splicing variants of TRPM2 are TRPM2-ΔC and TRPM2-ΔN. TRPM2-ΔC lacks a stretch of 34 amino acids (T1292–L1325) within the ADPR binding region of the C-terminus. TRPM2-ΔC is not sensitive to ADPR but is activated by H_2O_2, supporting the hypothesis of direct activation of TRPM2 by H_2O_2 [18]. TRPM2-ΔN shows a deletion of 20 amino acids (K538–Q557) in the N-terminus (TRPM2-ΔN). Neither ADPR nor H_2O_2 activates TRPM2-ΔN [18]. The third variant is an N-terminally truncated variant initially found in the striatum and thus named as the striatum shorter form (TRPM2-SSF), with the truncation of the first 214 residues. The TRPM2-SSF channel activity was markedly lower than that of TRPM2 [19]. Another isoform TRPM2-S is a quite short variant, lacking pore region, resulting from the termination of protein after the second transmembrane domain by an additional spliced stop codon. Co-expression of TRPM2-S with TRPM2 reduces the expression of functional TRPM2 channel. Thus, the variant may act as a dominant negative inhibitor of TRPM2 [20]. Other variants include two TRPM2 transcripts identified in melanoma, the melanoma-enriched antisense transcript TRPM2-AS and the

tumor-enriched transcript TRPM2-TE [21]. The expression of TRPM2 alternative splicing isoforms might represent adaptive mechanisms for the regulation of TRPM2 channel in different tissues, cells and physiological/pathophysiological states.

2.3 Expression pattern

The expression pattern of TRPM2 is widespread. TRPM2 is abundantly expressed in the brain. Its expression can be found in various brain regions such as cerebellum, cortex, hippocampus and medulla. Besides, TRPM2 is widely distributed in the immune system and many other tissues, including pituitary, lung, kidney, liver, bone marrow, pancreas, stomach, intestine, skeletal muscle, adipose, heart, blood vessel and placenta [5,22]. TRPM2 has been detected in a diversity of cells, including neurons, microglia, pancreatic β-cells, endothelial cells, smooth muscle cells, cardiomyocytes, and immune cells (such as neutrophils, megakaryocytes, monocytes/macrophages and lymphocytes) [3,5,22]. Although TRPM2 is usually described as a Ca^{2+}-permeable channel in plasma membrane, it has recently been found to also function as a Ca^{2+}-release channel in lysosomal membrane of pancreatic β-cells [8,23].

2.4 TRPM2 channel properties

ADPR is a key gating molecule of TRPM2 [11–13,24]. Upon binding of intracellular ADPR, the TRPM2 channel opens and allows the permeation of Na^+, Ca^{2+} and Mg^{2+} into the cell [5]. The relative permeability of P_{Ca}:P_{Na} is about 0.3 to 0.9 [4,13,24]. The Ca^{2+} permeability is of functional importance. TRPM2 activation brings a sizeable increase of $[Ca^{2+}]_i$. Whole-cell currents of TRPM2 show a nearly linear current-voltage relationship, with a reversal potential close to 0 mV and a weak outward rectification [5,13,24]. In physiological conditions, TRPM2 mediates inward cation currents mainly carried by Na^+ and Ca^{2+}. The single channel conductance is about 60 to 80 pS, with extremely long open time of many seconds [13,24,25]. Notably, TRPM2 channels properties are identical in cells heterologously expressing TRPM2 constructs and in native cells expressing endogenous TRPM2, suggesting that TRPM2 is likely to form homomultimers [13,26,27].

3 TRPM2 activators and inhibitors

ADPR is considered the primary gating molecule of TRPM2. Other channel activators of TRPM2 include reactive oxygen species (ROS, especially H_2O_2), nicotinamide adenine dinucleotide (NAD), cyclic ADP-ribose (cADPR), nicotinic acid-adenine dinucleotide phosphate (NAADP), Ca^{2+}, O-acetyl-ADP ribose (OAADPR), alloxan and arachidonic acid. Inhibition of TRPM2 channel can be achieved by blockers such as flufenamic acid (FFA), 2-aminoethoxydiphenyl borate (2-APB), and anti-fungal agents clotrimazole and econazole, none of which works selectively. No specific TRPM2 inhibitor has been available yet. Thus, development of TRPM2-specific inhibitors will facilitate the better understanding of TRPM2 properties and functions in future [5,22].

3.1 Activators

3.1.1 ADPR

ADPR is the first discovered and most efficient activator of TRPM2, with EC_{50} values of 1–90 μmol/L dependent on the cell types [13,24,26,28–31]. It is produced in the mitochondria and nucleus and is the major endogenous gating molecule of TRPM2 [3,13,24]. ADPR can be generated from the hydrolysis of NAD. NAD is a substrate of CD38, a multifunctional ectoenzyme with activities of NAD glycohydrolase, ADP-ribosyl cyclase and cADPR hydrolase. Thus, both ADPR and cADPR can be generated by CD38, and cADPR can be further converted to ADPR [32]. In TRPM2-transfected HEK-293 cells and endogenous TRPM2-expressing monocytic U937 cells, increasing intracellular concentration of ADPR induced large cationic currents characteristic of TRPM2 [13]. The ADPR-induced activation of TRPM2 is potentiated by cytosolic Ca^{2+}, NADPH and H_2O_2 [3,32,33].

3.1.2 NAD, cADPR and NAADP

In addition to ADPR, NAD and its metabolic products such as cADPR and NAADP can also activate TRPM2 channels. The EC_{50} values of NAD, cADPR and NAADP are 1–1.8 mmol/L, 0.7 mmol/L and 0.73 mmol/L, respectively [32]. The normal physiological concentration levels of these agents are much lower than those required for TRPM2 activation. However, these agents can synergize with ADPR to increase TRPM2 sensitivity to each other. Whether these agents bind directly to TRPM2 channels or are converted to ADPR, and how they synergize with ADPR is not well understood [3]. NAD can activate TRPM2 in inside-out patches, and has been suggested to bind to the Nudix box as ADPR, which indicates a direct effect of NAD without necessary conversion to ADPR [24,26,33]. However, incompatible data have shown that mutants of TRPM2

that are insensitive to ADPR can not be activated by NAD either [34]. Other compounds related to ADPR, including ATP, ADP-glucose, AMP and ribose-5-phosphate, are not able to activate TRPM2 [13].

3.1.3 H_2O_2 and oxidative stress

TRPM2 is a well established cellular sensor of oxidative stress. H_2O_2 induces TRPM2 activation and subsequent increase of $[Ca^{2+}]_i$ in various cell types [18,33]. The mode of TRPM2 activation by H_2O_2 has long been debated, and accumulating evidence suggests that H_2O_2 can activate TRPM2 channel either directly or indirectly [18,35]. Oxidative stress is well known to induce ADPR formation [36]. Thus, the H_2O_2-induced TRPM2 activation is often explained by formation of ADPR. However, H_2O_2 may also directly activate TRPM2. The TRPM2 splicing variant TRPM2-ΔC is insensitive to ADPR, but still responds to H_2O_2, suggesting the direct effect of H_2O_2 on TRPM2 [18].

3.1.4 Ca^{2+}

Ca^{2+} is critical for full activation of TRPM2 channels. Intracellular Ca^{2+} potently facilitates the activation of TRPM2 by ADPR, enhancing the channel sensitivity to ADPR and shifting the concentration-response curve to ADPR to the left [13,30,37]. Removing extracellular and/or intracellular Ca^{2+} markedly reduces the ADPR-induced currents [30,37]. The sensitization of TRPM2 activation by Ca^{2+} is probably due to the conformational changes evoked by $[Ca^{2+}]_i$-dependent interaction of calmodulin with the IQ-like motif in the N-terminus of TRPM2 [38]. Besides, Ca^{2+} even directly activates TRPM2 in a concentration-dependent manner, with an EC_{50} of 17 μmol/L, or 0.5 μmol/L in the presence of 10 μmol/L ADPR [37,38].

3.1.5 Other regulators

A novel acetyl-ADPR product, OAADPR has been reported to induce TRPM2 currents by directly binding to the Nudix box [39,40]. A pro-diabetic drug alloxan that is used to induce diabetes mellitus in animals activates TRPM2 to induce Ca^{2+} influx which mediates cell death [41]. Arachidonic acid has been suggested to be another activator of TRPM2, although the mechanisms for activation have not yet been elucidated [33].

3.2 Inhibitors

Blocker with desirable specificity to TRPM2 is not available yet. AMP cannot activate TRPM2. Instead, it antagonizes the channel activation by ADPR, cADPR, NAADP and NAD. The antagonism likely results from its interaction with the ADPR binding site in the Nudix box. 8-Br-cADPR inhibits or delays the channel activation by cADPR, NAD, NAADP and H_2O_2, but shows significant synergy with ADPR [5,22]. Several non-specific pharmacological blockers have been reported, including fenamates such as FFA, anti-fungal agents clotrimazole and econazole, N-(p-amylcinnamoyl) anthranilic acid, and 2-APB. FFA, a non-steroidal anti-inflammatory agent, shows anti-inflammatory effect in the central nervous system, probably by inhibiting a wide spectrum of cation influxes [42]. In TRPM2-expressing HEK-293 cells, FFA suppresses ADPR- or H_2O_2-induced currents and Ca^{2+} influx. The inhibition of TRPM2 by FFA is irreversible upon extended exposure (several minutes) [43]. Within the TRP family, TRPC5, TRPM4 and TRPM5 are also inhibited by FFA, whereas TRPC6 is stimulated by FFA [44–46]. Similarly to FFA, clotrimazole and econazole exert an irreversible open-channel block of TRPM2 channels activated by ADPR [47]. N-(p-amylcinnamoyl) anthranilic acid, a potent phospholipase A2 inhibitor, and 2-APB, a wide spectrum channel blocker, reversibly inhibit ADPR- or H_2O_2-induced currents and Ca^{2+} influx in a concentration-dependent manner [48,49].

4 Biological relevance of TRPM2

Research on biological relevance of TRPM2 in various cells and tissues have revealed several important roles of TRPM2 in physiological and pathological events, such as release of insulin from pancreatic β-cells, production of cytokines from inflammatory cells, increased endothelial permeability and oxidative stress-induced cell death [5]. Although TRPM2 is usually thought to serve as a Ca^{2+}-entry channel in the plasma membrane, its expression has recently been found in intracellular compartments as well, where it may be involved in lysosomal Ca^{2+}-release [3,8,23].

4.1 TRPM2 in insulin release

TRPM2 is abundantly expressed in pancreatic β-cells [5]. Numerous evidence has suggested that TRPM2 plays a key role in the constitutive and glucose-stimulated insulin release from pancreatic β-cells by mediating Ca^{2+} entry and depolarization [22,50]. Body temperature in synergy with cADPR can evoke TRPM2-mediated Ca^{2+} influx into pancreatic β-cells and consequent insulin release. Depletion of TRPM2 expression by TRPM2-specific siRNA strongly attenuated this thermal effect. The high glucose-stimulated insulin release is also reduced by FFA, econazole and TRPM2-

specific siRNA, and in *Trpm2* knockout mice [50,51].

4.2 TRPM2 in inflammation

During inflammation, ROS production is substantially increased at the sites of infection or injury. H_2O_2 and other ROS can stimulate the production of chemotactic cytokines from inflammatory cells such as neutrophils and monocytes, which is crucial for the recruitment of these cells [5,52,53]. It has been reported that, in human monocytic U937 cells, H_2O_2-induced CXCL8 production depends on the TRPM2-mediated Ca^{2+} influx and its signaling pathways, resulting in activation of nuclear factor-κB, which initiates CXCL8 transcription [54]. H_2O_2-induced $[Ca^{2+}]_i$ increase and CXCL2 production are strongly suppressed in monocytes of *Trpm2* knockout mice [54]. In a model of dextran sulphate sodium (DSS)-induced colitis, *Trpm2* knockout mice have a significantly reduced CXCL2 expression and neutrophil infiltration, and a remarkably attenuated severity of colitis [54]. TRPM2 is also required for the maturation and chemotaxis of dendritic cells [55]. A more recent study has suggested that immune cell activators such as lipopolysaccharide and tumor necrosis factor-α induce the up-regulation of TRPM2 mRNA and protein expression in both human primary monocytes and THP-1 cells, resulting in increased $[Ca^{2+}]_i$ and production of tumor necrosis factor-α, interleukin-6, interleukin-8 and interleukin-10, which can be mitigated by TRPM2 siRNA [56]. These studies suggest a key role for TRPM2 in the production of pro-inflammatory cytokines and the modulation of inflammatory responses.

4.3 TRPM2 in cell death

As an oxidative stress-sensitive Ca^{2+}-entry channel, TRPM2 confers susceptibility to cell damage in response to oxidative stress. Such TRPM2-mediated cell death has been reported by many studies in cells expressing recombinant TRPM2 channels as well as in cells expressing endogenous TRPM2 channels [3,5,22]. For example, in cultured rat striatal neurons expressing endogenous TRPM2, H_2O_2-induced cell death can be prevented by transfecting TRPM2-S as a dominant negative protein to suppress the activity of functional TRPM2 channels [20]. TRPM2 is also involved in cell death induced by inflammatory cytokines that are generated during oxidative stress, such as tumor necrosis factor-α-induced cell death of insulinoma RIN-5F and monocytic U937, and amyloid β-peptide-induced death of striatal neurons. As anticipated, such cell death can be reduced by TRPM2-S over-expression, TRPM2 siRNA, or inhibitor of ADPR formation [33,57–59]. Our lab have recently reported that anti-TRPM2 blocking antibody showed a protective effect against high concentration H_2O_2-induced apoptosis of vascular endothelial cells [60]. Together, the increasing evidence confirms a critical role of TRPM2 in oxidant stress-related cell death.

Ca^{2+} overload following TRPM2 activation and Ca^{2+} influx is the widely assumed mechanism to lead to cell destruction. A detailed study on oxidative stress-induced cardiomyocyte death has revealed that TRPM2 activation induces Ca^{2+} overload, followed by disruption of mitochondrial Ca^{2+} homeostasis, loss of mitochondrial membrane potential, release of cytochrome c, chromatic condensation or nuclear fragmentation, and eventually cell apoptosis [61].

4.4 TRPM2-mediated lysosomal Ca^{2+} release

In addition to the function as a Ca^{2+} influx channel in plasma membrane, studies provide new evidence that TRPM2 may also function as an intracellular Ca^{2+} release channel [3,8,23] (Fig. 1). Intracellular delivery of ADPR causes an increase in $[Ca^{2+}]_i$ in HEK293 cells that heterologously overexpressed TRPM2 and bathed in a Ca^{2+}-free extracellular medium, indicating that the ADPR-evoked $[Ca^{2+}]_i$ rise is due to Ca^{2+} release from intracellular stores. Such ADPR-evoked Ca^{2+} release has also been observed in a pancreatic β-cell line INS-1 and the primary mouse pancreatic β-cell expressing endogenous TRPM2. Intracellular TRPM2 in pancreatic β-cell is predominantly located in lysosomes, which suggests that TRPM2 mediates Ca^{2+} release from lysosomes [23]. In addition, the H_2O_2-induced cell death is found to be reduced in cells lacking TRPM2 even when the cells are bathed in Ca^{2+}-free medium, further supporting the notion that TRPM2 also serves as a lysosomal Ca^{2+}-release channel contributing to the oxidative stress-induced cell death [23]. Another study has showed that TRPM2 is present in late endosomes and lysosomes in dendritic cells and plays an important role in regulating dendritic cell chemotaxis [55].

4.5 TRPM2 and cardiovascular diseases

TRPM2 channels are likely to be involved in a number of pathophysiological processes in oxidative stress-induced cardiovascular injury [62,63]. In endothelial cells, TRPM2 mediates ROS-induced Ca^{2+} entry, altering endothelial barrier function, resulting in an increased endothelial permeability [63–65]. The increased

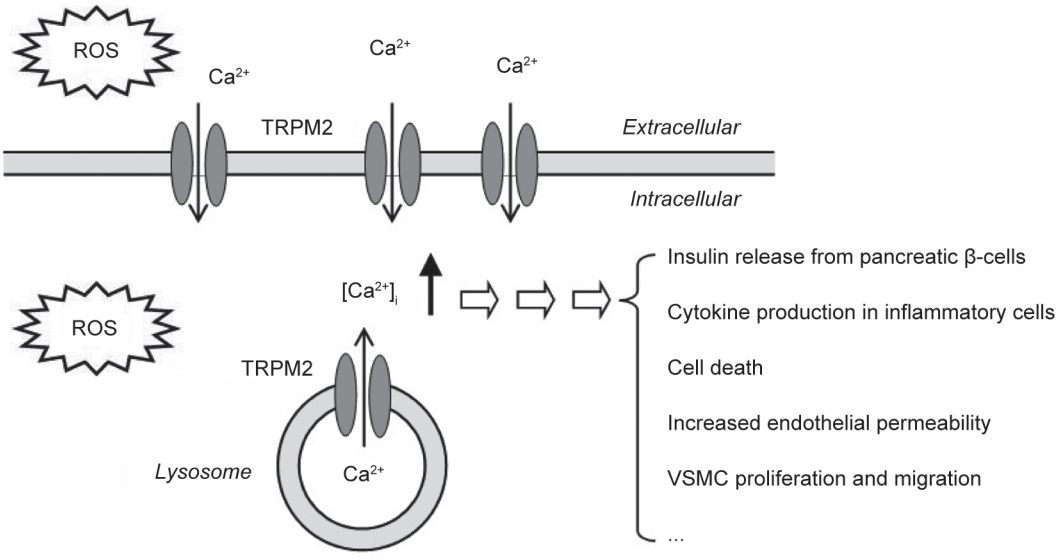

Fig. 1. Schematic representation showing the functional role of TRPM2 channel as an oxidative stress sensor. Oxidative stress-induced activation of TRPM2 mediates Ca^{2+} influx and/or lysosomal Ca^{2+} release. The increased $[Ca^{2+}]_i$, as a result, triggers several physiological and pathological events. ROS: reactive oxygen species; VSMC: vascular smooth muscle cell.

endothelial permeability plays a significant role in the pathophysiology of various cardiovascular diseases such as atherosclerosis, hypertension and heart failure, all of which involves oxidative stress and endothelial barrier dysfunction [65,66]. Our lab reported that H_2O_2 activates TRPM2 to induce Ca^{2+} influx and apoptosis of vascular endothelial cells [60]. It is widely known that endothelial injury is often the trigger for subsequent vascular pathologies. The functional role of TRPM2 in vascular smooth muscle cells has not been known. Recently, our lab found that TRPM2 contributes to neointimal hyperplasia by modulating ROS-stimulated vascular smooth muscle cell proliferation and migration (unpublished data). Neointimal hyperplasia, as a hallmark of arteriosclerosis, narrows vascular lumen, resulting in occlusive vascular diseases. We found that inhibiting TRPM2 substantially reduces the neointimal hyperplasia in both human and rodent blood vessels (unpublished data). In addition, TRPM2 has been suggested to play a critical role in mediating myocardial ischemia/reperfusion injury [61]. Neutrophil TRPM2 is implicated in the neutrophil accumulation in reperfused area and the exacerbation of myocardial ischemia/reperfusion injury [67].

5 Closing remarks

To conclude, TRPM2 is a Ca^{2+}-permeable cation channel that is activated by ADPR, ROS and Ca^{2+}, and serves as a cellular sensor for oxidative stress. Previous studies have implicated the involvement of TRPM2 in many physiological and pathological processes, such as insulin release, cytokine production, and oxidative stress-related cell death and cardiovascular injury. Oxidative stress causes cell damage and plays a major role in the development of numerous human diseases. The identification of TRPM2 as an oxidative stress-activated Ca^{2+} channel enables better understanding of the crosstalk between Ca^{2+} and oxidative stress, and the subsequent physiological responses and pathologies. Notably, *Trpm2* knockout mice are viable and fertile, and that there is no observable difference between *Trpm2* knockout and wild-type mice in general appearance, body weight, locomotion and behavior. Thus, inhibition of TRPM2 channel is expected not to be life-threatening, which indicates the feasibility of TRPM2 as a promising therapeutic target for oxidative stress-related disorders. For future research direction, it should be valuable to further explore the mechanisms that can modulate the expression and activity of TRPM2 channels, and to elucidate the signaling pathways upstream and downstream of TRPM2.

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